Onderstepoort J. vet. Res., 48, 235–238 (1981)

STUDIES ON *HAEMONCHUS CONTORTUS*. V. CHEMOPROPHYLAXIS AND ITS EFFECT ON WORM EGG COUNTS AND THE HAEMATOCRIT

R. K. REINECKE, CHRISTEL BRUCKNER(1) and I. L. DE VILLIERS, Faculty of Veterinary Science, P.O. Box 12580, Onderstepoort 0110, Republic of South Africa

ABSTRACT

REINECKE, R. K., BRUCKNER, CHRISTEL & DE VILLIERS, I. L., 1981. Studies on *Haemonchus contortus*. V. Chemoprophylaxis and its effect on worm egg counts and the haematocrit. *Onderstepoort Journal of Veterinary Research*, 48, 235–238 (1981).

A group of 12 10-month-old, worm-free Merino lambs were given a single injection of disophenol at 10 mg/kg. This group and another group of 12 worm-free lambs were infested with infective larvae of *Haemonchus contortus* 1–3 times a week with a maximum dose of 4000/week from 0–91 days. From 95–168 days the dose was increased to a maximum of 6000/week. Every 7 days faeces were examined for worm eggs and blood samples for haematocrit (H t). Worm egg counts in the controls rose from 21–56 days, fluctuated, and rose to another peak at 84 days, while the Ht fell from 28 days to rise again after 105 days. In the group treated with disophenol egg counts were negative until 161 days and Ht remained at normal values throughout.

Résumé

*ETUDES SUR L'*HAEMONCHUS CONTORTUS. *V. CHEMOPROPHYLAXIE ET SON EFFET SUR LES COMPTES EN OEUFS DE VER ET L'HAEMATOCRITE*

Un groupe de 12 *agneaux indemne d'infestation aux helminthes ont reçu une seule injection de disophenol à une dose de* 10 *mg/kg. Ce groupe et un autre groupe de* 12 *agneaux sans infestation, furent infestés avec des larves infectieuses de* Haemonchus contortus 1–3 *fois par semaine avec une dose maximum de* 4 000/*semaine de* 0 *à* 91 *jours. A partir de* 95–168 *jours, la dose fut accrue jusqu' à un maximum de* 6 000/*semaine. Chaque semaine les fèces furent examinés pour déterminer la présence d'oeufs de ver, et des échantillons de sang pour l'haematocrite (Ht). Le nombre d'oeufs monta de* 21–56 *jours chez les sujets témoins, fluctua et s'éleva jusqu'à une autre apogée à* 84 *jours tandis que le Ht tombait à partir de* 28 *jours pour s'élever à nouveau après* 105 *jours. Dans le groupe traité avec le disophenol les comptes d'oeufs furent négatifs jusqu'à* 161 *jours et le Ht demeura constamment à des valeur normales.*

INTRODUCTION

Some anthelmintics are not excreted immediately but residues remain in the host and may still be lethal for parasites weeks or even months after treatment. Rafoxanide 3',5 - diiodo - 3' - chloro - 4' - (p - chlorophenoxy)-salicylanalide is effective against adult *Haemochus contortus* 4 weeks after treatment and larvae of *Oestrus ovis* after 2 (Horak & Snijders, 1974). Two such compounds closantel N-{5-chloro-4-[alpha-(4-chlorophenyl)-alpha-cyanomethyl]-2 methyl phenyl}-2-hydroxy-3,5-diiodo benzamide and disophenol 2,6-diiodo-nitrophenol are registered for use as "residual anthelmintics" in sheep in the RSA. According to Van Wyk (1978), closantel is >80% effective against all stages of development of *Haemonchus contortus* and *Gaigeria pachyscelis* in >80% of sheep (Class A) up to 7 weeks after treatment. Reinecke (1980) and Reinecke, Bruckner & De Villiers (1981) showed that disophenol has the following residual efficacy against adult *H. contortus:* 31 days after treatment Class A; from 45–76 days after treatment it falls into Class B (>60% effective in >60% of sheep) and 91 days after treatment Class C (>50% effective in >50% of sheep). These results confirm those by workers in Brazil and Australia, who stated that disophenol is still effective 3 months after treatment (Rocha, Serra, Mendes, Rocha, Campos, Prucoli, Costa & Ribeiro, 1967; Gordon, 1974).

