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STUDIES ON *HAEMONCHUS CONTORTUS*. XI. THE EFFECT OF A BOVINE STRAIN OF *TRICHOSTRONGYLUS AXEI* IN MERINOS ON NATURAL PASTURES HEAVILY INFESTED WITH *H. CONTORTUS*

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ABSTRACT

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Sheep grazed on natural pastures heavily infested with infective larvae of *Haemonchus contortus*. Sixty-eight weaned Merinos were divided into 6 groups on Day 0 (23 November 1977), and on Day +14 (7 December) 79 Merinos were divided into 7 groups. There were 2 groups of undosed controls and other groups were either dosed with infective larvae of *Trichostrongylus axei* (bovine strain) only on Days 0 and +14, or in combination with *H. contortus*, or with subsequent doses of *H. contortus*, 28 days later. One group (Group 12) was dosed with *T. axei* and treated with a subcutaneous injection of di-iodonitrophenol (DNP) on Day +14. With the exception of 2 sheep, the sheep of the first 6 groups survived until slaughter in March and April 1978, while many sheep (43) of the latter 7 groups died or were killed *in extremis* from March–May. *T. axei* dosed on 23 November (Day 0) protected Group 2 by >50 % in >50 % of sheep. In the latter 7 groups the best results were achieved when DNP was combined with predosing with *T. axei*. The poor results were probably due either to delayed predosing with *T. axei* or a massive challenge in the wettest summer on record.

INTRODUCTION

It has been shown that if weaned Merinos were dosed with 40 000 infective larvae (L_3) of *Trichostrongylus axei* (ovine strain) early in November, they were able to withstand challenge in the following summer with *Haemonchus contortus* when they grazed on natural pastures in the Highveld (Reinecke, De Villiers & Brückner 1984). In the present trial a bovine strain of *T. axei* was used initially, either alone or mixed with *H. contortus* or in combination with an anthelmintic di-iodonitrophenol (DNP), and the results were compared with those of other groups which were dosed with *H. contortus* only, as well as with those of undosed controls.

MATERIALS AND METHODS

Infective larvae

Cattle were dosed with infective larvae (L_3) of *T. axei* harvested from cultures of sheep faeces. When the cattle became positive, cultures were made and all the infective larvae of *T. axei* used in the present trial were harvested from these bovines.

The L_3 of *H. contortus* used were harvested from a laboratory strain maintained in sheep at Onderstepoort for more than 15 years.

The experimental design is summarized in Table 1.

TABLE 1 Bovine strain of *T. axei* on natural pastures at the University of Pretoria's (UP) experimental farm. Experimental design showing the days on which sheep were dosed with anthelmintics, vaccinated, ear-tagged and dosed with infective larvae (L_3) either of *T. axei* or *H. contortus*.

Day	*Procedure*	
−29	59 Dorpers 4 months of age arrive at UP from Amersfoort	
−28	All Dorpers dosed with mebendazole at 30 mg/kg	
−26	Dorpers ear-tagged	
−19	150 Merinos 5 months of age arrive at UP from Amersfoort	
−15	All sheep vaccinated with enterotoxaemia, 150 Merinos vaccinated with supplementary bluetongue and 59 Dorpers with bluetongue vaccines All Merinos ear-tagged and dosed with mebendazole at 30–45 mg/kg	
−8	59 Dorpers vaccinated with supplementary bluetonge vaccine Seeders: 20 Dorpers each dosed with 5 250 infective larvae of *H. contortus*. Another 10 Dorpers retained for this trial and 29 transferred to another trial	
	23 November 1977	
0	Group 1:	10 Merinos Day 0 controls
	Group 2:	13 Merinos each dosed with 40 000 L_3 of *T. axei*
	Group 3:	13 Merinos each dosed with 40 000 L_3 of *T. axei* + 5 000 L_3 of *H. contortus*
	Group 4:	12 Merinos each dosed with 40 000 L_3 of *T. axei*
	Group 5:	10 Merinos each dosed with 5 000 L_3 of *H contortus*
+14	Group 7:	10 Merinos Day +14 controls
	Group 8:	13 Merinos each dosed with 40 000 L_3 of *T. axei*
	Group 9:	12 Merinos each dosed with 4 000 L_3 of *T. axei* and 5 000 L_3 of *H. contortus*
	Group 10:	13 Merinos each dosed with 40 000 L_3 of *T. axei*
	Group 11:	10 Merinos each dosed with 5 000 L_3 of *H. contortus*
	Group 12 :	11 Merinos each dosed with 40 000 L_3 of *T. axei* and injected subcutaneously with di-iodonitrophenol at 10 mg/kg
+28	Seeders:	10 Dorpers each dosed with 5 000 L_3 of *H. contortus*
	Group 4:	13 Merinos each dosed with 5 000 L_3 of *H. contortus*
	Group 6:	10 Merinos each dosed with 5 000 L_3 of *H. contortus*
+42	Group 10:	13 Merinos each dosed with 5 000 L_3 of *H. contortus*
	Group 13:	10 Merinos each dosed with 5 000 L_3 of *H. contortus*

