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STUDIES ON *HAEMONCHUS CONTORTUS*. II. THE EFFECT OF ABOMASAL NEMATODES ON SUBSEQUENT CHALLENGE WITH *H. CONTORTUS**

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ABSTRACT

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Fifty-thousand infective larvae of *Trichostrongylus axei* dosed to 7–9-month-old Merinos protected them against a subsequent challenge with 50 000 infective larvae of *Haemonchus contortus*.

Protection varied with the period between an initial dosage of *T. axei* and challenge with *H. contortus* as follows: After 90 days it was more than 80% effective in more than 80% of sheep; at 119 and 180 days there was still a significant reduction in the numbers of *H. contortus* recovered ($P<0,05$ and $P<0,001$, respectively).

Prior infestation with either *Ostertagia circumcincta* or with *Haemonchus placei* was ineffective against challenge with *H. contortus*.

Résumé

ÉTUDES SUR HAEMONCHUS CONTORTUS. *II. EFFET DES NÉMATODES DE LA CAILLETTE SUR UNE EPREUVE SUBSÉQUENTE À* H. CONTORTUS

L'administration de 50 000 *larves infestantes de* Trichostrongylus axei *à des Mérinos âgés de* 7 *à* 9 *mois les a protégés contre une épreuve subséquente avec* 50 000 *larves infestantes d'*Haemonchus contortus. *D'après le laps de temps entre l'administration initiale de* T. axei *et l'épreuve par* H. contortus, *la protection a varié comme suit: effective à plus de* 80% *chez plus de* 80% *des moutons après* 90 *jours; après* 119 *et* 180 *jours on a encore constaté une diminution significative des quantités d'*H. contortus *retrouvés (respectivement* $P<0,05$ *et* $P<0,001$).

Une infestation préalable avec soit Ostertagia circumcincta *soit* Haemonchus placei *n'a eu aucun effet sur l'épreuve avec* H. contortus.

INTRODUCTION

Turner, Kates & Wilson (1962) used 3-month-old lambs to test the interreactions of *Haemonchus contortus, Ostertagia circumcincta* and *Trichostrongylus axei* in the host by comparing single with double or triple infestations. They showed that the parasites interreacted to the particular detriment of *H. contortus* and, to a lesser extent, of *O. circumcincta*.

Subsequently, Reinecke (1966) attempted to obtain uniform worm burdens in anthelmintic tests by infesting sheep simultaneously with *H. contortus* and *O. circumcincta*. In 1 experiment there were 13 *H. contortus* and 1 215 *O. circumcincta*, while the other controls had from 984–1 798 *H. contortus* and 2 022–4 159 *O. circumcincta*, respectively.

The results obtained by Turner *et al.* (1962) and Reinecke (1966) under laboratory conditions offer a possible explanation for the epizootiology of *H. contortus* at the Outeniqua Experimental Farm, George, in the south-western Cape. The annual rainfall at George, according to Muller (1968), exceeds 820 mm. Rain falls throughout the year, but the driest periods occur during January and July. Temperatures in late spring and summer exceed a monthly mean of 20 °C, but vary from 14–17 °C in autumn and winter. Muller (1968) found that infective larvae of *H. contortus* were available throughout the year, but only minor peaks in adult worm burdens occurred in October, January and March. He cited Turner *et al.* (1962) and Reinecke (1966) in postulating that the dominance of *Ostertagia* spp. and reasonable numbers of *T. axei* in spring and summer suppressed the development of *H. contortus*.

In summer rainfall areas *H. contortus* is the mos important parasite of sheep. Lambs and weaners become heavily infested from December–March, develop a severe anaemia, and may die. Low burdens of 100–1 000 worms in winter continually deplete the iron reserves and, since compensatory erythropoeisis cannot occur, the animals eventually die (Allonby, 1973; Dargie, 1973).

Except on the Border and along the coast in the eastern and south-western Cape, *O. circumcincta* is either absent or present only in modest numbers (Barrow, 1964; Rossiter, 1964; Muller, 1968). *T. axei*, too, is present in small numbers in winter in summer rainfall areas (Reinecke, unpublished observations).

If either *T. axei* or *O. circumcincta* or both could be used as a vaccine to protect sheep against *H. contortus*, it would have the following advantages: deaths from *H. contortus* would cease; repeated drenching with anthelmintics throughout the summer and autumn would not be necessary and the farmer would thus be saved vast sums in capital and labour; annual vaccination for 2 or 3 years might possibly eliminate *H. contortus* from the property.

