Onderstepoort J. Vet. Res., 47, 35–44 (1980)

STUDIES ON *HAEMONCHUS CONTORTUS*. III. TITRATION OF *TRICHOSTRONGYLUS AXEI* AND EXPULSION OF *H. CONTORTUS**

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ABSTRACT

REINECKE, R. K., BRUCKNER, CHRISTEL, M. & 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 (1980).

Groups of Merino weaners were dosed with infective larvae of *Trichostrongylus axei* in numbers ranging from 20 000–50 000 and challenged 3 months later with 50 000 infective larvae of *Haemonchus contortus*. When half the dose of infective larvae of *T. axei* was given on Day 0 and the balance on Day +14, efficacy against *H. contortus* was > 60% in > 60% of sheep (P < 0,1). A single dose of 40 000 or 50 000 infective larvae of *T. axei* was > 80% effective against *H. contortus* in > 80% of sheep (P < 0,01).

Two doses of 20 000 infective larvae of *T. axei* followed by a challenge with *H. contortus* 31–33 days after the initial dose caused a reduction of > 50% in > 50% of sheep (P < 0,1). This rose to > 60% in > 60% of sheep if the doses of 25 000 infective larvae of *T. axei* were followed by a challenge with *H. contortus* 45 days after the initial dose of *T. axei*.

Most of the challenge do es of infective larvae of *H. contortus* were rejected within 3 days. Surviving worms were retarded in the 4th stage and only a few developed to the 5th or adult stage.

Résumé

ÉTUDES SUR HAEMONCHUS CONTORTUS. *III. TITRAGE DU* TRICHOSTRONGYLUS AXEI *ET EXPULSION DE* B. CONTORTUS

Des groupes de Merino en sevrage ont été traités avec des larves infectieuses de Trichostrongylus axei *en nombres se situant de* 20 000 *à* 50 000 *et ils furent ensuite soumis trois mois plus tard à une épreuve de* 50 000 *larves infectieuses d'*Haemonchus contortus. *Quand la moitié de la dose des larves infectieuses de* T. axei *fut administrée au Jour* 0 *et le restant au Jour* +14 *l'efficacité contre* H. contortus *fut de* 60% *chez* 60% *des moutons* (*P*< 0,1). *Une dose unique de* 40 000 *ou* 50 000 *larves infectieuses de* T. axei *fut de* 80% *effective contre* H. contortus *chez* 80% *des moutons* (*P*< 0,01).

Deux doses de 20 000 *larves infectieuses de* T. axei *suivies d'une épreuve avec* H. contortus 31 *à* 33 *jours après la dose initiale ont causé une réduction de* 50% *chez* 50% *des moutons* (*P* < 0,1). *Ceci augmenta jusqu'à* 60% *chez* 60% *des moutons quand les doses de* 25 000 *larves infectieuses de* T. axei *étaient suivies par une épreuve avec* H. contortus 45 *jours après la dose initiale de* T. axei.

La plupart des doses d'épreuve de larves infectieuses de H. contortus *furent rejetées dans les* 3 *jours. Les vers survivants furent retardés au* 4 *me stade et seuls quelques uns d'entr'eux se développèrent jusqu'au 5me stade ou stade adulte.*

INTRODUCTION

Previous studies have clearly shown that when sheep are dosed with infective larvae of *T. axei* there is a marked reduction in the establishment of a subsequent challenge with *H. contortus* administered 70; 90–92; 119–121 and 181–183 days later, respectively (Reinecke, 1974; Reinecke, Snyman & Seaman, 1979). The efficacy against *H. contortus* varied with the number of infective larvae of *T. axei* dosed. It was significant with either 16 000 or 25 000 larvae (P<0,05) and highly significant with 50 000 larvae (P<0,001).

At the highest level (50 000 *T. axei*), 4 out of 22 Merinos died from 60 days onwards after the initial larval dose, presumably of *T. axei* infestation (Reinecke *et al.*, 1979). It was therefore necessary to titrate the initial dose of *T. axei* to establish the lowest number of larvae that would protect sheep. In addition the fate of the challenge dose of *H. contortus* and the effect on this species, if administered in less than 90 days after dosing *T. axei*, was examined.

* Presented by the senior author in partial fulfilment of the requirements for the degree M.Med.Vet. (Parasitology), University of Pretoria

Received 2 January 1980—Editor

This paper describes the investigation of these aspects.

EXPERIMENT 1.—TITRATION OF THE DOSE LEVEL OF *T. AXEI*

Doses of 20 000; 30 000; 40 000 and 50 000 infective larvae of *T. axei*, either divided into 2 equal doses or given as a single dose, were compared. A dose of 50 000 larvae irradiated at 0,4 kGy before administration to 1 group of sheep was also included.

MATERIALS AND METHODS

A flock of 6-month-old Merino wethers, purchased at Vrede, Orange Free State, was brought to the laboratory, where the faeces were examined for worm eggs and the flock was treated with mebendazole at 10 mg/kg live mass. They were subsequently vaccinated with bluetongue and enterotoxaemia vaccines. The experiment commenced three months later.

One hundred and twenty of the sheep were divided at random into 10 groups of 12 sheep each. The numbers of infective larvae dosed to each sheep, the days on which they were dosed and on which the sheep died or were slaughtered are listed in the experimental design (Table 1).

TABLE 1 Experiment 1.—Experimental design showing the numbers of infective larvae dosed to each sheep, and the days on which the sheep that were dosed, died or were slaughtered

Day	No. of infective larvae dosed to each sheep				
	Group 1	Group 2	Group 3	Group 4	Group 5
0	0	*T. axei*	*T. axei*	*T. axei*	*T. axei*
+14	0	*T. axei*	0	*T. axei*	0
Total	0	20 000	20 000	30 000	30 000
+84	—	—	—	Sheep 156 died	—
+90	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
+91	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
+92	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
Total	50 000	50 000	50 000	50 000	50 000
+117	—	Slaughter	—	Slaughter	—
+118	—	—	Slaughter	Slaughter	Slaughter
+119	—	—	—	—	—
+120	Slaughter	—	Slaughter	—	—
Day	Group 6	Group 7	Group 8	Group 9	Group 10
0	*T. axei*	*T. axei*	*T. axei*	*T. axei*	**T. axei*
+14	*T. axei*	0	*T. axei*	0	**T. axei*
Total	40 000	40 000	50 000	50 000	50 000
+65	—	—	Sheep 33 died	—	—
+77	Sheep 53 died	—	Sheep 26 & 55 died	—	—
+90	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
+91	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
+92	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
Total	50 000	50 000	50 000	50 000	50 000
+117	—	—	—	—	—
+118	Slaughter	—	—	—	—
+119	—	Slaughter	Slaughter	—	Slaughter
+120	—	—	—	Slaughter	—

