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THE PROBLEM OF ESCALATING RESISTANCE OF *HAEMONCHUS CONTORTUS* TO THE MODERN ANTHELMINTICS IN SOUTH AFRICA

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ABSTRACT

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During the past decade in South Africa there has been a continual increase in sheep of strains of gastro-intestinal helminths resistant to the modern anthelmintics.

Five strains of *Haemonchus contortus* are described in this paper. Despite the fact that 2 of the 5 strains were tested for susceptibility only to ivermectin, a total of 10 instances of resistance were found. Four of the 5 strains were resistant to ivermectin, 2 to closantel, 2 to rafoxanide and 2 to the benzimidazoles. One of these strains was concurrently resistant to 3 different anthelmintic groups, namely, the ivermectins, the benzimidazoles and the salicylanilides.

Resistance to ivermectin developed in 2 strains of *H. contortus* after a history of only 3 treatments with this compound in one instance and 11 treatments in the other. In the latter case drenching with ivermectin was well interspersed with that of other anthelmintics. This rapid development of resistance suggests that there may be cross-resistance between ivermectin and another anthelmintic group.

Two of the ivermectin resistant strains were recovered from separate properties in the south-western Cape Province, where *Ostertagia circumcincta*, which is usually the dominant parasite in this region, was virtually eliminated by the anthelmintic treatment. On each of these properties it was apparently replaced by a resistant strain of *H. contortus*.

A serious threat to control is the dissemination of worm strains with multiple resistance to anthelmintics. The strain of *H. contortus* resistant to 3 anthelmintic groups has already been widely dispersed, as the farmer concerned suddenly decided to give up farming with sheep and to sell his flock.

INTRODUCTION

Anthelmintic resistance was apparently first suspected in South Africa more than half a century ago. Mönnig (1937) mentioned reports by farmers that a mixture of nicotine and copper sulphate was no longer as effective against *Moniezia* spp. as when the mixture was first introduced. However, these early cases of suspected resistance were apparently not investigated further.

The first report of resistance to the more modern anthelmintics in South Africa originated from a farm near Boshoff in the Orange Free State, where a heavy burden of *Haemonchus contortus* was observed by the farmer 4 days after a lamb had been drenched with parbendazole (Berger, 1975). This compound had been used on the farm to the exclusion of other anthelmintics for a period of 6 years. The strain of *H. contortus* showed "moderate to marked" resistance to all 3 of the benzimidazoles available at that time, but was susceptible to levamisole and haloxon (Berger, 1975). At much the same time strains of *H. contortus* at Onderstepoort and the adjacent experimental farm, Kaalplaas, showed resistance to parbendazole, mebendazole and thiabendazole (Van Wyk, personal observations, 1974 and 1976).

Thereafter, 1 case each of resistance of *H. contortus* against rafoxanide (Van Wyk & Gerber, 1980) and closantel was reported (Van Wyk, Gerber & Alves, 1982). This was followed by a report of 2 cases of *Ostertagia* spp. resistant to the benzimidazoles (Van Schalkwyk, Geyser & Resin, 1983) and a review listing 17 other occurrences of benzimidazole resistance concerning *H. contortus* (Van Schalkwyk, 1984). In 1986 an unpublished report appeared, indicating that resistance had been found to ivermectin in the south-western Cape Province (Anonymous, 1986), and Van Wyk, Malan, Gerber & Alves (1987) reported 2 further field strains of *H. contortus* concurrently resistant to rafoxanide and the benzimidazoles. The latter authors observed that of the anthelmintics registered for use in sheep at that time the anthelmintic efficacy of only disophenol, levamisole, morantel and trichlorphon had not been proved to be affected by nematode resistance in South Africa.

Resistance is not limited, however, to the nematodes of sheep. In 1987 mention was made of a property on which the Cyathostominae of horses were resistant to the benzimidazoles (Van Wyk, 1987), and Visser, Van Schalkwyk & Kotze (1987) recorded the occurrence of resistance of *Moniezia expansa* in sheep and possibly also in goats to this group of anthelmintics.

This paper describes experiments conducted with 5 strains of *H. contortus* to determine resistance to ivermectin, closantel, rafoxanide and the benzimidazole anthelmintics. Preliminary results have been reported by Van Wyk & Malan (1988). The purpose of the present paper is to report the detailed results for a full discussion of the observations and of their implications for control strategies.

GENERAL MATERIALS AND METHODS

With the exception of Strain 3 (Stellenbosch) and Strain 6 (susceptible laboratory strain), the various strains of *H. contortus* used in these investigations were collected on farms and isolated in our laboratories for anthelmintic testing. The infective larvae (L3), isolated from field samples of faeces, were used to infest worm-free donor sheep. The faeces of these sheep were cultured and the L3 used in the various experiments were obtained from these cultures. Consequently, these strains were passaged only once in the laboratory before the trials were commenced, and they were not selected with anthelmintics in the laboratory. The Stellenbosch strain was isolated by Messrs MSD (Pty) Ltd.