An obvious disadvantage would be the introduction of *T. axei* or *O. circumcincta* into flocks in which they are absent or of little significance. Fortunately, neither species is nearly as virulent as *H. contortus* (Horak & Clark, 1964; Ross, Purcell & Todd, 1969a, b) and, with the possible exception of hypobiotic larvae of *O. circumcincta*, both species are susceptible to treatment with benzimidazoles and morantel tartrate.

This paper describes 3 experiments which were carried out in sheep maintained under worm-free conditions to establish whether a vaccine can be developed.

EXPERIMENT 1. EFFECT OF *T. AXEI* and *O. CIRCUMCINCTA*

Worm-free sheep were infested with one or other of these species and subsequently challenged with *H. contortus*.

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MATERIALS AND METHODS

Forty-two Dorper yearling ewes, born, reared and housed worm-free, were used. The day on which each sheep was dosed with infective larvae and slaughtered is recorded in Table 1.

Worms were recovered at necropsy in Baermann traps in a modified water-bath and counted, as described by Reinecke (1973). Stages of development were identified according to the descriptions of Veglia (1915) for *H. contortus*, Douvres (1957) for *T. axei*, and Denham (1969) for *O. circumcincta*.

RESULTS

The numbers of worms recovered at necropsy from each group are summarized in Table 2.

Group 1 (Controls).—Despite a lapse of 40 days between infestation and slaughter, 8 out of 14 controls had more 4th stage larvae of *H. contortus* than adult worms, the total number ranging from 5–13 834 (mean 4 246) worms.

Group 2 (*T. axei*).—Sheep 17, 19 and 21 had worm burdens of 15 850, 12 193 and 11 664 *T. axei*, respectively. Sheep 17 had 1 430 4th stage larvae and 13 948 adult worms. The numbers of *H. contortus* varied from 6–2 987 (mean 1 401), which is considerably fewer than in the controls.

Group 3 (*O. circumcincta*).—Few *O. circumcincta* were recovered at necropsy, the burdens ranging from 575–4 213 worms. The numbers of *H. contortus* varied from 9–7 009 (mean 3 058), which constitutes only a slight reduction when compared with those of the controls.

ANALYSIS OF RESULTS

The numbers of 4th stage larvae, 5th stage larvae, adult and total worm burdens of *H. contortus* are ranked in Table 3. The wide range and the markedly skew distribution of worm burdens have been noted in anthelmintic tests, and the non-parametric method (NPM) of Groeneveld & Reinecke (1969) would give both Groups 2 and 3 an ineffective rating (Class X). This is also the case if the modification of Clark (1969, cited by Reinecke, 1973) is used, where the median rather than the lower limit (L_L) is used as an indication of the worm burden of the controls (Group 1). Nevertheless, it is apparent that Group 2 has fewer *H. contortus* than Group 1 and, at the suggestion of Groeneveld (personal communication, 1973), the Mann-Whitney U test was used to analyse the data (Table 4).

According to Siegel (1956), n_1=the number of cases in the smaller of the 2 independent groups, and n_2= the number of cases in the larger. To compute the value of U for fairly large samples, the rank of 1 is assigned to the lowest score of the combined value of (n_1+n_2) group of scores and rank 2 to the next lowest score, etc.

Then $$U=n_1n_2+\frac{n_1(n_1+1)}{2}-R_1$$

or, equivalently,

$$U'=n_1n_2+\frac{n_2(n+1)}{2}-R_2$$

Groeneveld (personal communication, 1976) states that the following formula may be used:

$$U=R_1-\frac{n_1(n_2+1)}{2},$$

Where R_1 is the sum of the ranks of the smaller groups and n_1 the numbers of animals in the smaller groups, equivalently, $U'=n_1n_2-U$, whichever is the smaller. Where both groups have the same number of animals, the same formula may be used.

The actual numbers of worms have been ranked in Table 3.

In Table 4 the controls in Group 1 are compared with those of either Group 2 or Group 3. Because each group has 14 sheep, the lowest number is assigned rank 1, the next rank 2, and so on, until rank 28 is reached. For example, the total worm burdens of *H. contortus* in Group 1 are compared with the total worm burdens of the same species in Group 2, and the sum of the ranks=254 and 152 respectively (Table 4). Using the formula mentioned above,

$$\begin{aligned} U&=152-\frac{14\times15}{2}\\ &=152-105\\ &=47 \end{aligned}$$

or, equivalently,

$$\begin{aligned} U&=14\times14-47\\ &=196-47\\ &=149 \end{aligned}$$

Because 47 is the smaller number, it is used as the test statistic.

