Onderstepoort J. vet. Res. 41 (4), 213—216 (1974)

STUDIES ON *HAEMONCHUS CONTORTUS*. I. THE INFLUENCE OF PREVIOUS EXPOSURE TO *TRICHOSTRONGYLUS AXEI* ON INFESTATION WITH *H. CONTORTUS**

R. K. REINECKE, Department of Parasitology, Faculty of Veterinary Science, University of Pretoria

ABSTRACT

REINECKE, R. K. Studies on *Haemonchus contortus* I. The influence of previous exposure to *Trichostrongylus axei* on infestation with *H. contortus*. *Onderstepoort J. vet. Res.* 41 (4), 213—216 (1974).

A group of Merino sheep, aged 6–7 months was infested orally with 50 000 infective larvae of *Trichostrongylus axei*. This group and a control group were challenged 90–92 days later with 50 000 infective larvae of *Haemonchus contortus*. Previous infestation with *T. axei* caused a reduction of more than 80% in the worm burdens of *H. contortus* in more than 80% of the sheep.

INTRODUCTION

There is some experimental evidence of inter-generic cross-reactions between abomasal helminths (Turner, Kates & Wilson, 1962; Durie, 1962; Reinecke, 1966). This has been confirmed in the field by Muller (1968), who noted: "that where *T. axei* was predominant the depression of *H. contortus* was notable, while the numbers of *Ostertagia circumcincta* were affected to a lesser extent".

The present experiment is an attempt to determine whether initial infestation of *T. axei* confers protection to a subsequent challenge with *H. contortus*.

TABLE 1 Experimental design

Day	No. of infective larvae dosed to each sheep	
	Group A Controls	Group B
0...	—	12 500 L_3* of *T. axei*
+2....	—	12 500 L_3 of *T. axei*
+10..	—	12 500 L_3 of *T. axei*
+13..	—	12 500 L_3 of *T. axei*
Total.	—	50 000
+30..	—	Sheep 22 died
+90..	16 000 L_3 of *H. contortus*	16 000 L_3 of *H. contortus*
+91..	16 000 L_3 of *H. contortus*	16 000 L_3 of *H. contortus*
+92..	18 000 L_3 of *H. contortus*	18 000 L_3 of *H. contortus*
Total.	50 000	50 000
+98..	Sheep 68 died.....	—
+114	—	Sheep 62 slaughtered
+118	—	Sheep 9 died
+126	10 sheep slaughtered....	2 sheep slaughtered
+127	—	7 sheep slaughtered

*L_3—third stage larvae

MATERIALS AND METHODS

Sheep

Twenty-three 5 month-old Merino wethers and ewes obtained from Vrede, Orange Free State were stabled under worm-free conditions and treated on 2 occasions with broad-spectrum anthelmintics prior to the commencement of the experiment.

Infective larva

The larvae used in this trial were harvested from the faeces of sheep with mono-specific infestations of *T. axei* and *H. contortus*.

Experimental design

This is summarized in Table 1. Sheep were divided as follows:

Group A (Controls)

Eleven worm-free sheep were dosed from Day +90 to Day +92 with a total of 50 000 infective larvae of *H. contortus*.

Group B

Each of the 12 sheep in this group received a total of 50 000 infective larvae of *T. axei* divided into 4 equal doses administered from Day 0 to Day +13 (Table 1).

The sheep in this group and the controls were challenged simultaneously on Days +90 to 92 with a total of 50 000 infective larvae of *H. contortus*. Emergency slaughter had to be performed on Sheep 62 which fractured a tibia on Day +114.

Worm recovery

At autopsy worms were recovered from 3 separate specimens: the ingesta of the abomasum and duodenum; the digested abomasal and duodenal wall; the ingesta of the first 7 m of the jejunum.

Specimens were heated to 60 °C to kill the worms, formalin was added to fix them and the specimens were sieved on to Endecott sieves (apertures 37 μm) and examined as described by Reinecke (1973).

Total counts were carried out on all the *H. contortus* recovered from the sheep in Group B as well as those of Sheep 25 and 28 in Group A (Table 2). The latter figures were essential for estimation of the lower limit (L_L) of the controls. Elsewhere worm burdens were estimated from aliquots using the methods of Clark, Tucker & Turton (1971). Larval stages of *H. contortus* were identified according to the descriptions of Veglia (1915).

RESULTS

The number of worms recovered is recorded in Table 2.

Group A: Controls

On Day +98 Sheep 68 died of bluetongue 8 days after it had received the last dose of infective larvae of *H. contortus* and this explains why 4th stage larvae were dominant in it. The survivors were killed on Day +126 when the youngest worm was 34 days old. The relatively large numbers of 4th stage larvae in these sheep cannot be explained. The sheep were susceptible, worm burdens in all of them varying from 156 to 9 358 (Table 2).

