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STUDIES ON *HAEMONCHUS CONTORTUS*. VI. ATTEMPTS TO STIMULATE IMMUNITY TO ABOMASAL TRICHOSTRONGYLIDS IN MERINO SHEEP

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ABSTRACT

REINECKE, R. K., DE VILLIERS, I. L. & BRÜCKNER, CHRISTEL, 1982, Studies on *Haemonchus contortus*. VI. Attempts to stimulate immunity to abomasal trichostrongylids in Merino sheep. *Onderstepoort Journal of Veterinary Research*, 49, 3–6 (1982).

Two doses of infective larvae of 20 000 *Trichostrongylus axei*, dosed to Merino lambs at an interval of 14 days and subsequently challenged with *Ostertagia circumcincta*, caused a significant reduction ($P < 0,01$) in the establishment of 5th and adult stages of the latter. *T. axei* was unable to protect Merino sheep against homologous challenge nor was *Haemonchus contortus* a successful vaccine against challenge with the same species. The vaccinated group showed a reduction ($P < 0,025$) only in 5th and adult *H. contortus*, but not in the total worm burdens.

INTRODUCTION

In previous experiments we have concentrated on dosing worm-free weaned lambs or yearlings with either *Trichostrongylus axei* or *Ostertagia circumcincta* in attempts to protect them against subsequent challenge with *Haemonchus contortus* (Reinecke, 1977; Reinecke, Snyman & Seaman, 1979). In this paper experiments are described to assess whether *T. axei* has any protective effect against *T. axei* or *O. circumcincta* and whether *H. contortus* can stimulate autochthonous immunity.

Experiment 1. The protective effect of *T. axei* against either *O. circumcincta* or *T. axei*

Materials and Methods

The Merinos used in this trial were purchased at the age of 4 months and were treated with anthelmintics. They developed coccidiosis, pasteurellosis and diarrhoea and were treated with Amprolium (MSD) tetracycline (Terramycin Pfizer) and other antibiotics. Some 18 out of 150 died and the survivors were used in this and other experiments.

Forty Merinos were divided into 4 groups, 2 of 9 sheep each (Groups A and C), one of 12 (Group B) and one of 10 sheep (Group D).

The experimental design is summarized in Table 1.

Each lamb in Groups B and D received 20 000 infective larvae of *T. axei* on Day 0 and a similar dose on Day +14.

Three sheep in Group B and 1 in Group D died before challenge. In both Groups B and D one sheep died after challenge.

On Days 90, 91 and 92 Groups A and B were challenged with a total of 50 000 infective larvae of *O. circumcincta*. On the same days each sheep in Groups C and D was dosed with a total of 100 000 infective larvae of *T. axei*. All the survivors were killed on either Day +118 or Day +119.

Results

Worms recovered are summarized in Table 2. With the exception of Sheep 539, which died on Day +66, worms were counted from all sheep that died and the results are included in Table 2.

Worm burdens are ranked in Tables 3 and 4 and the results analysed. Only when sheep vaccinated with *T. axei* were challenged with *O. circumcincta* did fewer worms of the challenge dose develop to adults ($P < 0,01$, Table 3). *T. axei* was unsuccessful as a vaccine against challenge with *T. axei*.

Experiment 2. Autochthonous immunity against *H. contortus*

Materials and Methods

The experimental design is summarized in Table 5.

Twenty-one 4-month-old Merino lambs from the same flock used in Experiment 1 were divided into:

Group E: 11 controls each dosed with 50 000 infective larvae of *H. contortus* from Day +90–Day +92.