TABLE 1 Experiment 1. Experimental design indicating the number of infective larvae dosed and the days on which the sheep were infested and slaughtered.

Days	No. of infective larvae dosed to each sheep		
	Group 1	Group 2	Group 3
0....	0	*T. axei*	*O. circumcincta*
+42....	0	*T. axei*	*O. circumcincta*
Total....	0	16 000	16 000
+70....	*H. contortus*	*H. contortus*	*H. contortus*
Total....	30 000	30 000	30 000
+110...	Slaughter	—	—
+111...	—	Slaughter	—
+112...	—	—	Slaughter

TABLE 2 Experiment 1. Worms recovered at necropsy

Sheep No.	*H. contortus* Stage of development			Total
	*L_4	**5	Adult	
Group 1: Controls				
1..........	0	0	5	5
2..........	48	0	648	696
3..........	1 759	1 041	3 465	6 265
4..........	0	216	1 907	2 123
5..........	3 862	0	1 889	5 751
6..........	1 141	327	940	2 681
7..........	3 142	0	140	3 282
8..........	6 363	528	3 701	10 592
9..........	3 356	103	2 249	5 709
10..........	5 622	286	7 926	13 834
11..........	849	8	384	1 241
12..........	2 750	0	1 053	3 803
13..........	658	18	547	1 223
14..........	2 176	3	61	2 240

*L_4=Fourth stage larvae
**5=Fifth stage

TABLE 2 (Continued)

Sheep No.	*H. contortus* Stage of development			Total	*T. axei* Stage of development			Total
	L_4	5	Adult		L_4	5	Adult	
	Group 2: Day 0 10 000; Day+42 6 000 L_3 of *T. axei*							
15..............	306	0	894	1 200	0	0	7 582	7 582
16..............	985	1	226	1 212	0	0	7 660	7 660
17..............	0	0	6	6	1 430	472	13 948	15 850
18..............	212	0	121	333	160	0	8 160	8 320
19..............	886	0	986	1 872	1	0	12 192	12 193
20..............	970	104	1 047	2 121	0	0	3 718	3 718
21..............	1 191	0	1 796	2 987	0	0	11 644	11 644
22..............	580	0	1 954	2 534	0	0	6 980	6 980
23..............	304	0	1 507	1 811	0	0	5 013	5 013
24..............	2 345	1	5	2 351	0	0	8 336	8 336
25..............	2 468	0	352	2 820	0	0	8 198	8 198
26..............	10	0	3	13	0	0	6 597	6 597
27..............	117	0	6	123	0	0	4 463	4 463
28..............	53	32	147	232	0	0	6 063	6 063

	H. contortus				*O. circumcincta*			
	Group 3: Day 0 10 000; Day+42 6 000 L_3 of *O. circumcincta*							
29..............	3	5	499	507	2	5	1 590	1 597
30..............	0	0	9	9	1 555	160	89	1 804
31..............	2 055	0	1 992	4 047	595	0	1 783	2 378
32..............	4 520	0	559	5 079	1 251	0	404	1 655
33..............	4 306	0	350	4 656	1 309	0	665	1 974
34..............	3 844	0	274	4 118	805	0	1 327	2 132
35..............	3 228	0	69	3 297	374	0	201	575
36..............	2 907	0	2 069	4 976	2 087	0	900	2 987
37..............	320	0	923	1 243	405	0	626	1 031
38..............	181	0	2 306	2 487	84	1	1 913	1 998
39..............	1 956	0	5 053	7 009	732	0	2 118	2 850
40..............	1 213	0	2 315	3 528	2 838	0	1 375	4 213
41..............	732	0	1 026	1 758	568	0	1 441	2 009
42..............	370	0	732	1 102	181	0	961	1 142