The sheep used in 5 of the 6 experiments were not raised under worm-free conditions, but were obtained from the field. Those used in the ivermectin control trial, in which a benzimidazole-susceptible strain of *H. contortus* was used, were raised on concrete floors under

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TABLE 1 Particulars of the anthelmintics used to assess resistance in various strains of *Haemonchus contortus*

Active ingredient	Trade name	Manufacturer	Dosage* (mg/kg)
Closantel	Flukiver/Seponver**	Janssen	10,0/5,0**
Fenbendazole	Panacur 2,5 %	Hoechst	5,0
Fenbendazole + trichlorphon	Panther	Hoechst	5,0 + 62,4
Ivermectin	Ivomec	MSD	0,2
Levamisole	Ripercol	Janssen	7,5
Oxfendazole	Synanthic	MSD	5,0
Rafoxanide	Ranide	MSD	7,5

* Recommended dosage rate for sheep in South Africa

** The active ingredient of both Flukiver and Seponver is closantel (5 % and 2,5 % concentration respectively), recommended at dosages of 10 mg/kg and 5 mg/kg respectively. We used Flukiver at a dosage of 5 mg/kg because no assayed Seponver was available to us at the time of the trial

conditions of minimal exposure to worms. Before commencement of each experiment the sheep were dewormed with either levamisole at a dosage of 15–20 mg/kg live mass (Strains 1–3 and 5–6) or with both fenbendazole at 10 mg/kg and levamisole at 7,5 mg/kg live mass (Strain 4). Subsequent faecal examinations of every sheep by total flotation of 5 g of faeces (Whitlock, 1959) failed to reveal any worm eggs. During the course of each trial the sheep were kept under conditions that precluded unintentional exposure to gastro-intestinal nematodes.

The number of larvae used for infestation was calculated from aliquots of larval suspension. However, it can be seen from the results that the number of larvae of Strain 6 dosed to the sheep were underestimated by this method.

Unless otherwise stated, the methods used for faecal egg counts, faecal cultures and infestation of animals were as described by Reinecke (1973). The abomasal ingesta were concentrated by serial sieving through mesh sizes 150 and 38 μm, whereafter the residues in both were retained for worm recovery. While the abomasal digests were examined *in toto* as suggested by Reinecke (1973), worm counts of the abomasal ingesta differed as follows from his recommendations: initially the worms were counted in a single 10 % aliquot of the ingesta of each abomasum; subsequently, total worm counts were done for—

(i) the control sheep with the median worm count,

(ii) the sheep with counts immediately above and below this count, and

(iii) some of the treated sheep, as indicated with asterisks in the tables.

All the anthelmintics used in the experiments (Table 1) had previously been assayed by the various pharmaceutical companies concerned in order to confirm the concentration of each active ingredient listed on the labels. The actual dosages were calculated from the assayed concentrations.

The same basic experimental design was used in each of the anthelmintic efficacy trials, and thus the details are tabulated only for the Sheepmoor strain (Table 2).

Starting 3 weeks after infestation, faecal worm egg counts were carried out on 3 occasions before the day of treatment. On the day of treatment the mass of each sheep was determined, and the sheep were ranked according to mean egg counts and allocated to the various experimental groups with the aid of tables of random numbers. The method of allocation in those trials in which unequal experimental groups were used is outlined by Van Wyk & Gerber (1980).

In those experiments where sufficient animals were used, the results were evaluated with the non-parametric method (NPM) of Groeneveld & Reinecke (1969), as modified by Clark (Reinecke, 1973).

1. Sheepmoor strain

History

The farm of origin of this strain of *H. contortus* is situated near Sheepmoor in the south-eastern Transvaal. Grazing consisted mostly of unimproved veld, with some patches of dryland improved grazing. Worm control consisted entirely of intensive drenching with anthelmintics.

The farmer sought help when it appeared to him that anthelmintic drenching did not reduce haemonchosis in accordance with the reputed efficacy of the remedies concerned. The worm strain involved was subsequently isolated in the laboratory from sheep faeces submitted by the farmer.

Materials and methods

Each sheep was infested daily with approximately 1 000 L3 of *H. contortus* on Days −29, −28 and −27; treatment occurred on Day 0 and the sheep were killed for worm recovery on Day +7 (Table 2).

TABLE 2 Experimental design of the anthelmintic efficacy trial on the Sheepmoor strain of *Haemonchus contortus*

Day	Treatment
−29	33 sheep each dosed with ± 1 000* L3 *H. contortus*
−28	33 sheep each dosed with ± 1 000 L3 *H. contortus*
−27	33 sheep each dosed with ± 1 000 L3 *H. contortus*
0	12 sheep drenched with closantel at 5,0 mg/kg 4 sheep drenched with rafoxanide at 7,5 mg/kg 4 sheep drenched with oxfendazole at 5,0 mg/kg 4 sheep drenched with levamisole at 7,5 mg/kg 9 sheep remained untreated as controls
+7	33 sheep killed for worm recovery

* Estimated from aliquots of larval suspension

Results

The mean efficacy of closantel was Class B (NPM), or 80,8 % (arithmetic mean reduction of the worm burdens compared with those of the untreated control sheep; Table 3). In the case of the other anthelmintics tested, too few sheep were used for analysis of the results by the NPM. The arithmetic mean efficacies of rafoxanide, oxfendazole and levamisole were 45,4 %, 79,0 % and 99,9 % respectively (Table 4).