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Animals

Fifty-nine 4-month-old Dorpers, purchased at Amersfoort in the eastern Transvaal, arrived at the University of Pretoria's (UP) Experimental Farm in the eastern suburbs of Pretoria on 25 October 1977 (Day −29). They were subsequently dosed with mebendazole* at 30 mg/kg (Day −28), ear-tagged (Day −26) and vaccinated with enterotoxaemia (Day −15) and bluetongue vaccines (Day −15 and Day −8). Thirty sheep were retained for this trial; the balance being used elsewhere.

Seeders (Table 1)

On Day −8 each of 20 Dorpers were dosed with 5 250 3rd stage larvae (L_3) of *H. contortus* and on Day +28 a further 10 Dorpers each received 5 000 L_3 of *H. contortus*. The 30 Dorpers were used to contaminate the pasture with *H. contortus* and are subsequently referred to as seeders.

Grazing

All the sheep grazed on natural pasture from 07h00–15h00 every day and were herded into kraals overnight. The grazing was a camp approximately 21 ha in extent at the experimental farm.

Infestation of Merinos (Table 1)

On Day −19, 150 Merinos arrived at UP from Amersfoort and on Day −15 each sheep was ear-tagged, dosed with mebendazole at 30–45 mg/kg and injected subcutaneously with enterotoxaemia and bluetongue vaccines.

This experiment can be divided into 2 parts, viz., Groups 1–6 and Groups 7–13. The original plan called for the trial to commence on Day 0 (23 November 1977) and for Merinos in Groups 1–6 to be slaughtered in March and April and Groups 7–13 in August 1978 (see below).

Sixty-eight sheep were divided into 6 groups (Groups 1–6) on Day 0 (23 November 1977) and 79 sheep into a further 7 groups (Groups 7–13) 14 days later. Three sheep were not used. The number of infective larvae (L_3) of either *T. axei* or *H. contortus* dosed to each sheep in the different groups is shown in Table 1.

Only 3 sheep died, but the remaining sheep in Groups 1–6 survived and were slaughtered either on Day +118 or Day +132 (Table 2).

It was our original intention to slaughter Groups 7–13 in August 1978. They started dying, however, from the end of March (Day +120) and April (Day +152) or were so weak they had to be transferred to pens, and those that had no chance of recovery were subsequently killed *in extremis*. The exception was Group 12 where only Sheep 200 died on Day +140. To enable us to compare worm burdens in Group 12 with those of the other groups we decided to slaughter another 3 sheep of this group on Day +167. The days on which sheep were removed from pasture, died, were killed *in extremis* or slaughtered are summarized in Table 2.

The 32 Merinos that survived from Groups 7–13 were not slaughtered, but remained on the grazing until discharges on 18 September 1978 (Day +339).

Faecal worm egg counts

(a) *Dorpers*

Faeces were collected every 5–14 days from the seeders from Day +20 until Day +133. When sheep had counts of either 25 000 eggs per gram of faeces (epg) or higher, they were dosed either with mebendazole, thiabendazole† or fenbendazole‡. Ten seeders were transferred to pens at the laboratory at Onderstepoort on 18 January 1978 (Day +64).

TABLE 2 Bovine strain of *T. axei* on natural pastures at UP. The days on which sheep were removed from pastures, died or were killed *in extremis*

Day	Group	Number of sheep
+ 75	4	1 Sheep died, no worm counts done
+100	8	1 Sheep died
+111	8	1 Sheep died
+114	1–6	66 Sheep removed from pasture
+117	13	1 Sheep died
+118	1–6	31 Sheep slaughtered
+120	10	1 Sheep killed *in extremis*
+124	13	1 Sheep removed from pasture
+124	10 & 11	3 Sheep died
+127	7–10	9 Sheep removed from pasture
+128	3	1 Sheep died
+131	3	1 Sheep died
+132	1–6	33 Sheep slaughtered
+132	9	1 Sheep killed *in extremis*
+133	7–11 & 13	9 Sheep removed from pasture
+133	10	1 Sheep killed in *extremis*
+135	11	1 Sheep killed *in extremis*
+138	7 & 10	2 Sheep died
+139	8	1 Sheep died
+140	9 & 12	2 Sheep died
+145	7 & 11	2 Sheep died
+146	7, 9 & 11	4 Sheep removed from pasture
+148	7 & 10	3 Sheep killed *in extremis*
+152	7–11 & 13	16 Sheep killed *in extremis*
+159	8	1 Sheep died
+161	7, 9, 10 & 12	8 Sheep removed from pasture
+167	7, 9 & 10	4 Sheep killed *in extremis*
+167	12	4 Sheep slaughtered
+168	8	1 Sheep died
+175	10	1 Sheep removed from pasture
+176	10	1 Sheep died