TABLE 3 Experiment 1. Ranked worm burdens of *H. contortus*

Group 1			Group 2			Group 3		
L_4	5 & *A	Total	L_4	5 & *A	Total	L_4	5 & *A	Total
0	5	5	0	3	6	0	9	9
0	64	696	10	6	13	3	69	507
48	140	1 223	53	6	123	181	265	1 102
658	392	1 241	117	6	232	320	350	1 243
849	562	2 123	212	121	333	370	504	1 758
1 414	648	2 240	304	179	1 200	732	559	2 487
1 759	1 053	2 681	306	227	1 212	1 213	732	3 297
2 176	1 267	3 282	580	352	1 811	1 956	923	3 528
2 750	1 889	3 803	886	894	1 872	2 055	1 026	4 047
3 142	2 123	5 709	970	986	2 121	2 907	1 992	4 118
3 356	2 352	5 751	985	1 151	2 351	3 228	2 069	4 656
3 862	4 229	6 265	1 191	1 507	2 534	3 844	2 306	4 976
5 622	4 506	10 592	2 345	1 796	2 820	4 306	2 315	5 079
6 363	8 212	13 834	2 468	1 954	2 987	4 520	5 035	7 009

*A=Adult.

TABLE 4 Experiment 1. The Mann-Whitney U test applied to *H. contortus* recovered from controls compared with *H. contortus* recovered from the vaccinated groups

Group 1 L_4	Group 2 L_4	Group 1 5 & A	Group 2 5 & A	Group 1 Total	Group 2 Total
2	2	2	1	1	2
2	4	6	4	7	3
5	6	8	4	10	4
12	7	12	4	11	5
13	8	13	7	15	6
18	9	14	9	16	8
19	10	17	10	19	9
20	11	19	11	22	12
23	14	22	15	23	13
24	15	24	16	24	14
25	16	25	18	25	17
26	17	26	20	26	18
27	21	27	21	27	20
28	22	28	23	28	21
244–105=139	162–105=57	243–105=138	163–105=58	254–105=149	152–105=47
	*P<0,05		*P<0,05		*P<0,025

Group 1 L_4	Group 3 L_4	Group 1 5 & A	Group 3 5 & A	Group 1 Total	Group 3 Total
2	2	1	2	1	2
2	4	3	4	4	3
5	6	5	6	6	5
10	7	8	7	7	8
11	8	11	9	10	9
13	9	12	10	11	12
14	12	16	13	13	15
17	15	17	14	14	16
18	16	18	15	17	18
20	19	21	19	23	19
22	21	24	20	24	20
24	23	25	22	25	21
27	25	26	23	27	22
28	26	28	27	28	26
213–105=108	193–105=88	215–105=110	191–105=86	210–105=105	196–105=91

* Significantly less than Group 1

To determine the critical values of U, the tables of Auble (1953, cited in the appendix to Siegel, 1956), are used, and 47 (Group 2) is significantly less than 149 (Group 1) ($P<0,025$). The numbers of both 4th stage and 5th stage larvae and adult *H. contortus* are significantly smaller in Group 2 than in Group 1 ($P<0,05$). There is no significant difference, however, in any stage of development nor in the total worm burdens of *H. contortus* when those in Group 3 are compared with those in Group 1.

The initial infestation of worm-free sheep with *T. axei* significantly decreased subsequent challenge with *H. contortus*, when total worm burdens and developmental stages were compared with those in the controls ($P<0,025$ and $P<0,05$ respectively). Because *O. circumcincta* appeared to have no effect on the subsequent infestation with *H. contortus*, no useful purpose would be served if this species were to be used as a vaccine in subsequent trials.

The first trial showed some defects in the infestation procedure for *T. axei*. Sheep 17, 19 and 21 were the only animals with more than 10 000 worms at necropsy. A lapse of 42 days between the 2 doses of infective larvae was possibly sufficient for the first dose to prevent the booster dose from developing at all, or, in 1 of the 3 sheep previously mentioned, to retard some of these worms as 4th stage larvae (Sheep 17). Sheep 18 had 160 4th stage larvae and 8 160 adults. These latter 4th stage larvae may also have been retarded for the same reason.

Therefore, in subsequent experiments, the period between the first and last dose(s) of infective larvae should be shorter than 6 weeks. Moreover, the effect of larger doses of infective larvae would have to be tested and an empirical decision made on the total number to be dosed. These were the main objectives of the following 2 experiments.

In addition, *Haemonchus placei*, an abomasal parasite of cattle, was included as an alternative method of control.

EXPERIMENTS 2 AND 3. *T. AXEI* AND *H. PLACEI*

These 2 experiments were run parallel, in both of which infective larvae of *T. axei* and *H. placei* were dosed to act as a vaccine.