Comment

Closantel was less effective than the registered claim in South Africa—Class A or more than 80 % effective in

TABLE 3 Sheepmoor strain—*Haemonchus contortus* recovered from untreated controls and from sheep treated with closantel

Numbers of *H. contortus* recovered	
Untreated (Control group)	Closantel (5,0 mg/kg)
1 477	32
1 484	53
1 873	73
2 269*	92
2 527*	114
2 580*	188
2 707*	400
2 808	562*
3 538	574*
	901*
	980
	1 467
Mean: 2 362	Mean: 453
Median: 2 527 × 0,4 = **1 011**	NPM: 1/12 > 1 011: Class B

NPM efficacy: Class B
Arithmetic mean efficacy: 80,8 %
* Total worm counts

TABLE 4 Sheepmoor strain—*Haemonchus contortus* recovered from untreated controls and from sheep treated with rafoxanide, oxfendazole and levamisole

Numbers of *H. contortus* recovered			
Untreated (Control group)	Rafoxanide (7,5 mg/kg)	Oxfendazole (5,0 mg/kg)	Levamisole (7,5 mg/kg)
1 477	904	100	0
1 484	1 228*	522*	0
1 873	1 383*	618*	2
2 269*	1 648	749*	23
2 527*	Mean: **1 290**	Mean: **497**	Mean: **6**
2 580*			
2 707*			
2 808			
3 538			
Mean: **2 362**			
Arithmetic mean efficacy:	Rafoxanide: 45,4 %	Oxfendazole: 79,0 %	Levamisole: 99,9 %

* Total worm counts

more than 80 % of the treated flock (Table 3). For an "A"-claim only one treated sheep may have a worm burden larger than the median burden of the untreated controls multiplied by 0,25 (Reinecke, 1973). In this trial, however, 3 sheep had worm counts higher than the reduced control median (2 527 × 0,25 = 632), with the result that closantel did not qualify for an A-claim. On the other hand, as fewer than 4 sheep in the treated group had worm burdens larger than the median of the controls × 0,4 (= 1 011), the drug did qualify for a B-claim (Table 3).

Taken at face value, the efficacy of closantel (geometric mean efficacy 89,4 %; arithmetic mean 80,8 %) does not appear to be much reduced. However, it must be kept in mind that closantel has a considerable residual action against *H. contortus*, with more than 80 % efficacy (NPM Class A) against new infestations acquired by sheep up to 21 days after a single treatment at 5 mg/kg live mass. In the present trial the anthelmintic failed to achieve Class A efficacy against an established infestation. Thus even the immediate efficacy is reduced, and although not tested, there can be no question of effective residual efficacy against this strain of *H. contortus*.

Although only 4 sheep were treated with rafoxanide at 7,5 mg/kg, it is clear from Table 4 that large numbers of worms were still present, giving a reduction on the arithmetic mean of only 45,4 %, compared to a registered efficacy in South Africa of Class A (>80 % effective in >80 % of the treated flock). Oxfendazole was likewise only 79 % effective compared to virtually 100 % efficacy in registration trials involving a susceptible strain of *H. contortus*. In contrast, the efficacy of levamisole was practically 100 % and it is obvious that this compound is not affected by the resistance exhibited by the Sheepmoor strain of *H. contortus*.

2. Cullinan strain

History

Ivermectin was drenched on only 3 occasions to the sheep on this farm: in October and December 1985, and during May 1986. However, the flock was not closed, as a stud ram was introduced from elsewhere shortly before the first drench with ivermectin, and another ram subsequently, and during 1984 emergency grazing was provided for 20 sheep from Warmbad.

The sheep grazed mainly on unimproved pasture, but for a short period each day they grazed in a vlei (marshy area) consisting mainly of kikuyu grass. As regards the epidemiology of gastrointestinal worm infestation, this area was thus similar to pasture under irrigation.

Drenching since 1984 consisted of rafoxanide[1] at intervals as short as 3 weeks during 1984, becoming less frequent thereafter. On occasions closantel[2] was alternated with the rafoxanide, and albendazole[3] was drenched a few times between the other treatments.

It was decided to investigate this strain of *H. contortus* when faecal worm egg counts remained reasonably high after the ivermectin treatment of May 1986.

TABLE 5 Cullinan strain—*Haemonchus contortus* recovered from untreated controls and from sheep treated with ivermectin

Numbers of *H. contortus* recovered	
Untreated (Control group	Ivermectin (0,2 mg/kg)
366	100
628	285
1 283*	303
1 560*	386
1 967*	409
2 017*	420
2 048	460
2 090	499
3 016	509*
	609*
	626*
	661*
	687*
Mean: 1 664	Mean: **458**
Median: 1 967 × 0,4 = **787**	NPM: 0/11 > 787: Class B

NPM efficacy: Class B
Arithmetic mean efficacy: 72,5 %
* Total worm counts

[1] Ranide (MSD)
[2] Flukiver (Janssen)
[3] Valbazen (SmithKline)

Materials and methods

Twenty-two sheep were each infested with about 1 100 L3 of *H. contortus* on Days −30, −29 and −28. Thirteen were treated with ivermectin on Day 0, and the remaining 9 sheep were left as untreated controls. All 22 sheep were killed on Day +7 for worm recovery.

Results

Ivermectin failed to obtain a Class A NPM efficacy in this trial, as 7 of the 13 treated sheep had worm burdens greater than the reduced control median (1 967 × 0,25 = 492; Table 5).

Comment

Despite only 3 previous drenches with ivermectin this strain of *H. contortus* exhibited a fair degree of resistance to the compound. The efficacy was only 72,5 %, compared with almost complete elimination of this worm species in the registration trials.

