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TWO FIELD STRAINS OF *HAEMONCHUS CONTORTUS* RESISTANT TO RAFOXANIDE

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ABSTRACT

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Two field strains of *Haemonchus contortus*, one from Pretoria on the Highveld and the other from White River in the Lowveld of the Transvaal, showed pronounced resistance to rafoxanide at a dosage rate of 7,5 mg/kg live mass *per os*. Both of these strains originated from kikuyu pastures under irrigation. Resistance in South Africa to rafoxanide and other anthelmintics, namely, closantel, ivermectin and the benzimidazoles is reviewed. It is concluded that apart from avoiding farming practices where integrated worm control is not possible, there is at present no solution to the problem of worm resistance in the country.

INTRODUCTION

The first report in South Africa of resistance of gastrointestinal nematodes to the modern anthelmintics appeared in 1975 (Berger, 1975). However, no systematic search has ever been conducted in South Africa for resistant strains, and in every case the resistance came to light because of complaints by consumers or during the course of routine investigations (Van Wyk & Gerber, 1980).

During the past decade resitance has escalated to the extent that many cases are occurring with ever increasing frequency, and we have already found resistance to an impressive array of anthelmintics and an increasing number of strains of *Haemonchus contortus*, *Ostertagia circumcincta* and *Moniezia expansa*.

This paper describes trials with 2 strains of *H. contortus* which were tested for resistance to rafoxanide and other anthelmintics.

MATERIALS AND METHODS

Unless otherwise stated, the methods used for faecal egg counts, faecal cultures and infestation of animals have been described by Reinecke (1973). The abomasum ingesta were concentrated by sieving through sieves having apertures of 150 μm onto sieves with apertures of 37 μm and retaining the residues in both for worm recovery.

EXPERIMENTAL INVESTIGATIONS

I. PRETORIA PTZR STRAIN

History

This strain was isolated from sheep grazing irrigated kikuyu pasture on a smallholding near Pretoria. From time to time between December 1983 and 1985, the flock was repeatedly enlarged by the introduction from elsewhere of a few sheep. Initially worm control consisted of anthelmintic treatments every 6–12 weeks. More recently, adult sheep were drenched every 5 weeks and lambs every 3 weeks.

Initially the anthelmintic used consisted of levamisole[1], but later this was regularly alternated with albendazole[2] and infrequently with rafoxanide[3], at intervals of more or less 6 months.

Materials and Methods

The strain was isolated in the laboratory to investigate the presence of considerable numbers of eggs of *H. contortus* in sheep after routine treatment with rafoxanide. It was passaged only once in the laboratory, the infective larvae (L3) for use in the trial originating from the first laboratory passage in donor animals. The sheep from which L3 of *H. contortus* were harvested for the trial was not dosed with rafoxanide; thus the strain was not selected with rafoxanide in the laboratory.

Thirty-one Dorper wethers, born and raised under conditions of minimal exposure to worms (concrete-floored pens swept twice per week for the removal of accumulating manure), were available for the trial. Subsequent to their introduction to the trial they were housed under worm-free conditions.

Prior to the commencement of the trial, faecal worm egg (epg) counts of the sheep were negative. Nevertheless, as a further precaution, the sheep were drenched with morantel[4] at a dosage range for the various sheep of 30–68 mg/kg.

Each of 31 worm-free Dorper wethers was dosed with 1 003 L3 of *H. contortus* on Day −28, Day −27, and on Day −26 (Table 1). On Days −5, −4, and −1 egg counts were carried out. On the day of treatment (Day 0) the sheep were mass-measured, ranked according to the mean egg counts, and allocated to the various trial groups with the aid of tables of random numbers. The method of allocation is outlined in detail by Van Wyk & Gerber (1980).

The groups were as follows: 11 sheep dosed with rafoxanide; 4 dosed with fenbendazole; 3 with levamisole; 3 with closantel; and 9 undosed controls (Table 1).

On Day +6 all the sheep were killed for worm recovery (Reinecke, 1973).