Group F: 12 animals each dosed with 5 000 infective larvae on Day 0 and a further 5 000 larvae on Day +14. From Day +90–Day +92 they were challenged with

TABLE 1 Experiment 1. Experimental design. The days on which sheep were dosed with infective larvae of either *T. axei* or *O. circumcincta* and slaughtered

Days	Number of infective larvae dosed to each sheep			
	Group A	Group B	Group C	Group D
0 +14	0 0	*T. axei* *T. axei*	0 0	*T. axei* *T. axei*
Total	0	40 000	0	40 000
+90 +91 +92	*O. circumcincta* *O. circumcincta* *O. circumcincta*	*O. circumcincta* *O. circumcincta* *O. circumcincta*	*T. axei* *T. axei* *T. axei*	*T. axei* *T. axei* *T. axei*
Total	50 000	50 000	100 000	100 000
+118 +119	— Slaughtered	Slaughtered —	— Slaughtered	Slaughtered —

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TABLE 2 Experiment 1. Worms recovered at necropsy

Sheep No.	*O. circumcincta* Stage of development				Total
	*L_3	*L_4	*5	Adult	
	Group A: *O. circumcincta* controls				
508	366	6 574	3 040	6 760	16 740
535	1 853	17 747	900	1 440	21 940
537	74	4 311	560	339	5 284
552	276	16 270	0	1 101	17 647
555	0	15 510	820	2 590	18 920
568	97	4 032	85	4 036	8 250
574	137	9 383	110	3 070	12 700
580	3	13 781	800	3 911	18 495
612	2 336	17 094	990	10 610	31 030

* L_3 = 3rd stage larvae
L_4 = 4th stage larvae
5 = 5th stage

TABLE 2 *(Continued)*

Sheep No.	*O. circumcincta* Stage of development					*T. axei* Stage of development				
	L_3	L_4	5	Adult	Total	L_3	L_4	5	Adult	Total
	Group B: 2 × 20 000 L_3 *T. axei*									
502	0	23 140	280	1 050	24 470	0	0	120	1 080	1 200
523	0	14 490	0	440	14 930	0	0	0	24 698	24 698
538	40	3 124	0	0	3 164	98	98	0	16 690	16 886
548	0	9 744	280	400	10 424	0	0	200	15 560	15 760
553	0	11 424	360	160	11 944	0	0	10	6 924	6 934
554	0	19 460	600	5 850	25 910	0	0	100	1 800	1 900
559	22	19 384	80	444	19 930	0	0	0	18 090	18 090
565	7	15 225	760	800	16 792	0	0	0	29 383	29 383
*601	0	3 216	0	0	3 216	0	0	0	13 194	13 194
Sheep 579 died on Day +55 before challenge						6	5	113	21 179	21 303
Sheep 518 died on Day +62 before challenge						39	253	57	12 973	13 322
Sheep 564 died on Day +66 before challenge						0	2	6	25 093	25 101

* Sheep 601 died on Day +114

TABLE 2 *(Continued)*

Sheep No.	*T. axei* Stage of development				Total
	L_3	L_4	5	Adult	
	Group C: *T. axei* controls				
503	1 300	510	4 780	21 850	28 440
517	572	581	2 123	38 919	42 195
581	807	3 156	5 009	31 200	40 172
591	2 770	7 330	2 940	26 810	39 850
613	745	8 070	4 445	18 210	31 470
617	1 670	1 880	640	24 880	29 070
624	1 780	4 220	4 330	32 950	43 280
627	794	2 965	4 860	34 090	42 709
632	4 570	11 620	5 690	31 100	52 980

TABLE 2 *(Continued)*

Sheep No.	*T. axei* Stage of development				Total
	L_3	L_4	5	Adult	
	Group D: 2 × 20 000 L_3 *T. axei*				
506	2 307	2 495	716	3 442	8 960
512	2 270	2 870	480	1 880	7 500
513	2 417	4 112	699	29 288	36 516
562	4 353	7 537	4 360	16 270	32 520
*566	18	409	964	32 693	34 084
567	520	1 064	323	42 925	44 832
585	1 013	5 766	1 362	42 665	50 806
587	12 990	6 960	5 890	7 820	33 660
606	991	49	1	8 567	9 608

* Sheep 566 died on Day +114

50 000 infective larvae of *H. contortus*. Four sheep died in this group, 2 before and 2 after challenge (Table 6).