(b) *Merinos*

Faeces were collected for worm egg counts every 14 days from 5–6 Merinos in each of Groups 3–6 from 21 December 1977 (Day +28) until removal for slaughter on 21 March 1978 (Day +118). Faecal samples for worm egg counts were collected from 5 sheep in each of Groups 9, 10, 11 and 13 every 14 days from 25 January 1978 (Day +63) until March or April. Faeces were collected from 32 survivors in Groups 7–13 for worm egg counts every 14 days from 14 June (Day +204) until the end of the experiment on 18 September 1978 (Day +339).

Weather data

The monthly rainfall, the number of days on which rain fell, the mean minimum and mean maximum temperatures recorded at UP are summarized in Table 3.

TABLE 3 Rainfall and mean monthly temperatures at UP experimental farm

	Total rainfall (mm)	Number of days on which rain fell	Mean monthly maximum (°C)	Mean monthly minimum (°C)
1977 November	52,8	6	28,5	14,2
1977 December	54,5	5	28,1	15,4
1978 January	486,9	14	26,4	15,4
1978 February	131,9	10	32,0	15,1
1978 March	36,1	7	25,9	14,2
1978 April	44,6	6	23,4	11,8
1978 May	—	—	21,8	6,0
1978 June	0,8	1	—	—

Analysis of results

The mean and range of the worm egg counts in the seeders and Merinos in Groups 9, 10, 11 and 13 were converted to the square root of each egg count. The data are illustrated in Fig. 1 and 2.

* "Multispec" Ethnor (Pty) Ltd
† Thibendazole MSD (Pty) Ltd
‡ Panacur Hoechst (SA) Ltd

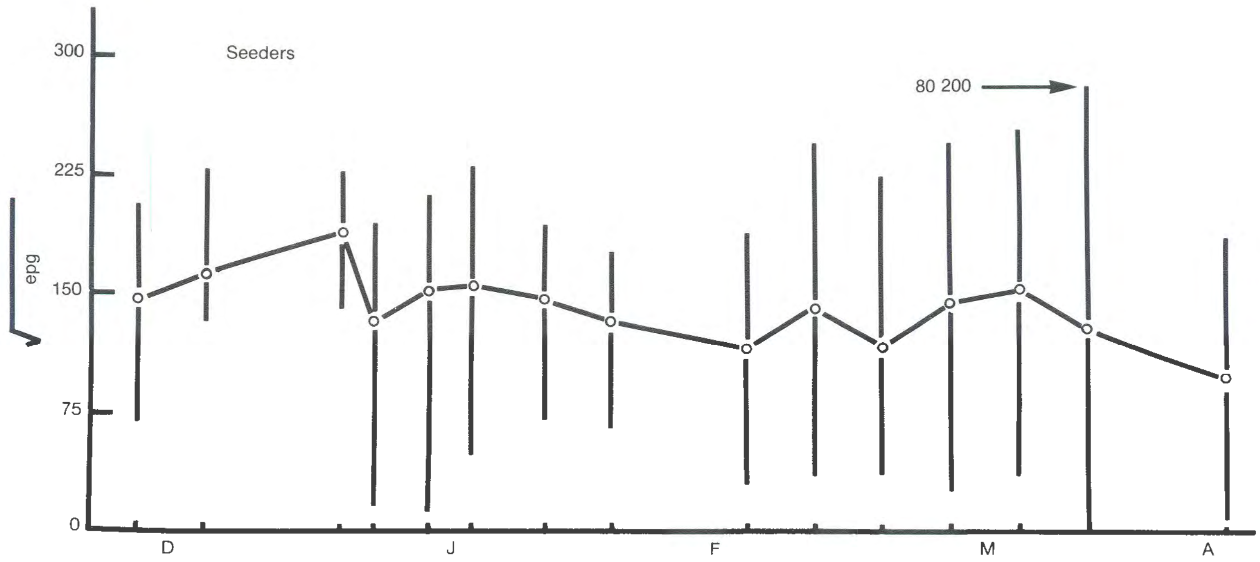

FIG. 1 Variations in the faecal worm egg counts (epg) in the seeders. The range and mean epg are converted to the square root of these values

