Onderstepoort J. vet. Res., 48, 229–234 (1981)

STUDIES ON *HAEMONCHUS CONTORTUS*. IV. THE EFFECT OF *TRICHOSTRONGYLUS AXEI* AND *OSTERTAGIA CIRCUMCINCTA* ON CHALLENGE WITH *H. CONTORTUS*

R. K. REINECKE, CHRISTEL BRUCKNER[1] and I. L. DE VILLIERS, Faculty of Veterinary Science, P.O. Box 12580, Onderstepoort 0110

ABSTRACT

REINECKE, R. K., BRUCKNER, CHRISTEL & DE VILLIERS, I. L., 1981. Studies on *Haemonchus contortus*. IV. The effect of *Trichostrongylus axei* and *Ostertagia circumcincta* on challenge with *H. contortus*. *Onderstepoort Journal of Veterinary Research*, 48, 229–234 (1981).

Worm-free Merino yearlings were dosed with either a mixture of infective larvae of *Trichostrongylus axei* and *Ostertagia circumcincta* or with *O. circumcincta* only, and challenged 90–93 days later with infective larvae of *Haemonchus contortus*. Neither of these methods protected sheep against challenge and slight protection was afforded sheep predosed with *T. axei* and *O. circumcincta* and challenged with a trickle dose of *H. contortus*.

Résumé

ETUDES SUR HAEMONCHUS CONTORTUS. *IV. L'EFFET DE* TRICHOSTRONGYLUS AXEI *ET DE* OSTERTAGIA CIRCUMCINCTA *SUR DES MOUTONS SOUMIS À UNE EPREUVE*

Des Merino d'un an indemnes d'infestation aux helminthes ont été traites avec soit, un mélange de larves infectieuses de Trichostrongylus axei *et d'*Ostertagia circumcincta *ou seulement avec* O. circumcincta; *ils ont été éprouves 90–93 jours plus tard avec des larves infectieuses d'*Haemonchus contortus. *Aucune de ces deux méthode ne procura une protection aux moutons contre cette épreuve et une légère protection fut acquise avec des moutons infestés auparavant avec* T. axei *et* O. circumcincta *et soumis à une épreuve avec une faible dose de* H. contortus.

INTRODUCTION

It has been shown that, if dosed with infective larvae of *Trichostrongylus axei*, weaned Merinos were protected against subsequent challenge with *Haemonchus contortus* (Reinecke, Bruckner & De Villiers, 1980). Infective larvae of *Ostertagia circumcincta*, dosed to yearling Dorper ewes, however, failed to protect them against challenge with *H. contortus* (Reinecke, Snyman & Seaman, 1979).

This paper describes 2 experiments in which Merinos were dosed with either infective larvae of *O. circumcincta* or with a combination of *O. circumcincta* and *T. axei* and subsequently or simultaneously with infective larvae of *H. contortus*. The object of the experiments was, firstly, to test the protective effect of *O. circumcincta* alone or in combination with *T. axei* against challenge with *H. contortus*; secondly, to ascertain the effect of challenge with *H. contortus* administered as a trickle dose over a period of 5 months.

Experiment 1.—*Trichostrongylus axei* and *Ostertagia circumcincta* as a possible vaccine against *H. contortus*

Materials and Methods

The experimental design is summarized in Table 1. Thirty-six 10-month-old Merinos were treated with anthelmintics, housed in worm-free pens, each labelled with an ear tag and divided into 3 equal groups of 12 sheep each. They were dosed and challenged with infective larvae and slaughtered, as summarized in Table 1.

At necropsy the ingesta of the abomasum and duodenum were washed on a sieve (38 μm apertures) and the residue on the surface of the sieve placed in a wide-mouthed 1 ℓ jar. Formalin was added as a preservative. The muscularis and mucosal layers of the abomasum and duodenum were digested in pepsin/HCl, sieved and preserved, as described by Reinecke (1973).