* Irradiated at 0,4 kGy

TABLE 2 Experiment 1.—Efficacy against *H. contortus* by the modified NPM. The numbers across the page (e.g. 2×10 000) indicate the number of infective larvae of *T. axei* dosed to each sheep before challenge with *H. contortus*

Controls 1	2×10 000 2	1×20 000 3	2×15 000 4	1×30 000 5	2×20 000 6	1×40 000 7	2×25 000 8	1×50 000 9	0,4 kGy irradiation 2×25 000 10
4 560	242	553	192	1 383	2	0	14	14	782
6 184	290	606	399	1 960	11	5	20	19	2 608
6 890	803	983	758	2 206	21	10	253	50	3 354
11 346	1 057	2 113	2 214	3 240	26	11	367	74	3 951
12 053	1 131	4 443	2 537	3 601	26	12	477	80	5 140
12 331	1 225	5 014	2 701	5 895	28	34	719	166	7 493
14 334	1 269	8 379	2 823	6 188	44	148	736	796	7 877
17 846	1 744	8 414	3 803	6 404	3 293	676	1 102	1 447	8 262
18 232	3 658	8 492	4 410	9 175	4 186	725	4 766	1 571	11 152
22 029	4 549	13 258	4 559	12 102	4 438	1 421	*Sheep 33	1 886	15 627
22 417	5 493	14 906	7 825	12 525	5 027	1 557	*Sheep 26	2 725	17 422
23 9(9	7 186	25 955	*Sheep 156	18 705	*Sheep 53	4 291	*Sheep 55	6 837	18 949
Median 13 332,5	2/12 >	6/12 >	1/11 >	4/12 >	0/11 >	1/12 >	0/9 >	1/12 >	7/12 >
×0,25	5 333	6 666	5 333	6 666	5 333	3 333	5 333	3 333	6 666
A 3 333,1	Class B	Class X	Class B	Class C	Class B	Class A	Class B	Class A	Class X
×0,4									
B 5 333,0									
×0,5									
C 6 666,25									

* Sheep 156, 53, 33, 26 and 55 died before challenge

Data were analysed by the modified, non-parametric method (NPM) (Clark, 1969, cited by Reinecke, 1973), which is widely used for anthelmintic tests and is easily adaptable to the analysis of these results. The analysis is as follows:

1. The median of the numbers of *H. contortus* in the controls indicates the worm burdens in the controls, i.e. 13 332,5 (Table 2).

2. More than 80% reduction in the controls is estimated by multiplying the median by 0,25. Simulation studies have shown that, despite a reduction of only 75%, i.e. median multiplied by 0,25, there is no chance that pre-dosing with *T. axei* will cause 80% reduction, i.e. it will cause >80% reduction in a subsequent challenge with *H. contortus*, and can be defined as such. Estimates of the various classes of efficacy at the 90% significance level are:

   Class A: >80% effective in >80% of the flock.

   At least 11 animals in the group were pre-dosed with *T. axei*, and 1 out of 11 sheep may have had more *H. contortus* than the control median multiplied by 0,25, i.e. 13 332,5×0,25=3 333,1 (Table 2).

   Class B: >60% effective in >60% of the flock. The control median is multiplied by 0,4 and 3 out of 11 animals may have had more *H. contortus* than this reduced control median, i.e. 13 332,5× 0,4=5 333,0 (Table 2).

   Class C: >50% effective in >50% of the flock. The control median is multiplied by 0,5 and 4 out of 11 animals may have had more *H. contortus* than this reduced control median, i.e. 13 332,5× 0,5=6 666,25.

   Class X: Ineffective. Results do not comply with Class C.

   Necropsy precedures and the estimate of worm burdens are described by Reinecke (1973).

RESULTS

Group 1 (Controls): The total worm burdens of *H. contortus* varied from 4 560—23 696 (median 13 332) worms.

The other results are described below.

COMMENT

Efficacy against H. contortus

The total worm burdens are ranked in Table 2 and the efficacy rating of the various groups compared with the controls given. Table 3 summarizes the efficacy rating obtained against 4th stage larvae and adult worms.

Neither 20 000 infective larvae of *T. axei* dosed on Day 0 (Group 3) nor 2 doses of 25 000 infective larvae of *T. axei* irradiated at 0,4 kGy (Group 10) were effective against challenge with *H. contortus*, if analysed by this method. These are denoted Class X (ineffective). If, however, the Mann-Whitney U test is used to compare the worm burdens, both groups have significantly lower worm burdens (P<0,025) than the controls (Siegel, 1956).

In Group 5 (30 000 infective larvae dosed on Day 0), efficacy rose to Class C. At the lower end of the series, dividing the dose in half was an improvement, and both Group 2 (2 doses of 10 000 *T. axei*) and Group 4 (2 doses of 15 000 *T. axei*) rose to Class B efficacy ratings.

When the original dose was a single infestation with either 40 000 (Group 7) or 50 000 infective larvae of *T. axei* (Group 9), this tendency was reversed, and both groups attained Class A. When the dose of infective larvae of *T. axei* was split in half, efficacy fell to Class B in both Group 6 (2×20 000) and Group 8 (2×25 000). It must be noted, however, that there were only 9 survivors in Group 8 and at least 11 results are essential before efficacy can be graded Class A; consequently with 8–10 results it is only possible to grade this Group as Class B.

If the Mann-Whitney U test is used and Group 2 and Group 4 are compared, there is no significant difference, but if Group 2 is compared with Group 6 the difference is significant (P<0,05). Thus, although both the latter groups are Class B by the modified NPM method, there are significantly fewer *H. contortus* in Group 6 than there are in Group 2.