The survivors of Group F and all the sheep in Group E were slaughtered on Day +119.

TABLE 3 Experiment 1. Ranked worm burdens of *O. circumcincta*

Group A			Group B		
$L_3 + L_4$	5 + A***	Total	$L_3 + L_4$	5 + A	Total
4 129	899	5 284	3 164	0	3 164
4 385	1 101	8 250	3 216	0	3 216
6 940	2 340	12 700	9 744	440	10 584
9 520	3 180	16 740	11 424	520	11 944
13 784	3 410	17 647	14 490	524	14 930
15 510	4 121	18 495	15 232	680	16 792
16 546	4 711	18 920	19 406	1 330	19 930
19 430	9 800	21 940	19 460	1 560	24 470
19 600	11 600	31 030	23 140	6 450	25 910
				($P <$ 0,01)**	*Sheep 518
					*Sheep 564
					*Sheep 579

* Sheep died before challenge
** Significantly fewer worms than Group A by the Mann-Whitney U test
*** A = Adult worms

TABLE 4 Experiment 1. Ranked worm burdens of *T. axei*

Group C			Group D		
$L_3 + L_4$	5 + A	Total	$L_3 + L_4$	5 + A	Total
1 153	22 655	28 440	427	2 360	7 500
1 810	25 520	29 070	1 040	4 158	8 960
3 550	26 630	31 470	1 584	8 568	9 608
3 759	29 750	39 850	4 802	13 710	32 520
3 963	36 790	40 172	5 140	20 630	33 660
6 000	36 209	40 640	6 529	29 987	34 084
8 815	37 280	42 195	6 779	33 657	36 516
10 100	38 950	43 280	11 890	43 248	44 832
16 190	41 042	52 980	19 950	44 027	50 806

TABLE 5 Experiment 2. Experimental design. The days on which infective larvae were dosed to each sheep and sheep were slaughtered

Days	Number of infective larvae dosed to each sheep	
	Group E	Group F
0	—	*H. contortus*
+14	—	*H. contortus*
Total	—	10 000
+90	*H. contortus*	*H. contortus*
+91	*H. contortus*	*H. contortus*
+92	*H. contortus*	*H. contortus*
Total	50 000	50 000
+119	Slaughtered	Slaughtered

Results

The previous infestation of *H. contortus* caused a highly significant reduction ($P <$ 0,025) in 5th and adult stages of the challenge dose of *H. contortus,* but not of total worm burdens (Table 7).

Four sheep died in Group F, 2 of them before challenge, probably because the initial dose of infective larvae of *H. contortus* was too high. The 2 that survived only to die after challenge had 7 907 (Sheep 524) and 11 805 (Sheep 569) *H. contortus* at necropsy respectively. Worm counts in Sheep 524 were less than the median of 8 075, while Sheep 569 had the highest worm burden in this group.

TABLE 6 Experiment 2. Worms recovered at necropsy

Sheep No.	*H. contortus* Stage of development			Total
	L_4	5	Adult	
	Group E: Controls			
509	302	113	6 340	6 755
525	39	0	6 767	6 806
531	122	100	9 628	9 850
533	35	40	12 989	13 064
575	256	170	7 991	8 417
578	344	445	12 330	13 119
590	41	410	6 728	7 179
594	192	312	13 680	14 184
595	75	281	5 151	5 507
598	219	293	10 973	11 485
608	195	223	10 511	10 929