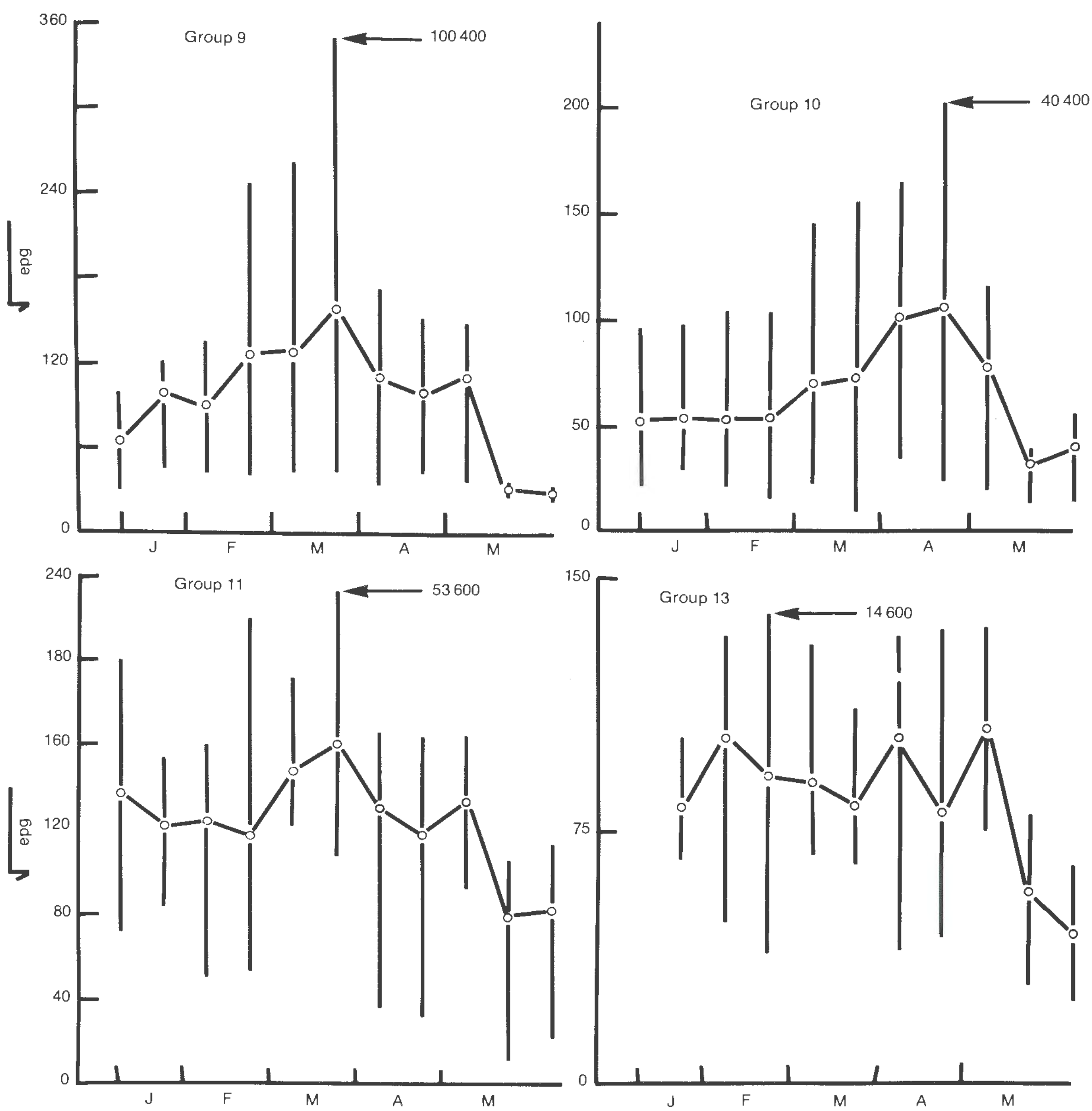

FIG. 2 Variations in $\sqrt{\text{epg}}$ of Groups 9, 10, 11 and 13

The worm burdens of controls were compared with those of other groups in Groups 1–6 either by the modified non-parametric method (Clark, 1968, cited by Reinecke, 1973) or the Mann Whitney U test (Siegel, 1956). The Kruskal-Wallis test described by Siegel (1956) or the non-parametric method described by Groeneveld & Reinecke (1969) was used to analyse data of worm burdens in Groups 7–13.

Worm recovery at autopsy

At necropsy the abomasum and duodenum were removed, the ingesta sieved through a 400 mesh sieve (apertures 38 μm) and the residue on the surface of the sieve transferred to glass jars and preserved in 10 % formalin. The gut wall was digested, sieved and preserved, as described by Reinecke (1973). Worm counts and identifications of larvae and adults were described by Reinecke (1973).

RESULTS

Weather

Eight hundred and seven comma six millimetres of rain fell from November 1977 until the end of June, and January 1978, when 486,9 mm fell in 14 days, was the wettest January on record (Table 3). This exceptionally wet summer with mean maximum temperatures ranging from 25,9–32 °C and mean minimum temperatures from 14,2–15,4 °C was optimal for the larval stages of *H. contortus* on the herbage.