At the higher dose of infective larvae of *T. axei*, however, a single dose of 40 000 or 50 000 larvae attained a rating of Class A against challenge with *H. contortus*, whereas, when this dose was divided into 2 equal doses at 14-day intervals, the rating was Class B efficacy (Groups 6, 7, 8 and 9).

If, however, Group 6 is compared with Group 7, and Group 8 with Group 9, there is no significant difference (by the Mann-Whitney U test) between the numbers of *H. contortus* recovered.

Retarded development

With the exception of Group 10, dosing with *T. axei* prevented the development to the adult stage of a large proportion of the subsequent infestations with *H. contortus*. In Groups 2, 4, 6, 8 and 9, efficacy against adult worms was always 1 or 2 classes higher than that against 4th stage larvae. The single exception (Group 7) could be excluded, because Class A was recorded against both 4th stage larvae and adults (Table 3).

TABLE 3 Experiment 1.—Efficacy against the stages of development of *H. contortus* by the modified NPM

Group	Class	
	L_4	Adult
2	C	A
3	X	C
4	C	B
5	X	B
6	B	A
7	A	A
8	C	B
9	B	A
10	X	X

Total worm burdens of T. axei *and* H. contortus (*Groups* 2–9)

When the total number of infective larvae of *T. axei* dosed to sheep exceeded 40 000 and 4th stage larvae in Group 8 (Class C) are excluded, efficacy against *H. contortus* was either Class A or B.

TABLE 4 Experiment 1.—Comparison of worm burdens of *H. contortus* with *T. axei*

H. contortus	*T. axei*	*H. contortus*	*T. axei*	*H. contortus*	*T. axei*	*H. contortus*	*T. axei*
Group 2		Group 3		Group 4		Group 5	
242	10 637	553	3 930	192	22 247	1 383	16 517
290	16 515	606	14 322	399	14 198	1 960	25 057
803	11 782	983	16 781	758	26 268	2 206	23 531
1 057	15 928	2 113	14 811	2 214	24 726	3 240	26 246
1 131	15 981	4 443	9 580	2 537	28 972	3 601	21 188
1 225	15 434	5 014	17 394	2 701	25 389	5 895	16 108
1 269	16 718	8 379	17 017	2 823	22 162	6 188	27 256
1 744	15 617	8 414	18 729	3 803	27 604	6 404	29 245
3 658	15 572	8 429	8 939	4 410	22 994	9 175	25 312
4 549	17 648	13 258	15 594	4 559	24 611	12 102	2 521
5 493	16 077	14 906	12 308	7 825	18 320	12 525	21 920
7 186	18 170	25 955	22 434	*		18 705	6 169
Group 6		Group 7		Group 8		Group 9	
2	2 987	0	24 703	14	36 638	14	32 042
11	28 285	5	2 300	20	45 536	19	26 863
21	21 189	10	26 256	253	10 293	50	29 230
26	28 888	11	502	367	32 932	74	27 884
26	31 071	12	24 399	477	40 653	80	30 608
28	29 619	34	28 205	719	38 563	166	37 049
44	34 497	148	31 227	736	28 811	796	35 236
3 293	26 194	676	20 362	1 102	27 647	1 447	35 083
4 186	39 620	725	33 710	4 766	26 044	1 571	27 305
4 438	29 387	1 421	22 632	(1)		1 886	34 889
5 027	28 992	1 557	31 610	(2)		2 725	41 255
**		4 291	31 467	(3)		6 837	7 064

	T. axei
(1) Sheep 33 died Day +65.......	23 280
(2) Sheep 26 died Day +77.......	26 380
(3) Sheep 55 died Day +77.......	19 507

* Sheep 156 died on Day +84. No worm count made

** Sheep 53 died on Day +77. No worm count made

In an attempt to determine the lowest number of adult *T. axei* established in the sheep that would be highly effective against *H. contortus*, the burdens of *H. contortus* were ranked and the *T. axei* present in the same sheep placed in a column next to the ranked *H. contortus* burdens (Table 4).

There is no apparent correlation between low *H. contortus* worm burdens and high residual numbers of *T. axei* in any group. Moreover, 2 sheep in Group 7 had very few worms of both species, viz:

Sheep	Numbers of worms recovered	
	H. contortus	*T. axei*
60........................	5	2 300
98........................	11	502

Possible explanations of these counts are—either

(i) that both sheep were resistant to both species before the experiment began;

(ii) or self-cure at challenge with *H. contortus* eliminated both species;

(iii) or some other mechanism not connected with self-cure eliminated both species after challenge.

To attain Class A there must be less than 3 333 *H. contortus*. If Sheep 60 and 98 are excluded and the number of *T. axei* are ranked in all the sheep with less than 3 333 *H. contortus* in Groups 7 and 9, Class A against *H. contortus* is attained with a median count of *T. axei* of 29 919, ranging from 20 362—41 225 worms.

This, however, fails in Group 7, where the highest burden of 4 291 *H. contortus* is accompanied by 31 467 *T. axei* in the same sheep.

Deaths

Five sheep died, 1 in Group 4, 1 in Group 6 and 3 in Group 8. The causes of these deaths cannot be explained. It is interesting to note, however, that these deaths were confined to groups that had been dosed twice with *T. axei* and did not occur in groups that had received a single dose of infective larvae.

EXPERIMENT 2.—THE FATE OF THE CHALLENGE DOSE OF *H. CONTORTUS*

Two trials were conducted to establish the rapidity with which sheep infested with *T. axei* expelled a subsequent challenge with *H. contortus*.

MATERIALS AND METHODS

Forty Merino sheep from the flock used in Experiment 1 were divided into 4 groups, 2 of 11 each and 2 with 10 and 8 sheep each. The day of infestation, the number of larvae dosed and the day of slaughter are summarized in Table 5.