TABLE 1 *Experiment* 1.—Experimental design showing the days on which infective larvae were dosed to each sheep and the day of slaughter

Days	No. of infective larvae dosed to each sheep		
	Group A	Group B	Group C
0	0	*T. axei*+*O. circumcincta*	*O. circumcincta*
+14	0	*T. axei*+*O. circumcincta*	*O. circumcincta*
Total	0	20 000+20 000	40 000
+90	*H. contortus*	*H. contortus*	*H. contortus*
+91	*H. contortus*	*H. contortus*	*H. contortus*
+92	*H. contortus*	*H. contortus*	*H. contortus*
Total	50 000	50 000	50 000
+119	Slaughter	Slaughter	Slaughter

[1] Department of Health, Private Bag X63, Pretoria 0001

Received 29 September 1981—Editor

TABLE 2 *Experiment* 1.—Worms recovered at necropsy from Group A (controls)

Sheep No.	*H. contortus* Stage of development			Total
	L_4	5	Adult	
Group A.—Controls:				
313	2 400	20	1	2 421
317	1 371	76	1 965	3 412
329	920	480	4 971	6 371
330	3 700	349	7 433	11 482
332	2 431	20	301	2 752
337	1 531	1 242	1 572	4 345
356	1 303	0	20	1 323
378	2 118	630	4 524	7 272
408	1 866	65	2 361	4 292
423	1 274	0	4 967	6 241
426	1 539	322	4 317	6 178
460	1 958	720	1 350	4 028

TABLE 2 (*Continued*). Worms recovered at necropsy from Group B (*T. axei*+*O. circumcincta*)

Sheep No.	*H. contortus*				*T. axei*				*O. circumcincta*			
	Stage of development			Total	Stage of development			Total	Stage of development			Total
	L_4	5	Adult		L_4	5	Adult		L_4	5	Adult	
Group B.—Day 0: 10 000 *T. axei*+ 10 000 *O. circumcincta*; Day+14: 10 000 *T. axei*+10 000 *O. circumcincta*:												
300	760	0	630	1 390	0	0	13 710	13 710	4 390	0	240	4 630
311	620	240	6 630	7 490	0	0	17 156	17 156	6 230	20	897	7 147
349	1 840	80	8 550	10 470	0	0	16 470	16 470	7 420	0	820	8 240
355	800	0	4 390	5 190	0	0	12 510	12 510	4 550	0	1 940	6 490
359	820	0	40	860	0	0	14 630	14 630	2 640	0	20	2 660
363	1 440	50	5 610	7 100	0	0	12 810	12 810	3 360	0	1 560	4 920
365	1 170	0	120	1 290	0	0	15 090	15 090	6 950	0	220	7 170
398	1 040	0	1 010	2 050	0	0	12 570	12 570	4 260	0	140	4 400
406	930	40	3 350	4 320	0	0	14 210	14 210	6 780	0	1 560	8 340
418	1 140	0	1 120	2 260	0	0	13 560	13 560	4 770	0	580	5 350
434	1 440	0	1 750	3 190	0	0	14 780	14 780	5 440	0	320	5 760
472	80	0	20	100	0	0	14 700	14 700	970	0	260	1 230

TABLE 2 (*Continued*). Worms recovered from Group C (*O. circumcincta*)

Sheep No.	*H. contortus*				*O. circumcincta*			
	Stage of development			Total	Stage of development			Total
	L_4	5	Adult		L_4	5	Adult	
Group C.—Day 0: 20 000; Day+14: 20 000 *O. circumcincta*								
306	1 731	468	1 572	3 771	10 714	120	1 446	12 280
350	4 880	4	3 731	8 615	11 540	160	1 186	12 886
352	1 526	0	181	1 707	12 310	0	10	12 320
360	1 240	220	860	2 320	7 919	80	2 970	10 969
362	340	0	3	343	2 971	1	25	2 997
368	1 720	390	6 340	8 450	10 900	0	2 950	13 850
371	2 100	0	42	2 142	14 765	0	322	15 087
400	20	0	1	21	3 463	0	2	3 465
412	240	20	3 034	3 294	4 931	20	4 117	9 068
431	540	120	2 360	3 020	7 604	0	4 340	11 944
444	5 120	0	180	5 300	14 785	0	342	15 127
471	3 320	40	281	3 641	11 673	0	181	11 854

TABLE 3 *Experiment* 1.—Ranked worm burdens of *H. contortus*. Only fourth stage larvae (L_4) of *H. contortus* in Group B (*T. axei*+ *O. circumcincta*) were significantly less ($P<0,001$) than the controls by the Mann-Whitney U test