General Materials and Methods

A flock of 5-month-old Merino wethers and ewes, purchased in Vrede in the Orange Free State, were transferred to the laboratory, where the faeces were examined for worm eggs. The flock was treated with levamisole at 15 mg/kg live mass and with parbendazole at 30 mg/kg live mass a few days later.

These sheep were infested and slaughtered as indicated in Tables 5 and 8.

Deaths occurred while the experiments were in progress in both Groups B and C in Experiment 2 and in Groups E and F in Experiment 3. As it was

TABLE 5 Experiment 2. Experimental design indicating the number of infective larvae used and the days on which the sheep were infested and slaughtered

Days	Group A	No. of infective larvae dosed to each sheep	
		Group B	Group C
0......	0	*T. axei*	*H. placei*
+ 2......	0	*T. axei*	*H. placei*
+10......	0	*T. axei*	*H. placei*
+13......	0	*T. axei*	*H. placei*
Total......	0	50 000	10 000
+41......	0	0	0
+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
+126.....	Slaughter	Slaughter	—
+127.....	—	Slaughter	Slaughter

TABLE 6 Experiment 2. Worms recovered at necropsy

Sheep No.	*Haemonchus* Stage of development			Total	*T. axei*
	L_4	5	Adult		Adult
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
77..........	1 419	484	5 227	7 130	60
Group B.—*T. axei* dosed 4 times from Day 0–Day +13					
2..........	6	9	579	594	16 842
3..........	0	5	34	39	26 965
9..........	0	0	0	0	40 340
16..........	0	2	20	22	14 840
19..........	0	18	134	152	41 880
21..........	0	5	11	16	23 175
39..........	0	7	37	44	14 315
52..........	0	5	38	43	27 060
62..........	0	0	0	0	27 800
73..........	0	3	275	278	18 670
78..........	1	1	9	11	36 300
Group C.—*H. placei* dosed 4 times from Day 0–Day +13					
4..........	5 157	2 241	10 450	17 848	—
12..........	3 674	55	311	4 040	—
24..........	5 992	9 449	10 514	25 955	—
26..........	18 926	503	4 719	24 148	—
32..........	3 554	2 493	12 453	18 500	—
47..........	2 926	789	4 860	8 575	—
49..........	3 688	843	2 696	7 227	—
53..........	1 521	0	386	1 907	—
57..........	2 519	5 767	1 141	9 427	—
60..........	4 236	693	9 783	14 712	—
65..........	2 823	2 794	18 420	24 037	—
74..........	9 795	1 390	836	12 021	—

desirable to have adequate numbers of animals for statistical analysis by the non-parametric method (NPM) of Groeneveld & Reinecke (1969), each group in Experiment 2 had to have at least 11 results. Thus dead sheep in Groups B and C in Experiment 2 were replaced by sheep from Groups E and F, respectively, in Experiment 3, so that at challenge with *H. contortus* in Experiment 2 there were 11 sheep each in Groups A and B, and 12 sheep in Group C. Although there were 11 controls in Group D, there were only 7 survivors in each of Groups E and F in Experiment 3.

EXPERIMENT 2. CHALLENGE 90–92 DAYS AFTER INITIAL INFESTATION

MATERIALS AND METHODS

The sheep were divided into 3 equal groups of 11 sheep each and replaced when deaths occurred, as described above. The days on which sheep were infested, treated and slaughtered are given in Table 5.

RESULTS

The numbers of worms recovered at necropsy are listed in Table 6.

Group A (Controls).—Sheep 68 died 6–8 days after infestation with *H. contortus*, 4th stage larvae being predominant at necropsy. The 10 survivors also had large numbers of 4th stage larvae despite the lapse of at least 34 days between the last infestation and slaughter.

Group B (*T. axei*).—*H. contortus* was unable to establish itself in 2 animals (Sheep 9 and 62), and in the other 9 sheep burdens ranged from 11–594 worms (Table 6), despite challenge with 50 000 larvae. When it became apparent that there were either no *H. contortus* or that they were present only in small numbers, all specimens were examined microscopically and total counts carried out. This was done to ensure that the worm counts were accurate.

Group C (*H. placei*).—More *Haemochus* spp. were recovered from this group than were present in the controls.

Total worm burdens of *Haemonchus* spp. for the 3 groups are ranked in Table 7. Analysis by the Mann-Whitney U test shows a highly significant difference ($P<0,001$) between the controls (Group A) and Group B.