TABLE 5 Experiment 2.—Experimental design showing the days on which infective larvae were dosed to each sheep, the numbers dosed, and day of slaughter

Day	No. of infective larvae dosed to each sheep	
	Group 11	Group 12
0	0	*T. axei*
+14	0	*T. axei*
Total	0	50 000
+45	*H. contortus*	*H. contortus*
+46	*H. contortus*	*H. contortus*
+47	*H. contortus*	*H. contortus*
Total	50 000	50 000
+52	5 sheep slaughtered	6 sheep slaughtered
+62	5 sheep slaughtered	5 sheep slaughtered
Day	**Group 13**	**Group 14**
0	0	*T. axei*
+14	0	*T. axei*
Total	0	50 000
+87	*H. contortus*	*H. contortus*
Total	50 000	50 000
+90	2 sheep slaughtered	3 sheep slaughtered
+91	2 sheep slaughtered	3 sheep slaughtered
+92	2 sheep slaughtered	3 sheep slaughtered
+93	2 sheep slaughtered	2 sheep slaughtered

TABLE 6 Experiment 2.—Groups 11 and 12. Worms recovered at necropsy

Sheep No.	Slaughtered on	*H. contortus* Stage of development		Total	*T. axei* Stage of development			Total
		L_4	5		L_4	5	Adult	
Group 11.—Controls: Infested from Day +45 to Day +47 with *H. contortus*								
201	Day +52	14 227	0	14 227	0	0	0	0
214	Day +52	22 617	0	*22 617	0	0	0	0
219	Day +52	17 343	0	17 343	0	0	0	0
221	Day +52	28 994	0	*28 994	0	0	0	0
228	Day +52	29 276	0	29 276	0	0	0	0
212	Day +62	22 918	1 278	*24 196	0	0	0	0
222	Day +62	22 273	7 047	29 320	0	0	0	0
223	Day +62	18 183	7 755	*25 938	0	0	0	0
225	Day +62	15 515	13 821	29 336	0	0	0	0
226	Day +62	18 708	4 477	*23 185	0	0	0	0
Group 12.—Challenged from Day +45 to Day +47 with *H. contortus*								
19	Day +52	9 740	0	9 740	8	720	36 108	36 836
76	Day +52	589	0	589	920	120	31 729	32 769
94	Day +52	1 964	0	1 964	1 556	1 257	33 120	35 933
158	Day +52	8 610	0	8 610	1 435	1 320	28 176	30 931
198	Day +52	196	0	196	180	2 920	24 822	27 922
199	Day +52	242	0	242	390	0	37 113	37 503
4	Day +62	250	1	251	0	0	34 693	34 693
77	Day +62	4 404	0	4 404	533	0	35 817	36 350
78	Day +62	0	0	0	160	0	34 623	34 783
89	Day +62	568	0	568	517	440	40 461	41 418
200	Day +62	614	0	614	607	0	23 534	24 141

* Total worm counts

Results

The number of worms recovered are summarized in Tables 6 and 7.

TABLE 7 Experiment 2.—Groups 13 and 14. Worms recovered at necropsy

Sheep No.	Slaughtered on	*H. contortus* *L₄ and total	*T. axei* *Adult and total
Group 13.—Controls: Infested on Day +87 with *H. contortus*			
206	Day +90	3 770	141
208	Day +90	13 252	296
210	Day +91	***25 665	84
216	Day +91	10 669	75
211	Day +92	***18 410	0
215	Day +92	34 002	573
213	Day +93	28 227	0
217	Day +93	29 145	14
Group 14.—Challenged on Day +87 with *H. contortus*			
2	Day +90	1 171	38 271
42	Day +90	**191	33 010
196	Day +90	859	10 259
12	Day +91	***4 578	1 502
45	Day +91	1 898	19 076
75	Day +91	180	17 432
16	Day +92	5 843	50 942
37	Day +92	1 942	37 237
186	Day +92	141	26 487
24	Day +93	191	33 050
193	Day +93	395	32 988

* Only fourth stage *H. contortus* and adult *T. axei* were recovered
** Including 2 third stage larvae
*** Total worm counts of *H. contortus*

Group 11.—Controls. Fourth stage larvae of *H. contortus* were either the only stage present or exceeded 5th stages because sheep were killed when the oldest worms were either 7 or 17 days of age, respectively.

Group 12.—Sheep 78 had no *H. contortus*. Nine sheep had only 4th stage larvae, and Sheep 4 250 4th stage larvae and a single 5th stage *H. contortus*.

With the exception of Sheep 4, which had neither 4th stage larvae nor 5th stage *T. axei*, all the sheep in this group had 4th stage larvae and 6 out of the remaining 11 sheep 5th stage worms of this species. Adult *T. axei*, however, were dominant, ranging from 23 534—40 461 worms.

Group 13.—Controls. Fourth stage larvae of *H. contortus* varied from 3 770–34 002. Contamination with *T. axei*, ranging from 14–573 worms, occurred in 6 of the 8 sheep.

Group 14.—The numbers of *H. contortus* ranged from 141–5 843 worms. This was a marked reduction in *H. contortus* when compared with Group 13.

The numbers of *T. axei* ranged from 1 502–50 942 worms. The latter number indicated that at least 1 animal (Sheep 16) was overdosed. No immature *T. axei* were present as was the case in the previous experiments.

Efficacy against H. contortus

Worm recoveries are ranked, and the analysis done by the modified NPM and the data are summarized in Table 8. Recounts were done of those figures with an asterisk, that is, of the totals close to the median of the controls. In Group 14, since the worm count below the reduced median had to be established, the *H. contortus* burden of Sheep 12 was also recounted.

TABLE 8 Experiment 2.—Efficacy against *H. contortus* by the modified NPM

Group 11 Controls	Group 12 45–47 days	Group 13 Controls	Group 14 87 days
14 227	0	3 770	141
17 343	196	10 669	180
*22 617	242	13 252	191
*23 185	251	*18 410	191
*24 196	568	*25 665	395
*25 938	589	28 277	859
*28 994	614	29 145	1 171
29 276	1 964	34 002	1 898
29 320	4 404	Median	1 942
29 336	8 610	=22 037,5	*4 578
Median	9 740	×0,25	5 843
=25 067	0/11	A=5 509,375	1/11
×0,25	>10 026		>5 509
A=6 266,75	Class		Class
×0,4	B		A
B=10 026			

* Total worm counts of *H. contortus*

Group 12 was compared with Group 11 and worm burdens of *H. contortus* were reduced by more than 60% in more than 60% of the sheep in Group 12 (Class B). This improved to more than 80% in more than 80% of the sheep in Group 14 (Class A).