Although these facts are interesting, it is of more practical importance to establish whether a 'residual anthelmintic' is a chemoprophylactic or merely another anthelmintic. This paper describes an experiment in which we treated a group of sheep with disophenol and monitored the faecal worm egg counts and haematocrit. The sheep were dosed repeatedly with infective larvae of *H. contortus* for 5 months. This treated group was compared with a group of untreated controls to establish whether the hosts would contaminate the environment and what the pathogenic effects would be under continuous challenge.

TABLE 1 Chemoprophylaxis. Experimental design. The days on which sheep were treated and infective larvae dosed, including the frequency and total number of *H. contortus* dosed and the day of slaughter

Days	No. of infective larvae dosed to each sheep			
	Frequency per week	Maximum per week	Group A Total	Group B Total
0.....	—	—	—	Disophenol 10 mg/kg (subcutaneously)
0–+91.....	1–3	4 000	50 000	50 000
+95–+154	1–3	6 000	50 000	50 000
+136.....	—	—	—	Sheep 389 slaughtered
+175.....	—	—	Slaughter	Slaughter

MATERIALS AND METHODS

Twenty-four 10-month-old Merinos were treated with anthelmintics and divided into 2 groups of 12 sheep each. The experimental design is summarized in Table 1. The 2 groups were treated, dosed and killed as follows:

Group A: Controls.—Trickle doses of infective larvae of *H. contortus* were dosed to each sheep from 1–3 times per week and a maximum dose of 4 000 larvae per week given from Day 0 – Day+91. From Day+95 – Day+154 the total number of larvae dosed did not exceed 6 000 per week.

(1) Medicines Control Board, Private Bag X63, Pretoria 0002

Received 21 September 1981—Editor

Group B: Disophenol at 10 mg/kg was injected subcutaneously on Day 0. Thereafter identical doses of infective larvae of *H. contortus* dosed to sheep in Group A were given to each sheep in Group B.

Sheep 389 in Group B was killed on Day+136. The other 23 sheep in both groups were killed on Day+175 for worm recovery post-mortem. From Day+21 faeces were collected from all sheep every 7 days up to and including Day +168. Eggs were separated from faeces in 40% sugar solution in flat medicine bottles and after 24 h incubation at 27 °C, 1st stage larvae (L_1) were identified according to the description of Whitlock (1959). Our technique of hatching eggs differed from that of Whitlock (1959) in that we placed the eggs in round glass Petri dishes ±20 mm in diameter in water to a depth of 3–4 mm and not in the special tubes described by Whitlock (1959). Blood was collected from the jugular vein in vacuum tubes containing anticoagulant every 7 days from Day+28 – Day+168 from each sheep in Group A and 6 sheep in Group B. Samples from each tube were centrifuged and the haematocrit (Ht) determined.

Results

The changes in the faecal worm egg count in Group A and fluctuations in Ht are illustrated in Fig. 1 & 2 respectively.

Group A controls (Fig. 1)

Worm egg counts rose steadily from 21 days to a peak at 56 days, fluctuated thereafter to another peak at 84 days and then fell steadily until the end of the experiment. The Ht fell from 28 days and rose again after 105 days (Fig. 2).

Group B disophenol

Egg counts were negative until Day+161 and Day +168 when 2 sheep only were positive, with counts ranging from 100 to 3 600 epg. The Ht fluctuated between mean normal values of 0,25 and 0,31, although one animal (Sheep 310) fell to 0,15 at 147 days but rose again to normal levels from 161 days (Fig. 2).

Post-mortem (Table 2)

Group A controls

Eight out of 12 sheep had more 5th and adult stages than 4th stage larvae (L_4) of *H. contortus*.