TABLE 7 Experiment 2. Ranked worm burdens of *Haemonchus* spp.

Group A Controls	Group B 50 000 L_3 *T. axei*	Group C 10 000 L_3 *H. placei*
156...............	0	1 907
1 551.............	0	4 040
3 905.............	11	7 227
5 732.............	16	8 575
6 070.............	22	9 427
6 846.............	39	12 021
7 010.............	43	14 712
7 130.............	44	17 848
7 448.............	152	18 500
9 358.............	278	24 037
14 894............	594	24 148
	*$P<0,001$	25 955

* Significantly fewer worms than in Group A (Mann-Whitney U test)

TABLE 8 Experiment 3. Experimental design indicating the number of infective larvae used and the days on which the sheep were infested and slaughtered

Days	No. of larvae dosed to each sheep				
	Group D	Group E	Group F	Group G	Group H
0..........	0	*T. axei*	*H. placei*	0	0
+2..........	0	*T. axei*	*H. placei*	0	0
+10.........	0	*T. axei*	*H. placei*	0	0
+13.........	0	*T. axei*	*H. placei*	0	0
Total........	0	50 000	10 000	0	0
+62.........	0	0	0	*T. axei*	*H. placei*
+64.........	0	0	0	*T. axei*	*H. placei*
+70.........	0	0	0	*T. axei*	*H. placei*
+72.........	0	0	0	*T. axei*	*H. placei*
Total........	0	0	0	25 000	5 000
+156........	0	0	0	0	0
+157........	0	0	0	0	0
+181........	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
+182........	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
+183........	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*	*H. contortus*
Total........	50 000	50 000	50 000	50 000	50 000
+217........	—	Slaughter	Slaughter	Slaughter	Slaughter
+218........	Slaughter	—	—	—	—

The results in Groups A and B can be analysed by a more stringent statistical test known as the non-parametric method (NPM) of Groeneveld & Reinecke (1969). The present data were subjected to this test by Reinecke (1974) with the result that prior dosing with *T. axei* reduced the worm burdens of the subsequent challenge with *H. contortus* by >80% in>80% of sheep (P<0,10).

EXPERIMENT 3. CHALLENGE 119–121 AND 181–183 DAYS AFTER INITIAL INFESTATION

Materials and Methods

The experimental design is given in Table 8. It has already been mentioned that deaths accounted for the low numbers of sheep in Groups E and F. The surviving 35 Merinos were divided into 5 groups as follows:

Group D. Controls: 11 sheep.

Group E. *T. axei* (50 000): 7 sheep.

Group F. *H. placei* (10 000): 7 sheep.

Group G. *T. axei* (25 000): 5 sheep.

Group H. *H. placei* (5 000): 5 sheep.

Results

The numbers of worms recovered post-mortem are summarized in Table 9.

Group D Controls.—The range of 1 997–17 116 *H. contortus* was more widely distributed than was the case in Experiment 2. There were also considerable numbers of 4th stage larvae despite the lapse of 35 days between the last larval dose and slaughter.

The worm burdens of *Haemonchus* spp. in each group are ranked in Table 10. The analysis of the data was done by the Mann-Whitney U test with the following results.

Group E.—50 000 *T. axei* challenged from Day +181–+183 with *H. contortus*. Highly significant difference (P<0,001).

Group F.—10 000 *H. placei* challenged with *H. contortus* from Day +181–+183. No difference.

Group G.—25 000 *T. axei* challenged 119–121 days later with *H. contortus*. Significant difference (P<0,05).

Group H.—5 000 *H. placei* challenged 119–121 days later with *H. contortus*. No difference.

Discussion

The favourable results obtained in Experiment 1 were greatly improved when the number of infective larvae of *T. axei* was increased from 16 000 to 50 000, the period of dosage reduced from 42 to 13 days and when 4 rather than 2 doses were given during this period. Two variables were investigated during challenge: firstly, the larval dose of 50 000 infective larvae of *H. contortus* was evenly spread over 3 days and, secondly, the challenge took place at either 3 months (90, 91 and 92 days) or twice as long at 6 months (181, 182 and 183 days), respectively.

As originally planned, each group consisted of 11 sheep, an arrangement which is adequate for the NPM at the 90% confidence level.