4. White River Krtz strain

History

The farm of origin of this strain of *H. contortus* is situated near White River in the Lowveld of the Transvaal, and has a mean monthly maximum temperature of 35 °C for the warmest month of the year. The annual rainfall is 720–1 500 mm, and grazing consisted of kikuyu grass under irrigation. The sheep were rotated every 3–4 days on camps rested for only about 3 weeks at the height of summer, which is also the most dangerous time for haemonchosis.

Sheep have been farmed here since 1983. After a complaint by the farmer, an investigation during 1985 showed resistance by *H. contortus* to rafoxanide and the benzimidazole anthelmintics (Van Wyk *et al.*, 1987). At that time there was no indication that resistance to ivermectin could have developed on this farm, with the result that it was not included in the initial investigations.

During subsequent routine monitoring of faecal egg counts, *Haemonchus* ova were encountered a few days after the sheep had been drenched with ivermectin, so it was decided to investigate the possibility of resistance to ivermectin.

The anthelmintics administered by the farmer during the previous 3 years are listed in Table 6.

Over the total period of 3,5 years vermifuges were drenched on average every 3,5 weeks (Table 6), increas-

TABLE 7 White River strain—*Haemonchus contortus* recovered from untreated controls and from sheep treated with ivermectin, closantel, fenbendazole, fenbendazole plus trichlorphon, and levamisole

Numbers of *H. contortus* recovered	
Untreated (Control group)	Ivermectin (0,2 mg/kg)
943	943
1 983	1 867
3 350	1 990
3 440	2 040
3 990	2 347
Mean: **2 741**	Mean: **1 837**
Levamisole (7,5 mg/kg)	Closantel (5,0 mg/kg)
0	840
0	1 320
0	1 527
0	1 955
0	2 407
Mean: **0**	Mean: **1 610**
Fenbendazole + trichlorphon (5 mg/kg) + (62,4 mg/kg)	Fenbendazole (5,0 mg/kg)
3	670
10	1 800
143	2 537
307	2 753
397	2 917
Mean: **172**	Mean: **2 135**
Arithmetic mean efficacy:	Ivermectin: 33,0 % Closantel: 41,3 % Fenbendazole: 22,1 % Fbz + trichl: 93,7 % Levamisole: 100,0 % Rafoxanide*: 13,9 %

* Van Wyk *et al.* (1987)

TABLE 6 Drenching history—White River Krtz farm

Date	Anthelmintic	Date	Anthelmintic
1983		1985/01/08	Closantel*
		02/04	Rafoxanide
Alternate every 30 days	Closantel	02/25	Levamisole
	Mebendazole	03/18	Oxfendazole
	Albendazole	04/08	Rafoxanide
		04/29	Rafoxanide
		05/20	Levamisole
		06/10	IVERMECTIN
1984/01/17	Closantel*	07/01	Levamisole
02/17	IVERMECTIN	07/15	IVERMECTIN
03/09	Levamisole	08/05	Rafoxanide
03/26	Levamisole	09/09	Levamisole
04/13	Levamisole	09/23	Levamisole
05/01	Levamisole	10/14	IVERMECTIN
05/24	IVERMECTIN	11/04	Levamisole
06/13	Levamisole	11/25	Levamisole
06/29	Levamisole	12/16	IVERMECTIN
07/16	Levamisole		
08/15	IVERMECTIN	1986/01/06	IVERMECTIN
09/11	Levamisole	01/20	Levamisole
10/08	Levamisole	02/10	IVERMECTIN
11/15	Levamisole	03/06	Levamisole
12/03	IVERMECTIN	03/31	Levamisole
		04/21	IVERMECTIN
		05/19	Closantel**
		06/05	Levamisole

* Dosage: 10 mg/kg
** Dosage: 5 mg/kg

ing during the last year of this period to an average of every 3 weeks. Ivermectin was drenched 11 times, levamisole 21 times, rafoxanide 4 times, and closantel and the benzimidazoles about 8–9 times each.

Materials and methods

Thirty 6–7-month-old Merino lambs were dewormed with both fenbendazole at a dosage rate of 10 mg/kg and levamisole at 7,5 mg/kg live mass. Subsequently they were each infested on Day −28 with about 5 000 L3 of the White River Krtz strain of *H. contortus* suspended in water. On Day 0 the sheep were divided into 6 similar groups consisting of 5 untreated control sheep, and 5 sheep for each of the following treatments: closantel, ivermectin, fenbendazole, fenbendazole + trichlorphon, and levamisole. The sheep were killed for worm recovery from Day +7 to Day +9.

Results

The efficacy (based on the reduction of the arithmetic mean worm burden compared with the controls) was 33,0 % for ivermectin, 41,3 % for closantel, 22,1 % for fenbendazole, 93,7 % for fenbendazole + trichlorphon, and 100 % for levamisole (Table 7).

Comment

It is obvious from these results that the White River strain of *H. contortus* is highly resistant to ivermectin, closantel and the benzimidazoles, while Van Wyk *et al.* (1987) showed it to be resistant also to rafoxanide.

3. Stellenbosch strain

History

As mentioned by Van Wyk *et al.* (1987), this strain of *H. contortus* was discovered by Visser and Schneider (Anon., 1986) on a farm in the south-western Cape. This is the first reported case involving resistance to ivermectin, and it was suspected because of poor reduction in faecal worm egg counts after treatment. This strain of *H. contortus* was included in the present series of investigations as it was considered essential to have further confirmation of this important discovery.