TABLE 1 Ptzr strain—experimental design: Numbers of L3 of *H. contortus* dosed to each sheep, the anthelmintics drenched, and the day of slaughter.

Day	Treatment
−28	31 sheep each dosed with 1 003 L3 *H. contortus*
−27	31 sheep each dosed with 1 003 L3 *H. contortus*
−26	31 sheep each dosed with 1 003 L3 *H. contortus*
0	11 sheep drenched with rafoxanide at 7,5 mg/kg
	4 sheep drenched with fenbendazole at 5 mg/kg
	3 sheep drenched with levamisole at 7,5 mg/kg
	3 sheep drenched with closantel at 5 mg/kg
	9 sheep remained untreated as controls
+6	31 sheep killed for worm recovery

At necropsy, worm recovery differed as follows from that of Reinecke (1973): Initially the worms were counted in only a single 10 % aliquot of the ingesta of

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[1] Ripercol (Janssen Pharmaceutica)
[2] Valbazen (SmithKline)
[3] Ranide (MSD)
[4] Banminth (Pfizer)

each abomasum. Subsequently, total worm counts were done of the control sheep having the median worm count, of the sheep with counts immediately above and below this count, and of the treated sheep having the 2nd highest to the 5th highest worm counts.

Results (Tables 2 & 3)

It is ovious that rafoxanide is ineffective because 5 of the treated sheep had worm counts higher than the reduced control median 1 213 × 0,5 = 615,5 or 616 (Table 2). For a Class C efficacy, only 4 treated sheep may have a worm count higher than that of the reduced control median (Reinecke, 1973).

TABLE 2 Ptzr strain—*H. contortus* recovered from untreated controls and from sheep treated with rafoxanide

Numbers of *H. contortus*	
Untreated (Control group)	Rafoxanide (7,5 mg/kg)
830	0
970	20
1 020	70
1 174*	80
1 231*	170
1 471*	180
1 550	215*
1 560	700*
1 880	719*
	824*
	840*
	1 190
Median: 1 231 × 0,5 = 615,5	Mean: 448 +/− 414
Mean: 1 298 +/− 340	5/11 > 616: Class X

NPM efficacy: Class X (ineffective)
Arithmetic mean efficacy: 65,5 %

* Total worm counts, and not only counts of aliquots, as in the other sheep

TABLE 3 Ptzr strain *H. contortus* recovered from untreated controls and from sheep treated with fenbendazole, levamisole, and closantel

Numbers of *H. contortus*	
Untreated (Control group)	Fenbendazole (5 mg/kg)
830	140
970	710
1 020	850
1 174*	1 180
1 231*	Mean: 720 +/− 434
1 471*	
1 550	Levamisole (7,5 mg/kg)
1 560	0
1 880	0
Mean: 1 298 +/− 340	0
	Closantel (5 mg/kg)
	0
	0
	0

Although only 4 sheep were treated with fenbendazole at 5 mg/kg (Table 3), it is obvious that large numbers of worms were still present, giving a reduction on the arithmetic mean of only 44,5 %.

That no worms survived treatment with closantel and levamisole indicates that these anthelmintics are still effective.

II. WHITE RIVER KRTZ STRAIN OF *H. CONTORTUS*

A farmer in the Lowveld region of the Transvaal complained that his sheep were showing signs of haemonchosis despite being treated with rafoxanide. The farm is situated on the north-eastern slope of a mountain, and is divided into numerous camps of about 1 ha each.

The kikuyu pasture is irrigated at regular intervals and grazed by a flock of about 2 000 South African Mutton Merino sheep. The sheep are divided into flocks of 100–200 head and graze each camp for 1 or 2 days, being moved when the top growth of kikuyu has been removed.

One of these sheep died from a heavy infestation of *H. contortus*, in spite of having been dosed a few days earlier with rafoxanide.