TABLE 2 Chemoprophylaxis. Worms recovered at necropsy

Sheep No.	*H. contortus*			
	Stage of development			Total
	L_4	5	Adult	
Group A: Controls:				
301	432	0	964	1 396
379	1 478	1	61	1 540
381	750	140	700	1 590
382	10 680	0	40	10 720
383	3 643	140	3 501	7 284
385	2 910	100	2 730	5 740
392	359	0	890	1 249
405	2 110	40	5 580	7 730
427	218	0	349	567
440	283	140	447	870
454	259	0	1 510	1 769
456	85	40	160	285
Group B: Day 0 Disophenol 10 mg/kg (sub. cut.):				
310	202	20	40	262
312	679	80	994	1 753
314	497	20	662	1 179
324	5 379	120	2 034	7 533
340	3 270	20	120	3 410
357	1 060	120	325	1 505
375	102	61	41	204
376	859	320	1 129	2 308
386	4 070	500	2 530	7 100
*389	10 509	760	404	11 673
401	273	80	580	933
414	714	164	427	1 305

* Sheep 389 slaughtered on Day+136

Group B

We killed Sheep 389 on Day+136 to determine if any worms were present, because egg counts were negative and 10 509 L_4, 706 5th and 404 adult *H. contortus* were recovered post-mortem respectively. Seven of 11 sheep killed on Day+175 had more L_4 than 5th stage and adult *H. contortus*.

Efficacy

When the worm burdens in Group A and Group B were compared, there was no significant difference between them (Table 3).

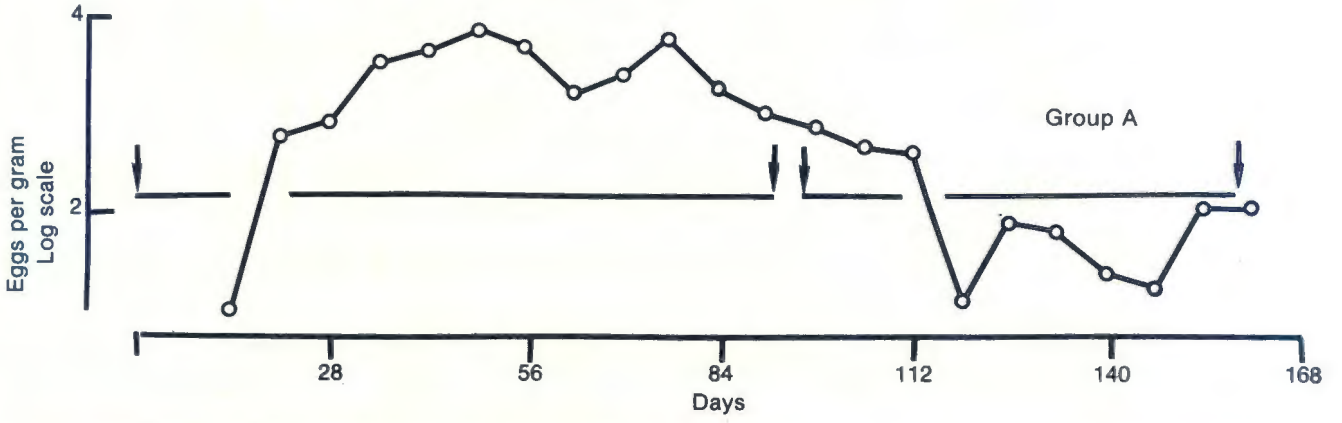

FIG. 1 Fluctuations in faecal worm egg counts of *H. contortus* in the controls (Group A). Arrows and lines indicate when infective arvae of *H. contortus* were dosed to each sheep (see Materials and Methods)

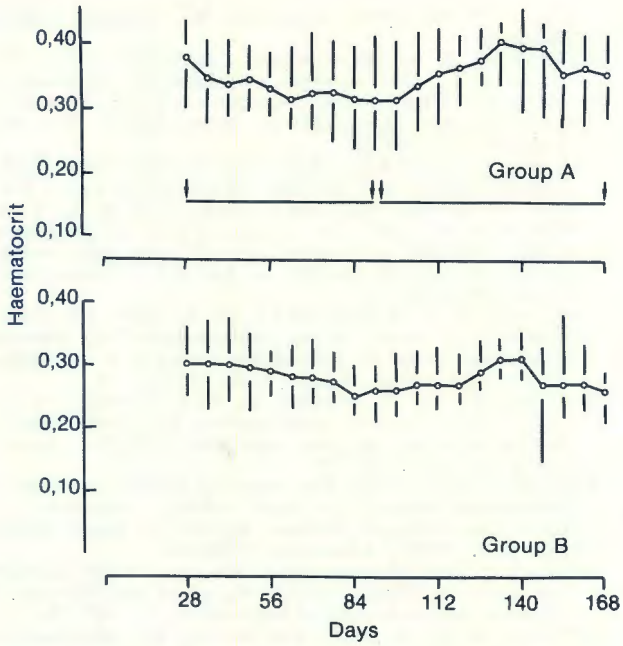

FIG. 2 Fluctuations in haematocrit in Groups A and B. Arrows and lines indicate periods when infective larvae of *H. contortus* were dosed to both groups (see Materials and Methods)