Group A			Group B			Group C		
L_4 (1)	5+A (2)	Total	L_4	5+A	Total	L_4	5+A	Total
920	20	1 323	80	20	100	20	1	21
1 274	21	2 421	620	40	860	240	3	343
1 303	321	2 752	760	120	1 290	340	42	1 707
1 371	2 041	3 412	800	630	1 390	540	180	2 142
1 531	2 070	4 028	820	1 010	2 050	1 240	181	2 320
1 539	2 426	4 292	930	1 120	2 260	1 526	321	3 020
1 866	2 814	4 325	1 040	1 750	3 190	1 720	1 080	3 294
1 958	4 639	6 178	1 140	3 390	4 320	1 731	2 040	3 641
2 118	4 967	6 241	1 170	4 390	5 190	2 100	2 480	3 771
2 400	5 154	6 371	1 440	5 660	7 100	3 320	3 054	5 300
2 431	5 451	7 272	1 440	6 870	7 490	4 880	3 735	8 450
3 700	7 782	11 482	1 840	8 630	10 470	5 120	6 730	8 615
			$P<0,001$					

(1) L_4=4th stage larvae
(2) 5+A=5th stage and adult worms

Results

Worms recovered are set down in Table 2 and ranked and analysed by the Mann-Whitney U test in Table 3. With the exception of 4th stage larvae (L_4) of *H. contortus* in Group B, which were significantly fewer than those in Group A ($P<0,001$), the other results showed no significant difference. *O. circumcincta* alone (Group C) was completely unsuccessful as a possible vaccine.

Experiment 2.—*T. axei* and *O. ostertagia* as a possible vaccine challenged with trickle doses of infective larvae of *H. contortus*

This trial differed from previous experiments in that challenge with infective larvae of *H. contortus* to both groups of sheep was administered at irregular intervals from Day 0 for a period of 5 months.

Materials and Methods

The experimental design is summarized in Table 4. This trial ran parallel with Experiment 1 and a further 24 Merinos were divided into 2 groups (D & E) of 12 sheep each. Group D served as controls and each sheep in Group E was dosed on Day 0 with 10 000 infective larvae of *T. axei* plus 10 000 infective larvae of *O. circumcincta*. This was repeated on Day +14. All the sheep in both groups were challenged with infective larvae of *H. contortus* from Day 0 to Day+154. Larvae were dosed on different days of the week varying from 1–3 times a week. From Day 0 to Day+91 the total number of larvae that were dosed in any week did not exceed 4 000, until each sheep had received 50 000 larvae. Thereafter the total number dosed per week rose to 6 000 per week until a further 50 000 larvae were dosed, i.e. from Day+95—Day+154.

Faecal samples were collected every 7 days from Day+21 and differential egg counts based on the identification of 1st stage larvae (L_1) were carried out (Whitlock, 1959). Blood samples for haematocrit (Ht) were collected from Day+28 onwards. All sheep were killed on Day+175.

Results

Fluctuations in worm egg counts and Ht are presented graphically in Fig. 1 and 2.

TABLE 4 *Experiment* 2.—Experimental design showing the days on which infective larvae were dosed to each sheep and the day of slaughter

Days	No. of infective larvae dosed to each sheep	
	Group D	Group E
0	—	*T. axei*+*O. circumcincta*
+14	—	*T. axei*+*O. circumcincta*
Total	—	20 000+20 000
0 to+91	*H. contortus*	*H. contortus*
Total	50 000	50 000
+95 to +154	*H. contortus*	*H. contortus*
Total	50 000	50 000
+175	Slaughter	Slaughter

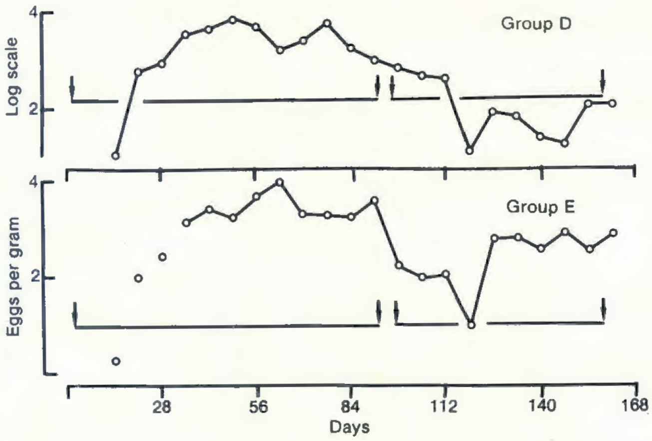

FIG. 1 Variation in faecal worm egg counts of *H. contortus* in Groups D and E. The first 3 egg counts in Group E were undifferentiated and therefore are not joined together with a line. Arrows indicate periods when infective larvae of *H. contortus* were dosed to both groups (see Materials and Methods).

Group D (Controls). Worm egg counts rose steadily from the 3rd week to reach a peak at 8 weeks, fluctuated to another peak at 12 weeks and then fell steadily to the end of the experiment at 24 weeks. The Ht, however, fell from the 4th week and rose after the 12th week.