Faecal worm egg counts

Seeders (Fig. 1)

Egg counts rose in January to a minor peak, fell and rose again to a peak in March. Sheep 41 had 80 200 epg on 22 March 1978.

Merinos (Groups 1–6)

Faecal samples were collected on only 2–7 occasions, the highest count being 31 800 in Sheep 94 (Group 3).

Merinos (Groups 7–13)

No faecal samples were collected from Group 7, 2 only from Group 8, and Group 12 remained negative until April. Variations in egg counts in Groups 9, 10, 11 & 13 are illustrated in Fig. 2. Mean egg counts reached a peak in Group 9 on 22 March, and Sheep 159 had 100 400 epg. In group 10 counts reached a peak on 19 April, and Sheep 166 had 40 400 epg. Sheep 188 in Group 11 had 53 600 epg and mean egg counts reached a peak on 22 March, but in Group 13 mean egg counts showed peaks in February, April and May, but the highest egg count was only 14 600 in Sheep 209 on 22 February. Of the sheep sampled for faecal egg counts in March or April, the sheep sampled for faecal egg counts in Groups 9, 10, 11 & 13, 4, 6, 3 and 3 animals respectively died or were killed *in extremis*.

Worms recovered autopsy

Groups 1–5 (Table 4)

Apart from the 3 sheep that died, the animals in Groups 1–5 were slaughtered either on 21 March 1978 (Day +118), or 4 April (Day +132).

TABLE 4 Bovine strain of *T. axei* Group 1–6. Worms recovered at necropsy

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 1								
Controls								
61	21 March	3 122	8 841	11 963	0	0	358	358
62	4 April	525	11 150	11 675	0	0	276	276
63	21 March	22 281	9 695	31 976	0	0	2 909	2 909
64	4 April	652	6 338	6 990	0	0	68	68
65	21 March	9 788	8 203	17 991	0	0	1 688	1 688
66	4 April	1 684	8 801	10 485	0	0	3	3
67	21 March	5 460	6 624	12 084	14	4	620	638
68	4 April	7 657	11 591	19 248	0	779	4 353	5 132
69	21 March	992	5 562	6 554	8	48	160	216
70	4 April	2 214	10 429	12 643	0	0	487	487

Median = 8 821 = 12 023,5
X 0,5 = 4 410,5 = 6 011,75
X 0,4 = 3 528,4 = 4 809,40

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 2								
T. axei Day 0								
71	4 April	589	40	629	0	137	14 269	14 406
72	21 March	563	0	563	83	255	38 051	38 389
73	4 April	1 040	100	1 140	65	65	30 791	30 921
74	21 March	1 060	762	1 822	0	0	3 647	3 647
75	4 April	4 339	2 563	6 902	0	0	1 030	1 030
76	21 March	370	19 850	20 220	0	0	2 440	2 440
77	21 March	875	40	915	132	0	20 823	20 955
78	21 March	728	30	758	0	1 042	27 739	28 781
79	4 April	115	5 724	5 839	3	0	2 330	2 333
80	21 March	520	40	560	4	0	7 143	7 147
81	21 March	80	20	100	0	40	16 058	16 098
82	4 April	2 858	3 234	6 092	0	0	7 349	7 349
127	4 April	122	250	372	8	8	18 648	18 664
			11/13 < 3 528 B	10/13 < 6 012 C				

TABLE 4 (contd)

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 3								
T. axei + *H. contortus* Day 0								
83	3 April	254	6 904	7 158	10	0	8 462	8 472
84	21 March	120	700	820	0	0	3 662	3 662
86	4 April	1 168	4 637	5 805	0	0	36 515	36 515
88	4 April	694	250	944	33	69	21 008	21 110
89	31 March	755	3 748	4 503	89	0	8 585	8 674
90	4 April	341	180	521	0	0	16 576	16 576
91	21 March	679	21 350	22 029	129	137	1 411	1 677
92	21 March	459	5 036	5 495	4	0	20 999	21 003
93	4 April	1 674	1 110	2 784	216	0	23 480	23 696
94	21 March	6 944	6 171	13 115	377	0	742	1 119
128	4 April	0	20	20	14	64	17 845	17 923
162	21 March	2 827	6 890	9 717	10	116	23 043	23 169