TABLE 3 Chemoprophylaxis. Ranked worm burdens of of *H. contortus*

Group A			Group B		
L_4	5+A	Total	L_4	5+A	Total
85	40	285	102	60	204
218	62	567	202	102	262
259	200	870	273	140	933
283	349	1 249	497	445	1 179
359	587	1 396	679	591	1 305
432	840	1 540	714	660	1 505
750	890	1 590	859	682	1 753
1 478	964	1 769	1 060	1 074	2 308
2 110	1 510	5 740	3 270	1 164	3 410
2 910	2 830	7 284	4 070	1 449	7 100
3 643	3 641	7 730	5 379	2 154	7 533
10 680	5 620	10 720	10 509	3 030	11 673

Discussion

Veglia (1915) showed that *H. contortus* caused anaemia and subsequently Allonby (1973), Dargie (1973) and Dargie & Allonby (1975) did detailed studies on the clinical signs and clinical pathology and divided the anaemia into various types:

(i) Severe loss of blood, with sheep losing 200–600 mℓ per day and death supervening before the worms are adult (10 000–35 000 L_4), is known as hyperacute haemonchosis.

(ii) Anaemia may not become worse (1 000–10 000 adult worms) accounting for a loss of 50–200 mℓ of blood a day, but Ht stabilize themselves at reasonable levels. Iron reserves, however, are continually lost and acute haemonchosis is recognized.

(iii) Chronic cases may be due to only a few adults (100–1 000) and as little as 5–50 mℓ blood per day being lost, but serum iron levels continually fall to levels below 40 $\mu g\%$ and this level of iron is unable to maintain erythropoeisis and animals die of anaemia.

(iv) If reinfestation takes place Ht suddenly falls and sheep may die.

The effect of L_4 of *H. contortus* was investigated by Sinclair & Prichard (1975), who showed that plasma, varying from 12–30 mℓ/day, leaked into the abomasum, the pH rose to a mean of 4,8 and plasma pepsinogen rose from the 3rd day and remained high for 55 days. These authors concluded at necropsy that L_4 caused some damage to the abomasal mucosa.

The adult worms are more pathogenic than the larval stages and in these present trials disophenol, by preventing anaemia from developing, prevented the harmful effects of haemonchosis developing in the lambs. It therefore fulfilled the main object of prophylaxis, that is, the prevention of pathogenic effects.

An interesting finding was that we were unable to recover eggs from faeces for nearly 4–5 months. In addition to normal egg counts by the McMaster method, we mixed faeces with 40% sugar solution, placed the mixture in flat medicine bottles and attempted to float eggs to the upper surface by the method described by Whitlock (1959). Despite examining faeces from each sheep in Group B by this method, not a single egg from any specimen was recovered from Day+77 – Day+119, and egg counts were negative until 2 sheep became positive 161 days after treatment. This is puzzling in view of our previous findings that a single dose of infective larvae of *H. contortus* 32 days after subcutaneous inoculation with 10 mg/kg disophenol was followed 20 days later by 100 epg in 2 out of 10 sheep. Moreover, larval dosage 91 days after treatment resulted in egg counts ranging from 50–400 epg, 111 days after disophenol treatment (Reinecke *et al.*, 1981).

It may be postulated that a trickle dose of infective larvae of *H. contortus*, which does not develop beyond L_4 for nearly 5 months (161 days), releases some antigen which prevents development of the larvae to the adult stage for a short period of 2 months (91–161 days).

Whatever the reason, the practical implication of this finding is extremely important. *H. contortus* is the most virulent pathogen of sheep in the summer rainfall areas in the RSA (Horak, 1978; Horak & Louw, 1977) and in Australia (Southcott, Major & Barger, 1976). The optimal conditions on both natural and irrigated pastures for the free-living stages are from December–May. Adult worms are dominant until February, and retarded larvae (L_4) dominant from March to October, reach a peak in May and June. These latter larvae are acquired from overwintering autumn infestations, since pasture is totally unsuitable for eggs to hatch and larvae to develop to the infective stage in the Highveld from June to December (Horak, 1978).