The grazing on the farm of origin consisted principally of improved kikuyu pastures under irrigation. Ivermectin was introduced into the dosing programme because it was suspected that the *Ostertagia* spp. on the farm were resistant to the benzimidazole group of anthelmintics.

TABLE 8 Stellenbosch strain—*Haemonchus contortus* recovered from untreated controls and from sheep treated with ivermectin

Numbers of *H. contortus* recovered	
Untreated (Control group)	Ivermectin (0,2 mg/kg)
11	0
133	0
204	31
500*	62
538*	117
645*	172
706*	198*
873	281*
1 239	281*
	290*
	302*
	372*
	437*
Mean: 539 Median: 538 × 0,5 = **269**	Mean: 196 NPM: 6/13 > 269: Class X

NPM efficacy: Class X (ineffective)
Arithmetic mean efficacy: 63,6 %
* Total worm counts

The farmer reported having drenched with ivermectin 7 times in the 10 months preceding the discovery of resistance, and a total of 19 times over a period of 2 years (Carmichael, personal communication, 1986).

Materials and methods

The 32 experimental sheep were individually infested with approximately 1 000 larvae of *H. contortus* per day on Days −30, −29 and −28. Nine sheep were left untreated as controls, and 23 were treated on Day 0 with either ivermectin, rafoxanide, oxfendazole, or levamisole. All of the sheep were slaughtered for worm recovery on Day +7.

Results

Ivermectin obtained an NPM efficacy rating of Class X, with an arithmetic mean efficacy of 63,6 % (Table 8).

Rafoxanide, oxfendazole and levamisole reduced the worm burdens by 99,9 %, 97,9 % and 99,1 % respectively (Table 9).

TABLE 9 Stellenbosch strain—*Haemonchus contortus* recovered from untreated controls and from sheep treated with rafoxanide, oxfendazole and levamisole

Numbers of *H. Contortus* recovered	
Untreated (Control group)	Rafoxanide (7,0 mg/kg)
11	0 0 1 Mean: **0,3**
133	
204	Oxfendazole (5,0 mg/kg)
500*	0 10 14 20 Mean: **11**
538*	
645*	
706*	Levamisole (7,5 mg/kg)
873	0 0 10 10 Mean: **5**
1 239	
Mean: 522	
Arithmetic mean efficacy:	Rafoxanide: 99,9 % Oxfendazole: 97,9 % Levamisole: 99,1 %

5. Swellendam strain

History

Two ewes from a flock of 254 sheep in the Swellendam district in the southern Cape died from haemonchosis 15 days after treatment with ivermectin by the owner. The worm egg count of one of these sheep was reported to be 24 000 eggs per g of faeces, with 22 000 *H. contortus* being recovered from its abomasum.

The pasture on which the ewes grazed consisted of improved dryland grazing, but as the farm is situated on the rainy, windward side of mountain range, the rainfall is regular and stable. During the preceding 2 years ivermectin had been drenched intensively to the exclusion of other remedies.

Materials and methods

Ten sheep were each infested daily on Days −30, −29 and on −28 with about 1 100 L3 of *H. contortus*.

On Day 0 half the sheep were treated with ivermectin, the remainder serving as untreated controls. All the sheep were killed for worm recovery on Day +7.

The worm counts were derived from an examination of 2 × 1/10 aliquots of abomasal ingesta, as well as an examination of the entire digested abomasal mucosa.

Results

Treatment with ivermectin resulted in an arithmetic mean reduction of 51,2 % in the worm burden (Table 10).

TABLE 10 Swellendam strain—*Haemonchus contortus* recovered from untreated controls and from sheep treated with ivermectin

Numbers of *H. contortus* recovered	
Untreated (Control group)	Ivermectin (0,2 mg/kg)
1 532	859
2 144	866
2 145	1 033
2 606	1 161
2 770	1 545
Mean: **2 239**	Mean: **1 093**

Arithmetic mean efficacy: 51,2 %

Comment

Too few sheep were treated for the results to be evaluated by the non-parametric method, but it is clear that the strain is highly resistant to a therapeutic dose of ivermectin. It can be calculated from the present data that the NPM efficacy classification is certainly lower than Class B[4]. Thus a maximum of Class C (moderately effective), or perhaps even Class X (ineffective) may be expected in a full trial in which sufficient sheep are used for NPM evaluation.

6. Onderstepoort reference strain

History

This strain of *H. contortus* was isolated by Reinecke prior to 1968 and has subsequently been maintained at the Veterinary Research Institute, Onderstepoort, as a susceptible reference strain for anthelmintic testing. It has also been distributed to the various private concerns conducting anthelmintic efficacy trials in this country. It has not been selected with anthelmintics in the laboratory.

Materials and methods

Eight sheep were used in the experiment. Each was infested with approximately 1 000 L3 *H. contortus* daily on Days −28, −27 and −26; on Day 0 half the sheep were treated with ivermectin and the rest remained untreated as controls. All the sheep were killed for worm recovery on Day +8.

Results

Compared with a mean worm buden of 3 266 for the untreated control animals, a total of 4 worms was recovered from the 4 sheep treated with ivermectin (Table 11). The efficacy (based on a reduction of the arithmetic mean burden) was thus 99,97 %.

[4] For a Class B efficacy only 3 of 11 treated sheep may have a worm count higher than that of the control median × 0,4 (in this trial 2 145 × 0,4 = 858). Not one of the 5 treated sheep in this trial had a worm burden lower than this reduced control median.