8/12
< 6 012
C

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 4								
T. axei Day 0. *H. contortus* Day +28								
95	21 March	0	110	110	5	136	23 632	23 773
96	4 April	409	9 046	9 455	0	0	773	773
97	21 March	2 110	210	2 320	40	0	4 834	4 874
98	4 April	1 193	5 647	6 840	112	0	6 254	6 366
100	4 April	492	1 820	2 312	10	0	2 400	2 410
101	4 April	261	3 648	3 909	0	0	10 335	10 335
102	21 March	5 340	2 810	8 150	0	0	12 662	12 662
103	4 April	1 624	7 402	9 026	0	0	4 027	4 027
104	21 March	81	43	124	0	489	26 895	27 384
105	4 April	704	10 536	11 240	0	0	6 095	6 095
106	21 March	272	756	1 028	0	0	18 877	18 877
129	4 April	756	7 330	8 086	0	36	318	354

P < 0,01

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 5								
H. contortus Day 0								
107	21 March	7 307	520	7 827	0	0	170	170
108	4 April	2 138	9 502	11 640	22	44	1 001	1 067
109	21 March	*4 162	3 252	7 414	0	0	91	91
110	4 April	501	1 640	2 141	82	124	889	1 095
111	21 March	3 464	8 828	12 292	0	266	935	1 201
112	4 April	*163	6 972	7 135	3	46	540	589
113	21 March	6 870	4 035	10 905	153	1 600	406	2 159
114	4 April	798	2 601	3 399	36	72	889	997
115	21 March	*2 141	5 381	7 522	91	89	12	192
116	21 March	5 132	8 265	13 397	22	249	573	844

P < 0,05

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 6								
H. contortus Day +28								
117	4 April	432	9 628	10 060	6	0	123	129
118	4 April	3 611	3 668	7 279	0	0	1 341	1 341
119	4 April	3 264	8 988	12 252	0	188	1 593	1 781
120	21 March	9 559	4 811	14 370	0	207	1 079	1 286
121	4 April	3 104	7 534	10 638	293	331	1 346	1 970
122	21 March	2 042	7 576	9 618	41	128	688	857
123	4 April	3 212	1 262	4 474	0	378	193	571
124	21 March	*4 648	3 596	8 244	486	0	483	969
125	4 April	4 834	8 768	13 602	0	216	2 130	2 346
126	21 March	4 480	11 568	16 048	0	0	1 239	1 239

* L_3 = parasitic 3rd stage larvae were also present

Group 1 (Controls)

Fifth stage and adults were dominant in 9 out of 10 sheep, and total worm burdens of *H. contortus* ranged from 6 554–31 976. These sheep, however, acquired only 3–5 132 T. *axei* from the grazing.

Group 2 (T. exei Day 0)

In 10 out of 13 sheep L_4 were dominant and total worm burdens of *H. contortus* ranged from 100–20 220. *T. axei* ranged from 1 030–38 389.

Group 3 (T. axei and *H. contortus* Day 0)

Fifth stage and adult *H. contortus* were dominant in 8 out of 12 sheep, and total worm burdens ranged from 20–22 029. Worm burdens of *T. axei* ranged from 1 119–36 515.

Group 4 (T. axei Day 0 *H. contortus* Day + 28)

Adults were dominant in 9 out of 12 sheep, and total *H. contortus* varied from 110–11 240, *T. axei* from 354–27 384.

Group 5 (H. contortus Day 0)

In 7 out of 10 sheep 5th stage and adult *H. contortus* were dominant, total worm burdens ranging from 2 141–13 397, and the number of *T. axei* acquired varied from 91–2 159. Three sheep also had 3rd stage larvae of *H. contortus*.

Group 6 (H. contortus Day + 28)

Sheep 124 had a few 3rd stage larvae of *H. contortus*, but most of them (7 out of 10 sheep) had more 5th stages and adults, and the total *H. contortus* worm burdens ranged from 4 474–16 048. Sheep acquired from 129–2 346 *T. axei* from the grazing.

Groups 7–13 (Table 5)

Forty-two sheep died or were killed *in extremis* between 3 March and 10 July (Table 2). In Group 12 only 1 sheep died and we decided to slaughter 4 sheep in this group on 9 May (Day + 167) to enable us to compare worm burdens in Groups 12 with those of the controls (Group 7).

Group 7 (Controls)

Massive worm burdens were present, ranging from 10 806–36 170 *H. contortus*, with 5th stages and adults dominant. From 384–11 445 *T. axei* were acquired from the grazing.

Group 8 (T. axei Day + 14)

Fifth stages and adults were dominant in only 4 out of 8 sheep, and total worm burdens of *H. contortus* ranged from a low 49 to 6 565. Total *T. axei* burdens ranged from 3 855–45 131.

Group 9 (T. axei + H. contortus Day + 14)

Total worm burdens of *H. contortus* rose from 3 096–10 799, and 4 out of 8 animals had more 5th stage larvae and adults than L_4. *T. axei* ranged from 1 515–33 681, compared with 3 855–45 131 in Group 8.