TABLE 6 *(Continued)*

Sheep No.	*H. contortus* Stage of development			Total
	L_4	5	Adult	
	Group F: 2 × 5 000 L_3 *H. contortus*			
*524	1 805	3 266	2 836	7 907
544	695	1 086	1 157	2 938
551	1 864	2 190	1 691	5 745
558	851	18	7 752	8 621
*569	640	5 971	5 194	11 805
572	1 933	3 058	1 558	6 549
586	832	121	7 291	8 244
602	1 004	5 481	3 528	10 013
611	1 081	222	1 234	2 537
620	604	655	8 207	9 466

Sheep 501 died on Day +40 before challenge. 7606 *H. contortus* recovered
Sheep 589 died on Day +66 before challenge. No count was done
* Sheep 569 died on Day +112
* Sheep 524 died on Day +118

TABLE 7 Experiment 2. Ranked worm burdens of *H. contortus*

Group E			Group F		
L_4	5 + A	Total	L_4	5 + A	Total
35	5 432	5 507	604	1 456	2 577
39	6 453	6 755	640	2 243	2 938
41	6 767	6 806	695	3 881	5 745
75	7 138	7 179	832	4 616	6 549
122	8 161	8 417	851	6 102	7 907
192	9 728	9 850	1 004	7 412	8 244
195	10 734	10 929	1 081	7 770	8 621
219	11 266	11 485	1 805	8 862	9 466
256	12 775	13 064	1 864	9 009	10 013
302	13 029	13 119	1 933	11 165	11 085
344	13 992	14 184		*$P <$ 0,025	

* Significantly fewer than Group E by the Mann-Whitney U test

DISCUSSION

We are indebted to Anderson (1972; 1973) for our knowledge of the epizootiology of *T. axei* and *Ostertagia* spp. in sheep in a winter rainfall area in Australia. *T. axei* is prevalent there from June to November and *Ostertagia* spp. from June to October. In non seasonal-rainfall areas in the Southern Hemisphere (Republic of South Africa, New Zealand and Australia) *T. axei* occurs from March to September and *Ostertagia* spp. from March to October (Muller, 1968; Brunsdon, 1970, 1973; Southcott, Major & Barger, 1976).

Muller (1968) has stated that retarded 4th stage larvae (L_4) of *Ostertagia* spp. are particularly abundant from May to September in a non-seasonal rainfall area (George, RSA). Anderson (1972, 1973) unfortunately did not distinguish between larval stages of *Ostertagia* spp. and those of *T. axei*.

In the present trials *T. axei* protected sheep against 5th stage and adult *O. circumcinta* ($P < 0{,}01$) but had no effect on L_4 of this species (Table 3). It is reasonable to assume that retardation of L_4 of *Ostertagia* spp. in the winter is due not only to the external environmental factors affecting the free-living stages with subsequent retarded development in the host, but to the fact that *T. axei* also prevents *Ostertagia* spp. from developing to 5th and adult stages under field conditions.

In the present trials vaccination with *T. axei* failed to immunize Merinos against *T. axei*. Possibly the 4-month-old Merinos we used were immunologically incompetent, since Ross (1970) showed that Blackface lambs up to 5 months of age could not develop immunity.

Another possible reason is that in young lambs 2 doses of 20 000 infective larvae of *T. axei* is too pathogenic, since 2 sheep died before the experiment was completed in Group D (*T. axei* challenged with *T. axei*) and 3 in Group B (*T. axei* challenged with *O. circumcincta*) respectively. Whatever the reason further investigations are necessary to explain our failure to immunize sheep successfully against *T. axei*.

In Experiment 2 we attempted to immunize Merino lambs against *H. contortus* by predosing them with *H. contortus*. There were fewer L_4 in the controls (Group E) ($P < 0{,}001$) compared with those in the vaccinated sheep (Group F), and this situation, combined with the poor reaction against 5th and adult stages ($P < 0{,}025$) is reflected in the total worm burdens of *H. contortus* when those of the vaccinated group are compared with those of controls. In the latter there is no significant difference. This confirms the finding by Lopez & Urquhart (1968) that Merinos are unable to develop immunity to *H. contortus*.

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