Comment

The results of Experiment 2 show that sheep infested with *T. axei* reject most of the challenge dose of infective larvae of *H. contortus* before they can develop, i.e. within 3 days (Sheep 2, 42, and 196, Group 14, Table 7). Surviving worms are retarded in the 4th stage; only 1 animal (Sheep 4) had a single 5th stage *H. contortus* and 4 sheep none (Group 12, Table 6). This may be compared with controls killed on the same day (Day + 62), in which 5th stage *H. contortus* ranged from 1 278–13 821 worms (Group 11, Table 6).

Douvres (1957) has shown that the 4th moult of *T. axei* takes place from the 10th to the 14th day. In the strain used in this laboratory eggs were noted in the faeces of infested sheep for the first time on the 24th day (De Villiers, 1976, personal communication). The larvae of *T. axei* dosed to the sheep in Group 12 on Day +14 should have completed the 4th moult by Day +28 and adults should have been present by Day +38. The presence of 4th stage larvae on Day +62 indicated a delay of at least 34 days before the 4th moult was completed.

In Group 14, the sheep were given a dose of infective larvae of *T. axei* similar to that given to Group 12, but they were killed from Day +90 to Day +93 (Table 5). Only adult *T. axei* were recovered post-mortem. Thus, 28 days after the slaughter of Group 12 (28+62=90), all the worms in Group 14 had developed into adults.

The presence of large number of *T. axei* from the first infestation was probably responsible for the retarded development of the worms from the booster dose given 14 days later. These retarded worms took longer to develop into adults than normal.

EXPERIMENT 3.—THE EFFICACY OF *T. AXEI* AGAINST A CHALLENGE WITH *H. CONTORTUS* ONE MONTH LATER

A trial was conducted to determine whether *T. axei* was able to protect sheep against a challenge 31–33 days later.

MATERIALS AND METHODS

Twenty-three six-month-old Merinos were purchased, transferred to the laboratory, ear-tagged, vaccinated and treated with anthelmintics. Two weeks later they were divided at random into one group (Group 15) of 11 and another of 12 (Group 16). The days on which they were dosed with infective larvae and slaughtered are listed in Table 9.

TABLE 9 Experiment 3.—Experimental design showing the days on which infective larvae were dosed to each sheep, the numbers dosed, and the days on which the sheep were slaughtered

Day	No. of infective larvae dosed to each sheep	
	Group 15	Group 16
0.........	0	*T. axei*
+15.........	0	*T. axei*
Total...	0	40 000
+31.........	*H. contortus*	*H. contortus*
+32.........	*H. contortus*	*H. contortus*
+33.........	*H. contortus*	*H. contortus*
Total...	50 000	50 000
+60.........	11 sheep slaughtered	12 sheep slaughtered

RESULTS

The number and developmental stages of the worms recovered are summarized in Table 10.

Group 15.—Controls. Adult *H. contortus* were dominant, but all sheep had 4th stage larvae, and 10 out of 11 sheep, 5th stages (Table 10).

Group 16.—Six sheep had either 3rd or 4th stage larvae or 5th stages of *H. contortus*, or combinations of these stages. Adult *H. contortus* was dominant in all the sheep, except in Sheep 530, which was negative for all stages.

Two sheep only had no larval stages of *T. axei* and the others either 3rd or 4th stage larvae, or both, and 9 sheep had 5th stage worms. The total number of larvae and 5th stage of *T. axei* ranged from 0–1 480 compared with the adult range of 7 940 to 31 580 worms.

Efficacy against H. contortus

The worm burdens of *H. contortus* are ranked in Table 11. The median of Group 15 (controls) was 9 850 which, if multiplied by 0,5, gives a result of 4 925. Only 3 out of 12 results in Group 16 (*T. axei* $2 \times 20\,000$) had more worms than the reduced control median of 4 925. This reduction can be classified Class C, >50 % effective in >50 % of the animals.

TABLE 10 Experiment 3.—Worms recovered at necropsy

Sheep No.	Slaughtered on	*H. contortus* Stage of development				Total	*T. axei* Stage of development				Total
		L_3	L_4	5	Adult		L_3	L_4	5	Adult	
Group 15 Controls											
509.......	Day +60	0	302	113	6 340	6 755	0	0	0	0	0
525.......	Day +60	0	39	0	6 767	6 806	0	0	0	0	0
531.......	Day +60	0	122	100	9 628	9 850	0	0	0	0	0
533.......	Day +60	0	35	40	12 989	13 064	0	0	0	0	0
575.......	Day +60	0	256	170	7 991	8 417	0	0	0	0	0
578.......	Day +60	0	344	445	12 330	13 119	0	0	0	0	0
590.......	Day +60	0	41	410	6 728	7 179	0	0	0	0	0
594.......	Day +60	0	192	312	13 680	14 184	0	0	0	0	0
595.......	Day +60	0	75	281	5 151	5 507	0	0	0	0	0
598.......	Day +60	0	219	293	10 973	11 485	0	0	0	0	0
608.......	Day +60	0	195	223	10 511	10 929	0	0	0	0	0
Group 16 2 × 20 000 *T. axei*—Challenged from Day +31–33 with 50 000 *H. contorus*											
507.......	Day +60	0	130	0	680	810	80	0	160	31 580	31 820
514.......	Day +60	20	70	20	1 882	1 992	150	300	220	16 370	17 040
516.......	Day +60	100	140	20	7 810	8 070	0	20	20	7 940	7 980
526.......	Day +60	0	0	0	161	161	150	130	160	27 790	28 230
530.......	Day +60	0	0	0	0	0	0	0	0	21 890	21 890
543.......	Day +60	120	0	60	699	879	0	0	0	14 450	14 450
547.......	Day +60	180	140	200	4 873	5 183	20	180	340	26 220	26 760
583.......	Day +60	5	15	0	7 715	7 735	10	0	0	15 450	15 460
604.......	Day +60	0	33	90	2 378	2 501	2	20	20	17 550	17 592
614.......	Day +60	0	0	0	62	62	164	40	920	25 920	26 414
625.......	Day +60	7	0	120	4 586	4 713	32	0	780	31 390	32 202
631.......	Day +60	0	0	0	5	5	60	140	1 280	24 750	26 230

TABLE 11 Experiment 3.—Efficacy against *H. contortus* by the modified NPM

Group 15 Controls	Group 16 31-33 Days
5 507	0
6 755	5
6 806	62
7 179	161
8 417	810
9 850	879
10 929	1 992
11 485	2 501
13 064	4 713
13 119	5 183
14 184	7 735
Median	8 070
=9 850	3/12
×0,5	>4 925
4 925	Class
	C

Comment

If these results (*T. axei* 2×20 000) are compared with those of Experiment 1, efficacy rises from Class C (50%:50%) at 1 month to Class B (60%:60%) after 3 months (compare Group 16 Table 11 with Group 6, Table 2). If the number of infective larvae is increased to 2 doses of 25 000 larvae of *T. axei*, efficacy against *H. contortus* rises from Class B (60%:60%) at 45–47 days to Class A (80%:80%) at 87 days (compare Group 12 with Group 14, Table 8). Mortalities were unacceptably high in the groups given split doses ranging from 30 000–50 000 infective larvae of *T. axei* in Experiment 1, and, since a single dose of 40 000 caused no mortalities and reached Class A (Group 7 Table 2), this would appear to be the optimum dose.