Group 10 (T. axei Day + 14 *H. contortus* Day + 42)

More sheep died or were slaughtered *in extremis* in this group than in any of the other groups. In 6 out of 10 sheep L_4 were dominant and total worm burdens of *H. contortus* ranged from 40–11 732. Large numbers of *T. axei* ranging from 3 994–38 908 were recovered, 6 of them having from 26 618–38 908 of this species.

Group 11 (H. contortus Day + 14)

In all 5 sheep 5th stages and adults were dominant and total *H. contortus* ranged from 4 532–18 612. *T. axei* acquired from grazing were few in numbers, ranging from 52–3 124.

Group 12 (T. axei + DNP Day + 14)

Sheep 200 that died had no *H. contortus* and the 4 sheep that were slaughtered harboured only 2 170–2 409 *H. contortus* with L_4 dominant, ranging from 1 710–2 359 and *T. axei* from 17 950–40 907.

TABLE 5 Bovine strain of *T. axei*. Groups 7–13. Worms recovered at necropsy

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 7								
Controls								
130	20 April	11 685	16 310	27 995	0	0	11 445	11 445
131	17 April	13 353	22 817	36 170	0	1 108	5 568	6 676
132	24 April	5 322	11 200	16 522	0	0	511	511
133	9 May	4 170	6 636	10 806	0	355	1 948	2 303
136	10 April	2 356	13 014	15 370	0	0	384	384
138	20 April	7 477	23 612	31 089	0	0	2 843	2 843

L_L = 15 151,80
X 0,4 = 6 056,72
X 0,5 = 7 570,90

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 8								
T. axei Day + 14 7 December 1977								
141	11 April	1 041	3 150	4 191	34	189	36 188	36 411
144	3 March	49	0	49	0	29	36 090	36 119
145	14 March	176	130	306	84	0	16 449	16 533
146	24 April	531	960	1 491	0	0	45 131	45 131
147	1 May	2	851	853	0	0	3 855	3 855
149	24 April	2 555	190	2 745	0	725	39 533	40 258
150	24 April	2 324	4 241	6 565	0	0	4 170	4 170
151	10 July	820	160	980	0	0	4 880	4 880

8/8 < 7 571
Reduction > 50 %

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 9								
T. axei + *H. contortus*								
155	24 April	2 774	808	3 582	0	0	33 681	33 681
156	4 April	3 721	7 078	10 799	0	0	16 406	16 406
157	9 May	3 900	4 090	7 990	0	0	1 515	1 515
159	12 April	1 761	8 606	10 367	0	0	9 354	9 354
160	9 May	8 230	290	8 520	0	0	25 480	25 480
161	24 April	4 396	3 280	7 676	0	0	5 613	5 613
163	24 April	1 513	1 583	3 096	0	0	2 191	2 191
165	24 April	3 231	1 310	4 541	0	0	4 222	4 222

P = 0,000

L_L = Lower limit of the medium

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 10								
T. axei Day +14 *H. contortus* Day +42 4 Jan 1978								
166	24 April	71	5 861	5 932	0	0	8 691	8 691
167	20 April	2 800	100	2 900	0	400	26 218	26 618
168	5 April	114	110	224	127	41	35 753	35 921
169	10 April	666	4 792	5 458	0	68	30 406	30 474
170	27 March	170	900	1 070	0	101	8 918	9 019
173	24 April	3 330	559	3 889	0	0	32 790	32 790
174	9 May	1 971	3 713	5 684	0	0	38 908	38 908
175	27 March	469	10	479	67	146	29 893	30 106
177	23 March	40	0	40	0	40	3 954	3 994
178	18 May	7 555	4 177	11 732	0	0	5 790	5 790

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 11								
H. contortus Day +14								
181	17 April	1 820	8 352	10 172	22	0	30	52
182	24 April	977	8 644	9 621	60	0	2 669	2 729
185	24 April	1 400	3 132	4 532	0	102	2 916	3 018
186	7 April	1 372	17 240	18 612	4	0	3 120	3 124
188	27 March	236	14 402	14 638	47	0	431	478

P = 0,063

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 12								
T. axei & DNP on Day +14								
189	9 May	2 158	130	2 288	244	488	40 175	40 907
191	9 May	2 359	0	2 359	0	202	35 409	35 611
192	9 May	1 710	460	2 170	0	0	17 950	17 950
197	9 May	2 329	80	2 409	0	407	27 940	28 347
200	12 April	0	0	0	0	0	31 750	31 750

5/5
< 6057
Reduction >60 %

Sheep No.	Date killed	*H. contortus* Stage of development			*T. axei* Stage of development			
		L_4	5 + A	Total	L_3	L_4	5 + A	Total
Group 13								
H. contortus Day +42								
207	20 March	2 607	6 720	9 327	0	0	983	983
208	24 April	1 475	1 560	3 035	111	111	483	705
209	24 April	2 148	2 354	4 502	0	0	613	613
212	24 April	1 775	2 832	4 607	0	0	1 583	1 583

P = 0,005

Group 13 (H. contortus Day + 42)

There were only 4 sheep in this group with a range of 3 035–9 327 *H. contortus*, and all the sheep had more 5th stages and adults than L_4. Low burdens of 613–1 583 *T. axei* were acquired from the grazing.