Group E (*T. axei*+*O. circumcincta*). Worm egg counts reached a peak at 10 weeks and minor peaks at 12 and 14 weeks respectively. Thereafter, as in Group D, they fell to low levels (Fig. 1). From the 4th to the 13th week Ht fell and thereafter rose to normal levels (Fig. 2). Again as worm egg counts rose Ht fell.

Worm recoveries and analysis by the Mann-Whitney U test are summarized in Tables 5 and 6 respectively.

Group D (Controls). Worm burdens of *H. contortus* ranged more widely than those in the controls of the previous trial (Experiment 1 Group A). Moreover there was no significant difference between Group D in the present trial and Group A (Experiment 1), although each sheep had received 100 000 infective larvae in the present experiment as compared with half the number (50 000 larvae) dosed to sheep in Group A.

Group E (*T. axei* and *O. circumcincta*). The total worm burdens of *H. contortus* by the Mann-Whitney U test showed a result of 44, only 2 more than 42 for this sized group at the confidence level $P<0,05$ (Table 6). H. T. Groeneveld (1976, personal communication) stated this was probably significant at $P<0,1$ which is not included in the tables in the reference of Siegel (1956).

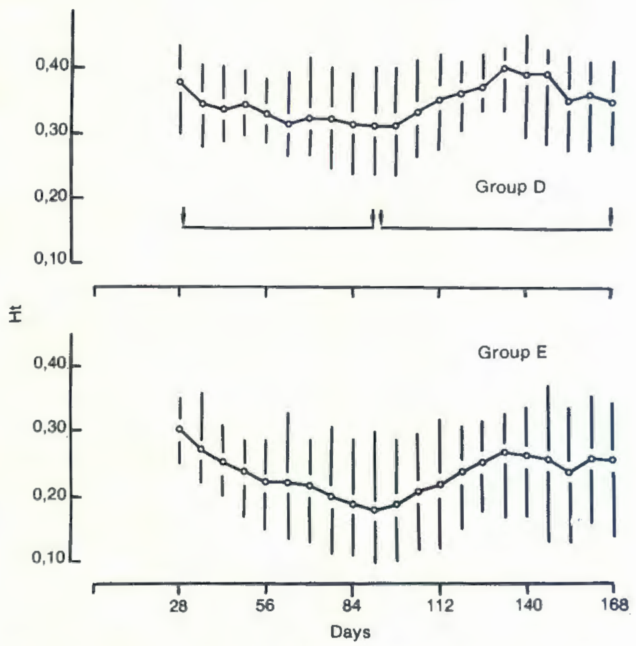

FIG. 2 Fluctuations in haematocrit in Groups D and E. Arrows indicate periods when infective larvae of *H. contortus* were dosed to both groups (see Materials and Methods)

Discussion

In a previous trial we showed that 2 doses of 10 000 infective larvae of *T. axei* dosed at an interval of 14 days was >60% effective in reducing the challenge by *H. contortus* in >60% of the sheep (Reinecke *et al.*, 1980).

TABLE 5 *Experiment* 2.—Worms recovered at necropsy from the controls (Group D)

Sheep No.	*H. contortus*			
	Stage of development			Total
	L_4	5	Adult	
Group D: 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

TABLE 5 (*Continued*). Worms recovered from Group E (*T. axei*+*O. circumcincta*)

Sheep No.	*H. contortus*				*T. axei*				*O. circumcincta*			
	Stage of development			Total	Stage of development			Total	Stage of development			Total
	L_4	5	Adult		L_4	5	Adult		L_4	5	Adult	
Group E.—Day 0: 10 000 *T. axei*+ 10 000 *O. circumcincta*; Day+14: 10 000 *T. axei*+10 000 *O. circumcincta*:												
307	615	0	280	895	0	0	15 850	15 850	9 995	0	250	10 245
319	2 794	0	9 220	12 014	0	0	14 980	14 980	8 726	0	920	9 646
328	120	0	260	380	0	0	5 210	5 210	200	0	60	260
347	258	0	160	418	0	0	11 760	11 760	3 862	0	340	4 202
353	478	0	280	758	0	0	16 690	16 690	2 742	0	1 140	3 882
377	0	0	400	400	0	0	13 400	13 400	6 160	0	190	6 350
404	2 765	0	2 370	5 135	0	0	14 180	14 180	6 305	0	320	6 625
410	52	0	40	92	0	0	10 630	10 630	2 218	0	120	2 338
417	1 682	0	2 660	4 342	0	0	15 390	15 390	8 028	0	370	8 398
419	40	0	80	120	0	0	1 010	1 010	0	0	80	80
421	1 306	0	890	2 196	0	0	8 040	8 040	1 104	0	60	1 164
435	23	0	20	43	0	0	12 440	12 440	427	0	20	447