For the past 3 years worm control on this farm consisted exclusively of monthly or more frequent drenching of different anthelmintics, which were fairly regularly alternated with one another on the basis of their modes of action. Rafoxanide was dosed only 4 times on the farm during these 3 years, viz., on 4 February, 8 April, 29 April and 5 August 1985.

This strain was not passaged at all in the laboratory, faecal cultures from the faeces of the dead sheep being used for infesting the trial animals.

MATERIALS AND METHODS (Table 4)

Nine yearling merinos were dewormed with fenbendazole[5] at a dosage rate of 10 mg/kg and levamisole[6] at 7,5 mg/kg live mass. They were subsequently housed under worm-free conditions before each of them was dosed *per os* with 5 000 L3 of the Krtz strain of *H. contortus* suspended in water. They were divided into 3 equal groups as follows: 3 untreated controls; 3 treated with rafoxanide[7] at 7,5 mg/kg; and 3 with fenbendazole[5] at 5 mg/kg.

TABLE 4 Krtz strain—experimental design: Numbers of L3 of *H. contortus* dosed to each sheep, the anthelmintics drenched, and the day of slaughter.

Day	Treatment
−18	9 sheep each dosed with 5 000 L3 *H. contortus*
0	3 sheep drenched with rafoxanide at 7,5 mg/kg
	3 sheep drenched with fenbendazole at 5 mg/kg
	3 sheep remained untreated as controls
+7	all 9 sheep killed for worm recovery

RESULTS (Table 5)

The efficacy (based on reductions of the arithmetic mean worm burden of the controls) was 13,9 % for rafoxanide, and 0 % for fenbendazole.

TABLE 5 Krtz strain—worms recovered from untreated controls and from groups of sheep treated with rafoxanide and fenbendazole

Numbers of *H. contortus*		
Untreated (Control group)	Rafoxanide (7,5 mg/kg)	Fenbendazole (5 mg/kg)
1 630	1 850	2 260
2 010	1 160	1 770
1 920	1 780	1 960
Mean: 1 853	1 597	1 997
Reduction*	13,9 %	0 %

* Reduction on arithmetic mean, treated compared to control worm burdens

DISCUSSION

With the exception of those in the rafoxanide treatment in Trial I, relatively few sheep were used in these trials. Nevertheless, it is plain that both Ptzr and Ktzr strains are markedly resistant to both rafoxanide and fenbendazole at recommended therapeutic doses. There

[5] Panacur (Hoechst)
[6] Ripercol (Janssen)
[7] Ranide (MSD)

is well-documented side resistance between the benzimidazoles (discussed by Martin, 1985), hence it can be accepted that none of the benzimidazoles will be effective against either strain.

Sheep had been introduced relatively recently to the farm of origin of the Ptzr (Pretoria) strain and they were dosed infrequently with rafoxanide. It therefore seems unlikely that the resistance to rafoxanide developed on this farm but was probably introduced with one or more of the groups of sheep that were brought in from elsewhere. Fortunately this strain was still susceptible to closantel and levamisole.

In the case of the Krtz strain, there are records of treatment since the beginning of 1983 and it seems unlikely that the resistant strain could have developed from a total of 4 exposures to rafoxanide over the ensuing period. It may have been resistant to rafoxanide prior to 1983, or else it may also have been introduced subsequently to the farm.

It is surpising that rafoxanide resistance took so long to develop in South Africa, since the following factors are favourable for the development of resistance to this anthelmintic:

1. Rafoxanide has been on the South African market for 15 years and has been used intensively during that time.
2. The compound has a residual efficacy of approximately 3 weeks' duration against *H. contortus* (Snijders, Horak & Louw, 1973), a characteristic that should theoretically predispose to relatively rapid development of resistance (reviewed by Le Jambre, 1985); and
3. It is highly effective against *H. contortus*, the most important helminth of sheep in the summer rainfall region, and is used very intensively—as frequently as every 3 weeks during periods of peak transmission in years of heavy rainfall. This means that, given the residual efficacy, exposure of the worm to the anthelmintic and hence also to selection for resistance was probably well-nigh continuous during these periods.