* This project was subsidised by a research grant from the Department of Agricultural Technical Services, Republic of South Africa

Received 12 August 1974—Editor

TABLE 2 Worms recovered *post mortem*

Sheep No.	*H. contortus*				*T. axei*	
	L_4	5	Adult	Total	Adult	Total
	Group A		Controls			
10	2 539	441	4 468	7 448	—	—
15	1 681	331	4 998	7 010	—	—
17	2 216	850	3 780	6 846	—	—
25*	837	327	2 741	3 905	—	—
28*	1 206	1 365	3 161	5 732	—	—
37	1 365	150	4 555	6 070	—	—
45	1 148	207	196	1 551	—	—
64	8 173	506	679	9 358	—	—
68	14 893	0	1	14 894	—	—
71	73	0	83	156	1	1
77	1 419	484	5 227	7 130	60	60
	Group B		Dosed with *T. axei* 90 days before challenge			
2	6	9	579	594	16 842	16 842
3	0	5	34	39	26 965	26 965
9	0	0	0	0	40 340	40 340
16	0	2	20	22	14 840	14 840
19	0	18	134	152	41 880	41 880
21	0	5	11	16	23 175	23 175
39	0	7	37	44	14 315	14 315
52	0	5	38	43	27 060	27 060
62	0	0	0	0	27 800	27 800
73	0	3	275	278	18 670	18 670
78	1	1	9	11	36 300	36 300

* Total worm counts were carried out

Group B: Dosed with T. axei *prior to challenge*

One sheep died from *T. axei* infestation on Day +30 and emergency slaughter had to be performed on Sheep 62 which fractured a tibia on Day + 114. Sheep 9 and 62 had no *H. contortus* while the numbers in the remainder varied from 11 to 594 (Tables 2 and 3). Adult worms were dominant and in only 2 animals were 4th stage larvae found: 6 in sheep 2 and 1 in Sheep 78.

The number of *T. axei*, however, varied from 14 315 to 41 880. The death of Sheep 9 was probably due to this species despite the fact that its worm burden fell within the range of *T. axei* recovered from the group.

Statistical analysis

The results were analysed by the non-parametric method of Groeneveld & Reinecke (1969) to determine the influence of previous exposure to *T. axei* on infestation with *H. contortus*.

The worm burdens of *H. contortus* in both groups are ranked numerically in Table 3. The first step is to estimate the lower limit (L_L) of the median of the controls. This lies between the 3rd (Sheep 25) and 4th (Sheep 28) lowest number of worms in this group (Groeneveld & Reinecke, 1969, Table 9). These numbers are essential for an accurate estimation of the influence of *T. axei* and the numbers listed in Tables 2 and 3 are totals of all 3 specimens of the respective sheep.

Using Tables 9 and 10 from Groeneveld & Reinecke (1969) the L_L of the controls can be calculated as being:

N_C	Probability	K′	K″
11	0,90	0,10	0,90

$$L_L = 3905\ (0,10) + 5732\ (0,90)$$
$$= 5549,3$$

Eighty % reduction on the L_L of the controls is therefore:

$$L_L\ (0,02) = 5\,549,3\ (0,2)$$
$$= 1\,109,86$$

TABLE 3 Level of reduction of *H. contortus* burden estimated by the non-parametric method

H. contortus	
Group A Controls	Group B
156	0
1 551	0
*3 905	11
*5 732	16
6 070	22
6 846	39
7 010	43
7 130	44
7 448	152
9 358	278
14 894	594
3 905 × 0,1 = 390,5	0/11
5 732 × 0,9 = 5158,8	exceed
L_L = 5549,3	1 109
5 549,3 × 0,2 = 1 109,86	

* Total worm counts were carried out

None of the treated animals had worm burdens exceeding 1 109. Therefore infestation with *T. axei* apparently caused more than 80% reduction in *H. contortus* in more than 80% of the infested population.

Discussion

The present experiment has shown that "infestation" with 50 000 infective larvae of *T. axei* dosed orally over a period of 13 days can protect sheep from a subsequent challenge of *H. contortus* 90 to 92 days later.

It was not the object of this investigation to determine the mechanism responsible for the protection that was induced. Self-cure does not appear to have taken place because the sensitizing dose of *T. axei* was not affected. It is therefore not clear whether a hypersensitivity reaction was involved. It is known that large worm burdens of *T. axei* cause a rise in

abomasal pH (Ross, Purcell & Todd, 1969) and Christie (1970) has suggested that a change in abomasal conditions, as exemplified by a rise in pH due to *H. contortus* infection, is directly responsible for preventing a massive challenge of this worm from overwhelming the host.

The major defect of this method of "vaccination" is that 50 000 infective larvae of *T. axei* were lethal in 2 out of 11 sheep (18,1%) within the experimental period of 127 days.

To apply this method of control in practice it would be essential not only to reduce the dose of infective larvae of *T. axei* to non-pathogenic levels but also the frequency of their administration to 1 or, at the most, 2 doses.

ACKNOWLEDGEMENTS

The Director, Veterinary Research Institute, Onderstepoort is thanked for the facilities to carry out these trials and The Dean, Faculty of Veterinary Science for allowing the time to do this work. Mrs. Helga Seaman, Miss Elizabeth Lategan and Mr N. J. Meintjies are thanked for their valuable technical assistance.

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