Obviously sheep should be treated in December or early January with disophenol and this would be adequate to protect them for the entire summer.

Our trials at the University of Pretoria's Experimental Farm, however, showed that a disophenol injection in December protected sheep until March, when eggs were present in faeces for the first time. Therefore a second injection of disophenol in March is also advocated (Reinecke *et al.*, 1981).

Acknowledgements

We wish to thank the University of Pretoria for providing financial assistance for us to do these trials and the Director, Veterinary Research Institute, for his assistance with sheep pens for housing our animals.

REFERENCES

ALLONBY, E. W., 1973. Ovine haemonchosis, epidemiology, clinical signs and diagnosis. *In*: Helminth deseases of cattle, sheep and horses in Europe. *ed.* G. M. URQUHART & J. ARMOUR, 59–63. Glasgow: University Press.

DARGIE, J. D., 1973. Ovine haemonchosis. Pathogenesis. *In*: Helminth diseases of cattle, sheep and horses in Europe *ed.* G. M. URQUHART & J. ARMOUR, 63–71. Glasgow: University Press.

DARGIE, J. D. & ALLONBY, E. W., 1975. Pathophysiology of single and challenge infections of *Haemonchus contortus* in Merino sheep: studies on red cell kinetics and 'self-cure' phenomenon. *International Journal of Parasitology*, 5, 147–157.

GORDON, H. McL., 1974. Disophenol (2,6 diiodo-4-nitrophenol) a remarkable anthelmintic. *Proceedings of the International Congress in Parasitology*, 3, Munich, August 25–31, 1974; Vol. 3 Sect. E. 7, 1392–1393.

HORAK, I. G., 1978. Parasites of domestic and wild animals in South Africa. V. Helminths of sheep on dryland pasture in the Transvaal Highveld. *Onderstepoort Journal of Veterinary Research*, 45, 1–6.

HORAK, I. G. & LOUW, J. P., 1977, Parasites of domestic and wild animals in South Africa. IV. Helminths in sheep on irrigated pasture on the Transvaal Highveld. *Onderstepoort Journal of Veterinary Research*, 44, 261–270.

HORAK, I. G. & SNIJDERS, A. J., 1974. The effect of *Oestrus ovis* infestation in Merino lambs. *Veterinary Record*, 94, 12–16.

REINECKE, R. K., 1980. Development of the larval anthelmintic test for parasitic nematodes in ruminants. D.S.c. Thesis, Potchefstroom University for Christian Higher Education.

REINECKE, R. K., BRUCKNER, CHRISTEL & DE VILLIERS, I. L., 1981. A residual anthelmintic 2,6-diiodo-4-nitrophenol (Disophenol). Methods of testing its anthelmintic efficacy. *Journal of the South African Veterinary Association*, 52, 195–200

ROCHA, U. F., SERRA, RACHEL G., MENDES, MARGARIDA DE F. M., ROCHA, CLAUDIA A. DA, CAMPOS, M. S. DE, PRUCOLI, J. O., COSTA, J. W. & RIBEIRO, R., 1967. The residual activity of disophenol in the treatment of nematodiasis of ovine and bovine: *Boletim de Industria Animal*, 24, 105–110 (in Portuguese). English summary.

SINCLAIR, K. B. & PRICHARD, R. K., 1975. The use of disophenol in studies of the pathogenicity of the arrested fourth-stage larvae of *Haemonchus contortus* in the sheep. *Research in Veterinary Science*, U.K., 19, 232–234.

SOUTHCOTT, W. H., MAJOR, G. W. & BARGER, I. A., 1976. Seasonal pasture contamination and availability of nematodes for grazing sheep. *Australian Journal of Agricultural Research*, 27, 277–286.

VAN WYK, J. A., 1978. The control of internal parasites in domesticated animals in South Africa. Department of Agricultural Technical Services, Republic of South Africa. ISBN 0 621 04708 2 Addendum 1978/07/10.

VEGLIA, F., 1915. The anatomy and life-history of the *Haemonchus contortus* (Rud.). *Annual Report of the Director of Veterinary Research Union of South Africa*, 3/4, 347–500.

WHITLOCK, H. V., 1959. The recovery and identification of the first stage larvae of sheep nematodes. *Australian Veterinary Journal*, 35, 310–316.

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