Veglia (1915) showed that the 3rd moult of *H. contortus* takes place 36 hours after infestation. Slaughter of sheep in Experiment 3 took place when *H. contortus* varied in age from 27–29 days. The presence of 3rd stage larvae of *H. contortus* in 6 sheep pre-dosed with *T. axei* cannot be explained (Group 16 Table 10). Similarly, 3rd stage larvae of *T. axei* were recovered from 9 sheep in Group 16, despite the fact that the 3rd moult normally takes place on the 3rd day (Douvres, 1957).

Since at this stage larvae of the latter species were 45 days old, it could be postulated that pre-dosing with *T. axei* interfered with the development of the subsequent challenge with *H. contortus* as early as the 3rd stage, were it not for the fact that only 4 of these sheep had 3rd stage larvae of both species at slaughter (Sheep 514, 547, 583 and 625, Group 16, Table 10).

In Experiment 2, 6 sheep were killed when the 2nd dose of *T. axei* was 38 days old and *H. contortus* 5–7 days of age (Group 12 Table 5). Neither *T. axei* nor *H. contortus* had any 3rd stage larvae in Group 12 (Table 6). No explanation can be given for the difference in the results of Experiments 2 and 3.

Discussion

Pathogenesis of H. contortus

Sinclair & Prichard (1975) infested 5 Clun sheep with 175 000 infective larvae of *H. contortus*. Seven days later these sheep were given an injection of disophenol at 10 mg/kg which prevented the worms from developing into adults but had no effect on early 4th stage larvae. The sheep were killed about 8 weeks after infestation. Plasma pepsinogen rose from the 3rd day and remained high for 55 days. Plasma, which leaked into the abomasum, varied in volume from 12,5–20,3 mℓ (mean 15,9 mℓ) per day, and the dry matter in the faeces fell. At necropsy, the abomasal pH ranged from 3,8–5,4 (mean 4,8). From these findings they concluded that 4th stage larvae cause some damage to the abomasal mucosa. Blood cell counts were carried out, but the results are not recorded. At necropsy only 38 adults were recovered from 1 sheep while 4th stage larvae ranged from 4 656–22 264.

In the present trials, only 4 out of 44 sheep that had previously been dosed with 40 000 or 50 000 infective larvae of *T. axei* had more than 4 000 4th stage *H. contortus* larvae (range 4 271–6 832) in Groups 6, 7, 8 and 9 in Experiment 1. This number was at the lower limit of the range of 4th stage larvae that were pathogenic in the experiment of Sinclair & Prichard (1975).

Fouth stage larvae can cause some loss of blood, since blood has been found in faeces as early as 6–12 days after infestation (Clark, Kiesel & Goby, 1962).

In Experiment 2 in the present series, it was shown that most of the challenge dose is expelled within 3 days (Table 7) and only moderate numbers of 4th stage larvae of *H. contortus* survive (Table 6). Therefor the pathogenic effects mentioned by Sinclair & Prichard (1975) and Clark *et al.* (1962) would most probably not occur in animals previously infested with *T. axei*.

Allonby (1973) has shown that sheep die within a week from a massive haemorrhage which he has called "hyperacute haemonchosis". The sheep lost from 200–600 mℓ of blood a day and carried burdens of 10 000–35 000 5th stage and young adult worms.

In the acute syndrome, 50–200 mℓ of blood is lost daily as a result of the activities of 1 000–10 000 adult worms. Signs are noted from 1–6 weeks after infestation, but finally a massive drainage of albumin and serum iron reserves results in anaemia and deaths (Dargie, 1973).

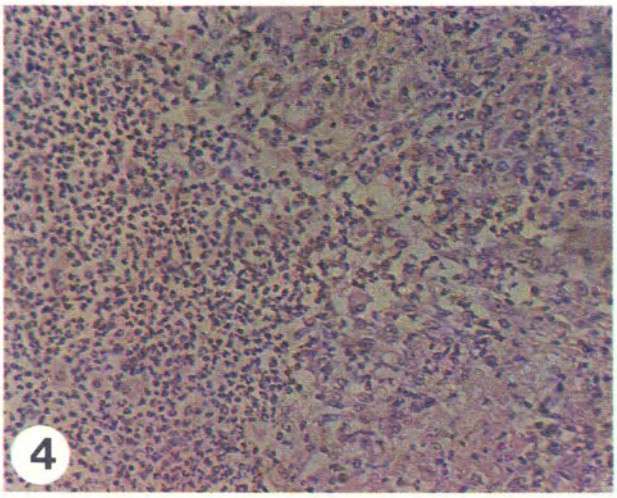
Chronic haemonchosis lasts for 40–100 days and, although there are only a few adult worms (100–1 000) present, the chronic blood loss stimulates compensatory erythropoiesis. This, however, depletes serum iron reserves to a level below which erythropoiesis cannot be maintained, i.e. less than 40 μg%.