The unexpected deaths of 4 sheep in Groups B and E necessitated the re-allocation of animals in the various groups in the following ways:

(a) If the data from Group B were to be analysed by the NPM, it was essential that it should consist of 11 sheep. Thus 2 of the 9 survivors in Group E were drafted to Group B, giving 11 sheep in Group B, and a sufficient number of sheep in Experiment 2 for Group B to be compared with the controls (Group A) by the NPM. Naturally, the 7 survivors in Group E were insufficient for the NPM and the less sensitive Mann-Whitney U test had to be used.

TABLE 9 Experiment 3. Worms recovered at necropsy

Sheep No.	*Haemonchus* Stage of development			Total	*T. axei*
	L_4	5	Adult		Adult
Group D Controls					
1..........	2 844	223	4 327	7 394	—
6..........	2 078	1	10 502	12 581	—
20..........	1 062	753	5 320	7 135	—
27..........	121	1	1 875	1 997	—
30..........	1 071	651	11 913	13 635	—
31..........	506	36	1 751	2 293	—
34..........	881	970	8 516	10 367	—
56..........	2 841	387	1 926	5 154	—
72..........	1 406	0	15 710	17 116	—
85..........	2 606	320	4 800	7 726	—
89..........	2 712	682	9 165	12 559	—
Group E *T. axei* dosed 4 times from Day 0–Day +13					
23..........	0	0	457	457	17 145
40..........	198	66	195	459	36 968
51..........	0	0	1 993	1 993	29 639
66..........	0	0	7 119	7 119	17 942
67..........	121	157	209	487	22 574
75..........	146	162	244	552	34 723
81..........	333	27	367	727	20 884
Group F *H. placei* dosed 4 times from Day 0–Day +13					
29..........	2 596	793	13 746	17 135	—
33..........	472	6	11 403	11 881	—
38..........	4 626	327	1 473	6 426	—
46..........	1 506	200	5 753	7 459	—
55..........	1 685	668	11 420	13 773	—
69..........	5 368	1 224	7 030	13 622	—
83..........	337	15	740	1 092	—
Group G *T. axei* dosed 4 times from Day +62–Day +72					
14..........	1 173	35	7 375	8 583	10 281
61..........	0	0	55	55	7 233
76..........	1 078	24	2 832	3 934	10 376
80..........	1 829	514	198	2 541	11 811
88..........	946	320	421	1 687	10 747
Group H *H. placei* dosed 4 times from Day +62–Day +72					
7..........	2 521	163	3 783	6 467	—
54..........	5 315	354	11 046	16 715	—
70..........	1 292	151	13 573	15 018	—
79..........	1 676	211	3 385	5 272	—
87..........	1 008	484	7 023	8 515	—

TABLE 10 Experiment 3. Ranked worm burdens of *Haemonchus* spp.

Group D Controls	Group E 50 000 *T. axei*	Group F 10 000 *H. placei*	Group G 25 000 *T. axei*	Group H 5 000 *H. placei*
1 997	457	1 092	55	5 272
2 293	459	6 426	1 687	6 467
5 154	487	7 459	2 541	8 515
7 135	552	11 811	3 934	15 018
7 394	727	13 622	8 583	16 715
7 726	1 993	13 773	—	—
10 367	7 119	17 135	—	—
12 559	—	—	—	—
12 581	—	—	—	—
13 365	—	—	—	—
17 116	*P< 0,001	—	*P< 0,05	—

* Significantly less than in Group D (Mann-Whitney U test).

(b) When sheep dosed with 50 000 infective larvae of *T. axei* began to die, something might be recovered from Experiment 3 if half this dose was used. Thus 5 worm-free Merinos from the same source were dosed with 4 equal doses of infective larvae in a period of 10 days (Day +62—Day +72) until each sheep had received 25 000 *T. axei* and was challenged with *H. contortus* along with the other sheep from Days +181, +182 and +183, respectively.

Fifty-thousand infective larvae of *T. axei* undoubtedly gave the best protection at 3 months when it reached Class A by the NPM; in other words, *T. axei* at this dosage was 80% effective against *H. contortus* in 80% of sheep and after 6 months caused a highly significant reduction ($P<0,001$). Even half of this dose (25 000 infective larvae) still caused a significant reduction ($P<0,05$) to challenge with *H. contortus* 4 months (119–121 days) later.

The deaths of sheep in Groups B and E, probably due to *T. axei*, was unacceptably high and some method or modification has to be evolved to overcome this.

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