TABLE 6 *Experiment* 2.—The Mann-Whitney U test applied to *H. contortus* recovered from controls compared with the vaccinated group (Group E)

Group D	Group E	Group D	Group E	Group D	Group E
L_4	L_4	5+A	5+A	Total	Total
5	1	2,5	1	4	1
7	2	4	2,5	8	2
9	3	7	5	10	3
10	4	11	6	12	5
11	6	13	8	13	6
12	8	14	9	14	7
15	13	15,5	10	15	9
17	14	17	12	16	11
19	16	18	15,5	20	17
22	18	21	19	21	18
23	20	22	20	22	19
24	21	23	24	23	24
174–78 =96	126–78 =48	168–78 =90	132–78 =54	178–78 =100	122–78 =44

This analysis by the modified NPM (Reinecke, 1973) is more sensitive than the Mann-Whitney test, but in Experiment 1, if the sheep are predosed twice with 10 000 *T. axei* plus 10 000 *O. circumcincta* at 14 day intervals, there is a reduction in L_4 ($P<0,001$) only, and none in total worm burdens of *H. contortus*. Moreover, 2 doses of 20 000 *O. circumcincta* alone had no effect on subsequent challenge with *H. contortus*. Thus in Group B (*T. axei*+*O. circumcincta*) we were unable to confirm the results of Turner, Kates & Wilson (1962) that these 2 species had a deleterious effect on the establishment of *H. contortus*. In addition, Turner *et al.* (1962) and Reinecke (1966) stated that simultaneous infestation with *O. circumcincta* and *H. contortus* blocked particularly the establishment of *H. contortus* and, to a lesser extent, that of *O. circumcincta*. We were unable to confirm the deleterious effect of *O. circumcincta* on *H. contortus* in Experiment 1 if sheep were predosed with *O. circumcincta* before challenge with *H. contortus*.

In Experiment 2 the mixture of *T. axei* and *O. circumcincta* was possibly able to reduce the challenge of *H. contortus* ($P<0,05$) (Groeneveld, 1976, personal communication) with a trickle challenge, but tended to give the same result that a challenge with *H. contortus* did after a period of 90–93 days in Experiment 1.

It is reasonable to assume that a mixture of *T. axei* and *O. circumcincta* has less protective effect against challenge with *H. contortus* than *T. axei* alone, as was shown in previous experiments by Reinecke *et al.* (1980). Mixing the species is not cumulative as the experiments of Turner *et al.* (1962) show. In these experiments lowest worm burdens of *H. contortus* resulted if *T. axei*, *O. circumcincta* and *H. contortus* were dosed simultaneously; a better result than when either *T. axei* and *H. contortus* or *O. circumcincta* and *H. contortus* were dosed simultaneously. The reasons for these conflicting results in the present trials are not known.

Acknowledgements

We wish to thank the Department of Agriculture and Fisheries for the financial assistance to enable us to carry out these experiments and the Director, Veterinary Research Institute, for the facilities to do the work.

References

REINECKE, R. K., 1966. The value of uniform worm burdens in the larval anthelmintic test. *Journal of the South African Veterinary Medical Association*, 37, 133–142.

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

REINECKE, R. K., BRUCKNER, CHRISTEL & DE VILLIERS, I. L., 1980 Studies on *Haemonchus contortus*. III. Titration of *Trichostrongylus axei* and expulsion of *H. contortus*. *Onderstepoort Journal of Veterinary Research*, 47, 35–44.

REINECKE, R. K., SNYMAN, MARIE H. & SEAMAN, HELGA, 1979. Studies on *Haemonchus contortus*. II. The effect of abomasal nematodes on subsequent challenge with *H. contortus*. *Onderstepoort Journal of Veterinary Research*, 46, 199–205.

SIEGEL, S., 1956. Non-parametric statistics for the behavioural sciences. New York: McGraw Hill Book Co. Inc.

TURNER, J. H., KATES, K. C. & WILSON, G. I., 1962. The interaction of concurrent infections of the abomasal nematodes *Haemonchus contortus*, *Ostertagia circumcinta* and *Trichostrongylus axei* (Trichostrongylidae) in lambs. *Proceedings of the Helminthological Society of Washington*, 29, 210–216.

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