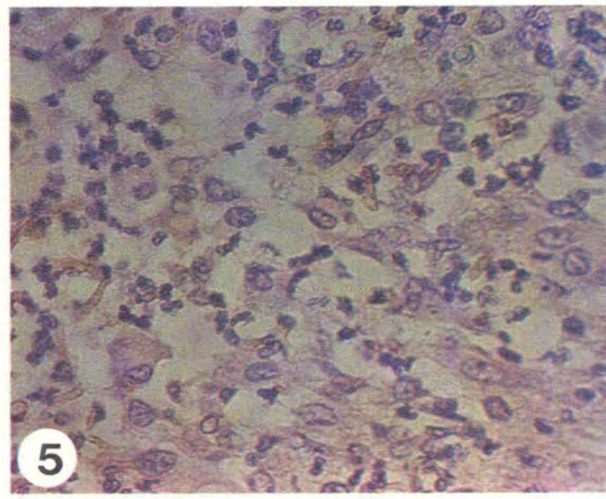
Epizootiology of H. contortus

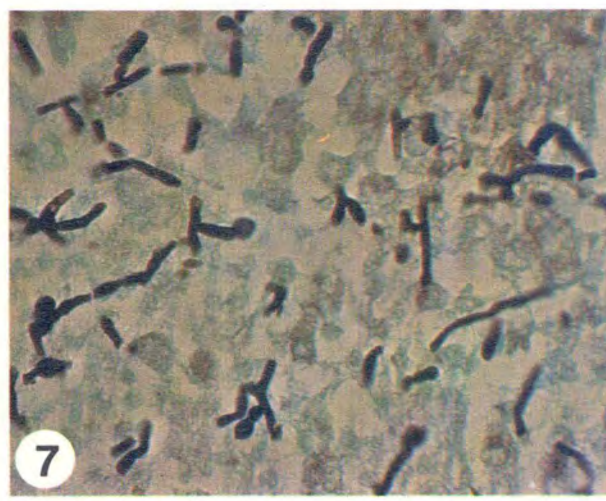
These worms occur in summer rainfall areas throughout the Republic (Barrow, 1964; Rossiter, 1964; Viljoen, 1964; Thomas, 1968). Infective larvae increase in summer after the rains under the influence of temperatures above 17 °C and a monthly rainfall in excess of 15 mm.

Animals become heavily infested from December–April and young lambs may die if they are not treated. If rains in summer are frequent, older sheep can also die from hyperacute or acute haemonchosis. Self-cure takes place, yet some animals have persistent adult worm burdens which cause hypoalbuminaemia and dwindling iron reserves. Poor quality grazing, with very little protein, aggravates the chronic anaemia and sheep die in an emaciated condition with only 100–1 000 adult worms.

A successful vaccine should prevent *H. contortus* from infesting the host, but this is probably impossible. If the worm does survive, it should not develop to the more virulent adult stage. In these experiments *T. axei* was the most successful method employed in reducing the numbers of *H. contortus* and limiting the survivors to early 4th stage larvae.







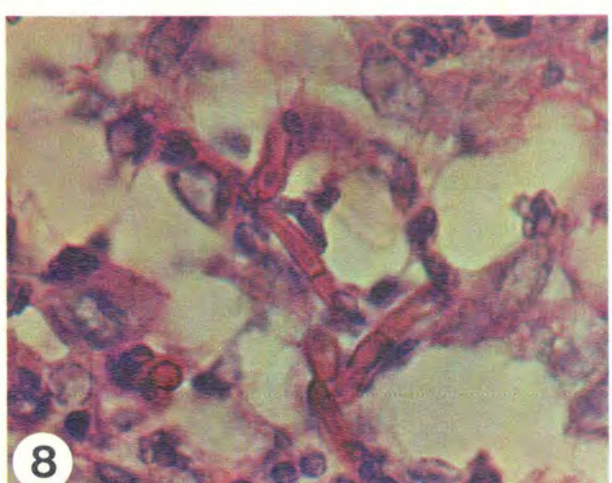


FIG. 4 Part of cerebellar lesion, showing central area (left) containing neutrophils and macrophages and part of the surrounding layer (right) of astrocytes and macrophages; HE × 100

FIG. 5 Higher magnification of the above, showing the cell reaction. A giant cell is present to the right of the field. Several dematiaceous hyphae are present; × 400

FIG. 6 Unstained section of the central area, showing dematiaceous hyphae and structures resembling conidia; × 400

FIG. 7 Hyphae and structures resembling conidia in central area; G.M.S. × 400

FIG. 8 Septate hyphae in close association with neutrophils in the central area of the lesion; P.A.S. × 1 000

The effect of T. axei *on subsequent challenge with* H. contortus

T. axei is the most effective of the species tried in controlling subsequent challenge with *H. contortus*. Neither *O. circumcincta* nor *H. placei* is able to do this (Reinecke *et al.* 1979).

Optimum efficacy of pre-dosing with *T. axei* depends on at least 2 factors:

(i) Forty-thousand or more infective larvae of *T. axei* must be dosed.
(ii) Efficacy varies with time, from a moderate level at 31 days, reaching a high level at 45 days, with a peak at 90 days, to decrease to a lower level from 120–180 days after infestation.

The effect on *H. contortus* is—

(1) that many worms are rejected within 3 days of infestation, i.e. either as 3rd stage or early 4th stage larvae, and
(2) those worms that do survive are arrested in the abomasum as 4th stage larvae.

Michel (1974) gave 2 causative factors for arrested development of nematodes:

(i) "Seasonal factors acting on the free-living stages, such as changes in temperatures and, probably, photoperiod.
(ii) Factors affecting the suitability of the host as an environment for worm development. Among these are the unsuitability of abnormal hosts, innate resistance due to age or sex of the host and the individual or breed characteristics, and the effects of previous infection, the concurrent presence of mature worms or very large inocula".

The writer's hypothesis is an amplification of the ecological suitability of the host for worm development, with particular reference to the last 2 clauses of (ii) above, viz:

The presence of mature worms of one genus that results from a large infestation competes with the subsequent infestation of another genus and prevents both its establishment and development of surviving larvae in the host. The original genus either renders the environment unsuitable or deprives the subsequent infestation of its physiological requirements.

Is there any evidence that this reaction can be explained on physiological or ecological grounds rather than as an immune reaction?

Ross, Purcell & Todd (1969a) reared 3 Blackface-cross lambs parasite-free and inserted abomasal cannulae at 6 months of age. Seven to 14 days later they dosed the lambs with 150 000–250 000 infective larvae of *T. axei*. In the lamb that received 250 000 larvae and developed an acute infestation, the pH of the abomasal fluid rose from the 14th day to reach a level of 6,0. This was associated with an increase in the Na concentration of the abomasal fluid, and a decrease in K concentration and in the dry matter content. In the other 2 lambs, both of which developed chronic infestations, the changes which occurred were not as marked, since the pH increased above 5,0, and changes in the abomasal iron concentrations were erratic. The 3 lambs had burdens that varied from 75 700–96 600 worms, figures which are considerably higher than in any of the present trials.