TABLE 11 Onderstepoort reference strain—*Haemonchus contortus* recovered from untreated controls and from sheep treated with ivermectin

Numbers of *H. contortus* recovered	
Untreated (Control group)	Ivermectin (0,2 mg/kg)
2 630	0
3 278	1
3 536	1
3 618	2
Mean: **3 266**	Mean: **1**

Arithmetic mean efficacy: 99,9 %

Comment

It is obvious that the numbers of infective larvae dosed to the experimental sheep were underestimated, as the mean number of worms recovered was higher than the estimate of the total number of larvae administered.

This trial was conducted to assess the efficacy of the batch of ivermectin used throughout the present series of experiments against a susceptible strain of *H. contortus*. Clearly, the efficacy is exceptionally good, as the initial registration trials showed.

DISCUSSION

Two of the 5 resistant strains of *H. contortus* were tested for susceptibility only to ivermectin. Among the remaining 3 strains 8 instances of resistance were found: to ivermectin in 2 strains; to closantel in 2; to rafoxanide in 2; and to the benzimidazoles in 2; with 1 strain resistant to 3 separate anthelmintic groups. The White River strain is apparently the first that is less than 50 % susceptible to closantel at a dosage rate of 5 mg/kg live mass.

Closantel

As discussed by Van Wyk *et al.* (1987), it was to be expected that resistance should have developed to closantel since:

1. It has a marked residual effect against *H. contortus*, probably leading to longer and more severe selection for resistance;
2. It has been marketed in South Africa for more than 9 years; and
3. It is chemically related to rafoxanide which has been used widely and at times intensively in South Africa for more than 15 years.

Ivermectin

The numbers of strains of *H. contortus* resistant to ivermectin merit careful consideration. Four of the 5 strains investigated by us showed various degrees of resistance to this drug. In addition, and despite the fact that no organised search has been made, we are aware of 5 other strains for which preliminary results strongly indicate resistance (Table 12). These 9 strains originate from 3 of the 4 provinces of South Africa and resistance is thus widely disseminated, although it cannot be said to be common. There are, however, likely to be more strains than these that have come to light more or less by chance.

Why should resistance to ivermectin have been expressed so rapidly in so many strains of *H. contortus*?

Swellendam and Stellenbosch strains

There are some obvious factors that may have favoured the development of resistance to ivermectin in some of the 5 worm strains investigated. As discussed by Van Wyk & Malan (1988), anthelmintics are often used at intervals of 3 weeks or less in sheep on irrigated pastures in South Africa, the most modern compound often being used to the exclusion of other remedies.

TABLE 12 A summary of information on the spectra of resistance of 7 strains of *Haemonchus contortus*†

Strains of *H. contortus*	Efficacy (%)		
	Ivermectin	Rafoxanide	Benzimidazoles
White River	33	14	22
Pietermaritzburg#	51	87	0
Cedara††	94	77	16
Swellendam	51	98**	NI*
Cullinan	73	95	NI
Stellenbosch	64	99	98
EVW (Pta)	RE***	RE	RE

† Resistance to ivermectin has also been shown in 2 further strains of *H. contortus* (Ceres and Caledon) but the results are the confidential property of the firm concerned, and we have not been able as yet to repeat the trials for making the efficacy spectra known

* NI: no information

** Hyman (Ciba Ceigy): unpublished information, 1988

*** RE: reduced efficacy, based on preliminary faecal egg counts in the field

\# Based on reduction of the faecal egg count (Anon., 1987; Bath, Personal communication, 1987)

†† Unpublished results (Van Wyk, Gerber, Visser, Alves & Bath, personal observations, 1988)

H. contortus has a minimum prepatent period of 18–21 days (Reinecke, 1973) and ivermectin has a residual action of a few days when drenched *per os* (Van Wyk, personal observation, 1985). Thus drenching with ivermectin at intervals of 21 days does not allow susceptible *H. contortus* the opportunity to produce ova and to propagate susceptible individuals. This results in a very high selection pressure.

In the light of these facts it is understandable that the strains of *H. contortus* from Stellenbosch and Swellendam developed resistance, as ivermectin was used intensively and to the exclusion of other anthelmintics. What was surprising, however, is that it was *H. contortus* that became resistant in these districts which fall in the winter-rainfall region and normally have few if any problems with haemonchosis. *Ostertagia* spp. infestations in sheep predominate to the extent that Reinecke (1983) states: "*H. contortus* . . . is absent in winter-rainfall areas . . .". It is known that prior infestations with *Ostertagia* spp. have a suppressive effect on *H. contortus*, which may explain this phenomenon (Turner, Kates & Wilson, 1962).

Not a single *Ostertagia* spp. worm was recovered from sheep artificially infested with either of these field isolates for the 2 anthelmintic efficacy trials, this despite the fact that in the case of the Stellenbosch isolate the farmer had initially switched to drenching with ivermectin because resistance of *Ostertagia* spp. was suspected.

The *Ostertagia* strains present before the introduction of ivermectin were apparently unable to develop resistance to ivermectin, and, being virtually annihilated by the intensive drenching, released their suppressive effect on *H. contortus*. In contrast, the 2 strains of *H. contortus* expressed resistance to ivermectin and developed unhinderedly to the extent that some sheep died from haemonchosis when the anthelmintic became ineffective. This observation was first made by Carmichael (personal communication, 1986).