Despite these factors, only 1 case of slight resistance to rafoxanide has been described before (Van Wyk & Gerber, 1980). It is true that no organized search for resistance has ever been made in South Africa, and this may account for so few reported cases. But, on the other hand, pharmaceutical companies investigate numerous complaints from consumers on a routine basis, and this should have unearthed at least some cases of resistance, if resistance was common (Berger, 1975; Van Schalkwyk, Geyser & Rezin, 1983; Van Schalkwyk, 1984).

At present we are investigating 2 more strains with apparent resistance of *H. contortus* to rafoxanide, and another 2 strains have been investigated by a pharmaceutical company.

Resistance in South Africa (Table 6)

The most disquieting aspect to resistance in South Africa is the recent discovery of resistance of *H. contortus* to ivermectin[8], an anthelmintic which has only been registered for use in sheep in South Africa since 1983. This strain was discovered by Visser & Schneider (Anon., 1986) on a farm in the South-western Cape, where ivermectin was used at very short intervals. Suspected resistance of *O. circumcincta* to the benzimidazoles had caused the farmer to change to ivermectin, and unfortunately very few other anthelmintics were subsequently used on the farm. This resistance developed under intensive grazing conditions on irrigated pasture. At least 2 further strains of *H. contortus*, strongly suspected of being resistant to ivermectin, are being investigated at the moment, both of which also originated from sheep intensively grazing irrigated pastures.

TABLE 6 The present state of resistance of nematodes of sheep to anthelmintics in South Africa

1. *Haemonchus contortus*
Benzimidazoles
Rafoxanide
Closantel
Ivermectin
2. *Ostertagia circumcincta*
Benzimidazoles

Equally disquieting is the fact that some cases of resistance of *H. contortus* to closantel[9] are under investigation at the moment (Malan & Van Wyk, unpublished observations, 1986). In the light of the substantial residual efficacy of this remedy against *H. contortus* (Van der Westhuizen, personal communication, 1979) it was not surprising that resistance developed relatively quickly. Nevertheless, this residual efficacy is very useful in a dosing programme and increased resistance will rob the sheep farmer of yet another anthelmintic.

This state of affairs means that, of the anthelmintics at present registered for use against nematodes of sheep in South Africa, only disophenol, levamisole, morantel and trichlorfon have not as yet been proved to be affected by nematode resistance in South Africa. Of these, levamisole and morantel are in the same group (according to the classification of Arundel, 1985), and disophenol has not been used on a large scale.

Almost without exception the cases of resistance of the more modern anthelmintics in South Africa have been associated with intensive sheep farming involving irrigated pastures, or improved pastures in regions having relatively high or consistent rainfall. This is probably the modern trend—intensive sheep farming on permanent pastures (Van Wyk, unpublished observations, 1985; P. C. van Schalkwyk, personal communication, 1986; P. Botha, personal communication, 1986; T. Taljaard, personal communication, 1986).

Under intensive grazing conditions worms are controlled exclusively by frequent drenching with anthelmintics, often without alternation of the compounds or with alternation of chemically related compounds. Heavy selection pressure is caused because these anthelmintics are dosed as often as every 3 weeks at times of peak transmission, and this pressure may be increased when the first signs of resistance occur, since the farmer often tends to increase the frequency of treatment still further.

The devastating effect of this intensive farming is evident in the rapidity with which ivermectin has become involved in resistance. This remedy is one of the most efficient against *H. contortus*, and it was hoped that resistance would not occur. Perhaps the short residual effect of ivermectin aggravated the development of resistance.

If resistance to other new compounds follows the same trend as ivermectin, we may reach a crisis in the treatment of *H. contortus*. The modern systems of integrated control, such as provision of safe pastures at critical times, are not applicable to irrigated pastures and many farmers might have to abandon sheep farming under such conditions.

[8] Ivomec (MSD)

[9] Cantel (SmithKline); Flukiver (Janssen); Seponver (Janssen)

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