In subsequent experiments, however, Ross, Purcell & Todd (1969b) infested 6-month-old Blackface lambs with 25 000–250 000 infective larvae of *T. axei*. Six lambs died 5–16 weeks later with burdens of 13 800–57 100 worms, that is, 7–78% of the larvae dosed. These burdens fall within the range of the experiments reported in this paper.

Reinecke *et al.* (1979) recorded deaths in 4 out of 22 sheep dosed with a total of 50 000 infective larvae of *T. axei* on 4 occasions during a 13-day period. In the present experiments most deaths occurred in those sheep dosed with 50 000 infective larvae of *T. axei* on 2 occasions during a 14-day period (Group 8, Experiment 1). In Group 8, 3 sheep died before challenge with burdens varying from 19 507–26 380 worms, that is, 39,0%–52,7% of the number of larvae dosed. There was, however, no correlation between the number of worms recovered and mortalities, because a further 35 sheep in Experiment 1 and 7 sheep in Experiment 2 survived with more than 26 380 *T. axei* each.

Nevertheless, it is reasonable to assume that *T. axei* had physiological effects in Merinos similar to those in Blackface sheep, as reported by Ross *et al.* (1969a).

Christie, Angus & Hotson (1975) dosed lambs with infective larvae of *H. contortus* as follows: 2 000 on Day 1; 1 000 000 on Day 31. Forty-eight to 72 hours after dosing the pH rose, and sheep killed 3, 4, 5 and 27 days after challenge had a mean rise in abomasal pH to 5,68 (lowest 3 values 3,8; 4,6 and 5,0). Adult worms of the original infestation were expelled from the 4th or 5th day onwards.

The rise in pH of the abomasal fluid reported by Ross *et al.* (1969a) in lambs infested with *T. axei* could also have occurred in Merinos in the present trials. The rise in pH is probably one of the factors which make the abomasum unsuitable for the establishment and development of *H. contortus*.

The positive factors supporting the ecological or physiological postulate are presented in the last few paragraphs. There is also evidence to justify the rejection of an immunological basis for these reactions.

Simultaneous infestation

To determine the effect of simultaneous infestation with 2 or 3 species of abomasal nematodes, Turner, Kates & Wilson (1962) divided 12 Shropshire and 8 Columbia Southdale lambs aged 3 months into 4 separate groups. The lambs were paired off and then infested with either *T. axei*, *H. contortus* or *O. circumcinta* (Group I); 3 additional pairs were given 2 species in all possible combinations (Group II); 4 lambs were given all 3 species (Group III) and Group IV were uninfested controls.

Slaughter 3 months later showed that *H. contortus* disappeared in all but 1 lamb in the triple combination and was severely reduced in dual combinations. *O. circumcincta* decreased to a lesser extent when combined with 1 or 2 species, but *T. axei* maintained itself and might even have become established in greater numbers when combined with other species.

This represents competition between the different abomasal parasites, and *T. axei* is obviously the dominant species. There is no evidence that this is an immune reaction.

Merinos as hosts

Lopez & Urquhart (1968), who carried out experimental and field observations in Kenya on the immune response of Merinos to *H. contortus*, concluded that East African Merinos only become immunologically responsive to *Haemonchus* spp. at some point between 7 and 24 months of age. In other experiments prior to their trials on weaned lambs, they attempted to vaccinate adult male and female Merinos with X-irradiated larvae of *H. contortus*, but without success, judging from the faecal egg counts. They also treated 2 groups of 12 adult male Merinos in the field with thiabendazole 7 and 30 days, respectively, before each sheep was challenged with 10 000 larvae and then brought indoors. A third group of 24 wethers was brought indoors on the day of challenge. The half of them that were slaughtered immediately had a mean worm burden of 850 ± 480; the remaining 12 sheep were each challenged with 10 000 larvae. At slaughter 30 days later the mean burdens of the groups were 1 979 ± 633, 1 664 ± 583 and 1 918 ± 1 570 worms, respectively.

Because there were no adult control sheep reared worm-free from birth, it was difficult to assess the precise degree of immunity that had developed in the latter 3 groups. By comparison with worm-free adult Blackface sheep, however, in which a similar number of worms develop from a single dose of 10 000 larvae, the Merinos had no acquired immunity despite repeated exposure to infestation from birth.

In the present trials, weaned Merino lambs aged 6–9 months were dosed with infective larvae of *T. axei*. Challenge with infective larvae of *H. contortus* was complete when the sheep were 8–12 months of age.

Obviously, these Merinos were fully susceptible to *H. contortus*, since the controls were heavily infested. The median counts were 9 850 in Group 15 (Experiment 3), rising to 25 067 in Group 11 and 22 037 in Group 13, respectively (Experiment 2).

The evidence that there is no cross-immunity between *T. axei* and *H. contortus* is that—

(i) 40 000 or more infective larvae of *T. axei* must be dosed to sheep for optimum protection against *H. contortus*;
(ii) *T. axei* probably causes physiological changes in the abomasal fluid, including a rise in pH which may be detrimental to the establishment and development of *H. contortus*;
(iii) simultaneous infestation of *T. axei* and *H. contortus* prevents the development of *H. contortus*;
(iv) neither a related species, *H. placei*, nor another abomasal parasite, *O. circumcincta*, is able to protect sheep against *H. contortus* (Reinecke *et al.* 1979);
(v) *T. axei* can protect weaned Merinos against *H. contortus*. This does not occur in the field wi h natural infestations of *H. contortus* nor wi h the dosing of irradiated infective larvae of the latter species to weaned Merinos.

ACKNOWLEDGEMENTS

We wish to thank the Director, Veterinary Research Institute, Onderstepoort, for housing the sheep, and the Secretary of Agricultural Technical Services for the financial assistance which enabled us to carry out these experiments.

Miss Elisabeth Lategan and Dr N. Meintjies are sincerely thanked for the diligent manner in which they assisted in carrying out the experiments.

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