Cullinan and White River strains

In the case of the strains of *H. contortus* from Cullinan and White River in the Transvaal, the situation was very different from that described above.

The history of a total of only 3 treatments with ivermectin on the Cullinan farm and 11 treatments well interspersed with other anthelmintic groups on the White River farm excluded continual, intensive selection with ivermectin to the exclusion of other compounds.

One or more of the following factors could have given rise to the resistance to ivermectin on the 2 Transvaal farms:

1. The strains were naturally tolerant to ivermectin;
2. The resistant strains were imported from elsewhere;
3. Resistance developed independently of resistance to other compounds; or
4. There is cross-resistance between ivermectin and another compound or compounds.

1. *Natural tolerance to ivermectin*

As the susceptibility of neither the Cullinan nor the White River strain was tested when ivermectin was used for the first time, the possibility of tolerance from the outset cannot be excluded. The same applies to almost every case of resistance that has been recorded worldwide. However, this seems to be most unlikely in the case of ivermectin in view of the consistent, well-nigh 100 % efficacy recorded thusfar globally against *H. contortus* (Armour, Bairden & Preston, 1982; Wescott & Leamaster, 1982; Yazwinski, Greenway, Presson, Pote, Featherstone & Williams, 1983), as also against the Onderstepoort strain in the present investigations.

2. *The resistant strains were imported from elsewhere*

The possibility exists that the resistant strains of *H. contortus* did not develop on the farms from which they were isolated, but were introduced by bringing in animals from other farms where the worm strains were resistant.

Cullinan. This farmer did not maintain a closed flock as he supplied emergency grazing for 20 sheep from the Warmbad district during 1984 and brought in 2 stud rams during 1985.

Stud breeders are inclined to deworm more intensively than breeders of commercial flocks, often opting for the more modern vermifuges (Van Wyk, unpublished observations, 1986), and hence the 2 rams could have harboured a resistant worm strain. It is questionable, however, whether worms introduced by only 1 or 2 animals (that arrived on the farm in the spring and summer of 1985) could have given rise to generalized resistance on the property within a single worm season: ". . . when resistant worms are transferred from one farm to another, the parasites may lose their survival advantage unless similar selection pressure is maintained." (Waller, 1985.) It is important to note that according to the drenching history, this farmer did not drench very intensively, except for a time during 1984, before the rams were introduced.

The other possible source of resistance is the group of 20 sheep given emergency grazing in 1984. However, it seems most unlikely that these sheep were the source of the resistant strain. They arrived on the farm less than a year after ivermectin had been registered for use in sheep, and there was no history of intensive drenching with this compound beforehand. Had there been, it is probable that the owner of the sheep would have continued with intensive drenching with this anthelmintic on the emergency grazing and that the Cullinan farmer would have been aware of ivermectin, which at the time was regarded by many as the ultimate answer to worm control. Instead, he did not use ivermectin until it was recommended by his advisers in October 1985, more than a year after the 20 sheep had arrived on the farm.

White River. Van Wyk *et al.* (1987) discussed the development of resistance to rafoxanide on this farm after only 4 drenches. They speculated that this strain of *H. contortus* was either already present on the farm when the farmer started the enterprise in 1983, or was introduced subsequently, as it appeared most unlikely that resistance could have developed from so few treatments. In the case of resistance to closantel the following must be considered: it is chemically related to rafoxanide; it has been on the South African market for a relatively long time; and resistance could have developed elsewhere and been introduced to the farm. This probably does not hold true for ivermectin, which was introduced to the South African market for use in sheep only in 1983. It seems well-nigh impossible that resistance to this compound could have developed on another property and then been introduced to the White River farm, thereafter managing to replace the local worm strain within such a short period of time, despite such regular drenching with particularly levamisole, which apparently retained full efficacy against this strain.

3. *Resistance developed independently of the resistance to other compounds*

There appear to be no convincing grounds for accepting this hypothesis.

Cullinan. According to the farmer, ivermectin was used only 3 times—in October and December 1986 and in May 1987. It appears unlikely that selection for resistance could have occurred under field conditions from only 3 treatments spread over 7 months, unless ivermectin resistance is coupled with resistance to other anthelmintics. This possibility is dicussed more fully below.

White River. The white River farmer, who kept meticulous records of drenching, used ivermectin more often, but with a single exception he consistently alternated anthelmintic groups (Table 6). The approximate intervals between drenches with ivermectin between 1984 and June 1986 when the investigation was conducted, were 14, 12, 15, 28, 5, 13, 9, 3, 5 and 10 weeks. If all the anthelmintics used are taken into account, then the mean interval between drenches over this period was only 2,9 weeks.

The anthelmintic that was alternated most often with ivermectin was levamisole. This remedy which was still fully effective when the investigation was conducted, was used 21 times during the 112 weeks, giving a mean interval between levamisole drenches of only 5,4 weeks. This should in itself have been almost sufficient to severely limit *H. contortus*, particularly if the distribution of the levamisole drenches is more closely scrutinized.

In the fall, winter, spring and early summer of 1984 levamisole was used a mean of once every 3,5 weeks. This was certainly sufficient to control *H. contortus*, even disregarding the 2 ivermectin drenches used additionally during this period. Thereafter, no levamisole was drenched for 27 weeks during the summer and fall of 1984/1985, during which time ivermectin was used only once. It thus seems highly unlikely that much selection for resistance to ivermectin could have occurred prior to the winter of 1985.

What remains therefore, is the period of 12 months from the winter of 1985 until isolation of the strain of *H. contortus* in June 1986. During this time the sheep were drenched a mean of every 21 days, and with 2 exceptions only ivermectin and levamisole were alternated, ivermectin being drenched 7 times and levamisole 9 times. The chances were therefore better that selection for resistance to ivermectin could have occurred during this period. However, levamisole was alternated with ivermectin and this, coupled with the fact that there are probably only about 3 main generations of *H. contortus* annually in a mild region like White River, also seems to rule out the possibility that sufficient selection could have occurred during 1985/1986 to manifest as a high degree of resistance by June 1986.

In summary, there seems to be little evidence supporting the hypothesis that ivermectin resistance developed independently of resistance to other compounds on the White River farm.

4. *There is cross-resistance between ivermectin and another compound or other compounds*

If it is accepted that the 2 strains were not naturally tolerant to ivermectin; that selection for ivermectin resistance did not occur independent of resistance against other compounds; and that the strains were not imported from elsewhere, then practically the only explanation that remains is that there is cross-resistance between ivermectin and another drug or drugs.

Although insufficient work has been done to confirm this hypothesis, there is some evidence to support such a possibility.

Table 12 is a summary or information on the spectra of resistance of 7 strains of *H. contortus* showing reduced susceptibility to ivermectin. Some of the results are, however, of a preliminary nature, and in the case of the Cedara strain the reduction in susceptibility to ivermectin is only marginal. Of these 7 strains, 4 also show evidence of reduced susceptibility to rafoxanide, and 4 of the 5 strains for which information is available are also resistant to the benzimidazole group of anthelmintics.

The resistance of these strains to the benzimidazoles is much more severe than that to rafoxanide. This may indicate that if there is indeed a link between ivermectin resistance and that of another anthelmintic group it is more likely to be to the benzimidazoles than to rafoxanide. Benzimidazole resistance is more common and of longer standing in South Africa than that against rafoxanide, against which only a few cases of resistance have been found. Thus, if resistance did develop independently, one would not expect a large percentage of worm strains to be resistant concurrently to rafoxanide and ivermectin. In fact, while there is too little information to confirm this, it seems possible that, depending upon the region from which the strains originated, there may be a difference in the simultaneous occurrence of resistance to the 2 drugs. In the south-western Cape (Stellenbosch and Swellendam), where there is relatively little haemonchosis and where rafoxanide is thus probably drenched less frequently than in the summer-rainfall regions, both of the ivermectin-resistant strains showed undiminished susceptibility to rafoxanide. In contrast, 4 of the 5 strains of *H. contortus* from the summer-rainfall regions which showed signs of reduced susceptibility to ivermectin, were also less susceptible to rafoxanide.

Resistance to each group of anthelmintics may have developed independently from a common cause, namely, intensive drenching, with one anthelmintic after the other becoming less effective, necessitating repeated changes to other drugs. In such a situation, it seems possible that by the time a new compound comes on the market, a farmer may have had a worm strain resistant to most of the previously available remedies, and may already have been on the look-out for a new, effective chemical when the new arrival appeared on the scene. In such a case it would not be surprising if it appeared as if there was a link in the development of resistance between the various groups. This would not, however, explain the very rapid development of resistance to ivermectin in the Cullinan and the White River strains of *H. contortus*.

Clearly, the possibility of links between the development of resistance to the 3 anthelmintic groups requires further investigation before the hypothesis can be accepted or rejected.

CONCLUSIONS

The most disturbing results from these investigations are, firstly, that ivermectin resistance was found in 4 of the 5 strains of *H. contortus* investigated; and, secondly, that one of the strains was highly resistant to 3 of the 4 anthelmintic groups used most frequently in South Africa, only the levamisole/morantel anthelmintic group remaining unaffected.

It is probable that the problem will be compounded by the dissemination of strains that are resistant to so many anthelmintic groups that the farmers concerned fear that nothing will be effective and hence sell their stock. Already the owner of the White River farm has sold all his sheep without consulting his advisers. These sheep have been widely dispersed, and with them the *H. contortus* with the resistant genes. It is possible that on farms of destination where drenching is intense these genes will give rise to resistance to all 3 groups of anthelmintics.

The slaughter of sheep and quarantining of farms may be a means of eradicating resistant worm strains and preventing dissemination. However, resistant strains need not to be dispersed for resistance to develop elsewhere; on every farm where the resistant genes are present, resistant strains develop when drenching becomes intense. There is no test for determining worm populations that have resistant genes in low concentrations. Thus, unless resistance can be monitored everywhere, new strains will develop and become disseminated before regulating authorities can detect them and prevent their spread.

What would happen if in the future the remaining effective anthelmintics should also fail in South Africa! A strain of *H. contortus* with suspected resistance to levamisole and morantel is at present being investigated in our laboratories. In Australia, some milch-goat farmers have had to be informed that there were no anthelmintics left with which resistant worm strains could be controlled on their properties (Waller, 1985). At that stage neither closantel nor ivermectin was available in Australia, and there was still hope that these compounds could buy considerable time for the search for ways of overcoming resistance to anthelmintics. The evidence from the White River strain certainly seems to dispel such hopes, and the pressure is mounting to find a solution timeously.

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