Analysis of the results

Groups 1–6 (Table 4)

The reduction in total worm burdens of *H. contortus* when either Group 2 *(T. axei* Day 0) or Group 3 *(T. axei + H. contortus* Day 0) was compared with Group 1 (controls) reached Class C or >50 % efficacy in >50 % of sheep, according to the modified non-parametric method (Clark, 1968, cited by Reinecke, 1973). The significance of the reduction in Group 4 *(T. axei* Day 0 *H. contortus* Day +28) and Group 5 *(H. contortus* Day 0) was P <0,01 and P <0,05 respectively if the Mann-Whitney U test was used (Siegel, 1956). Group 6 *(H. contortus* Day +28), when compared with the controls, showed no significant difference.

Group 7–13 (Table 5)

The number of sheep in these groups varied from 4 (Group 13) to 10 (Group 10), which is inadequate for analysis by the non-parametric method. When compared with the controls (Group 7), Groups 9, 10, 11 and 13 showed reductions in the worm burdens of *H. contortus* at the following levels of significance by the Mann-Whitney U test:

Group 9 *T. axei + H. contortus* Day + 14) P = 0,000

Group 10 *(T. axei* Day +14 *H. contortus* Day +42) not significant

Group 11 *(H. contortus* Day +14) P = 0,063 and

Group 13 *(H. contortus* Day +42) P = 0,005.

Groups 7, 8 and 12

Groeneveld & Reinecke (1969) described a very stringent, non-parametric method for comparing worm burdens in 2 groups of sheep. This method may be used in this experiment if the worm burdens of either Group 8 *(T. axei* Day +14) or Group 12 *(T. axei* + DNP Day +14) are compared with those of the controls.

Groeneveld & Reincecke (1969) used the lower limit (L_L) of the worm burdens of the median of the controls to indicate the worm burdens of the controls. At least 5 control animals are necessary to estimate the L_L of the median. Groeneveld & Reinecke (1969) give constant values between the lowest and 2nd lowest worm burdens of the controls (Table 9 p. 293) and the values for interpolation of L_L (Table 10 p. 293). In the present trial this estimate in the controls (Group 7) was carried out as follows:

(a) The position of L_L for a 90 % confidence limit in the total worm burdens of *H. contortus* of the controls (Group 7 Table 5) lies between:

10 806 (the lowest) and
15 370 (the 2nd lowest worm burden of the controls), if there are 6 controls.

(b) The values for interpolation of L_L at the 90 % confidence limit are:

$K' = 0{,}05$ and $K'' = 0{,}95$. Hence
$L_L = 10\,806 \times K' + 15\,370 \times K''$
$= 10\,806 \times 0{,}05 + 15\,370 \times 0{,}95$
$= 15\,372$

The L_L can be reduced by 60 % and 50 % by multiplying it by either 0,4 or 0,5 to give the following results:

15 372,2 × 0,4 = 6 148,8 or 6 149 and 15 372,2 × 0,5 = 7 686,1 or 7 686.

At the 90 % confidence limit the minimum number of animals required to be treated for 50 % reduction is 4 and at 60 % reduction at least 5, according to Table 12 (p. 294) in Groeneveld & Reinecke (1969). All these animals must have less than the reduced L_L of the controlled median (Groeneveld & Reinecke, 1969). Data in Table 5 in the present trials show that the total worm burdens of *H. contortus* of 8/8 sheep in Group 8 have fewer then 7 686 *H. contortus*, and this means that predosing with *T. axei* on Day +14 caused >50 % reduction in *H. contortus* (P <0,01). This improved to >60 % reduction in *H. contortus* in Group 12, when *T. axei* on Day +14 was combined with an injection of DNP at 10 mg/kg on the same day (P <0,01).

Kruskal-Wallis test

Groups 7–13 were compared and analysed by the Kruskal-Wallis test described by Siegel (1956).

With the exception of Group 11, all groups had fewer *H. contortus* than Group 7 (controls), and Group 12 *(T. axei* + DNP on Day +14) had in addition fewer worms than either Groups 9, 11 or 13 (P <0,05).

Discussion

When compared with the results of the experiment done on the same farm in the previous year (Reinecke *et al.*, 1984), the results of this field trial were most disappointing for various reasons.

Firstly, predosing with *T. axei* should take place early enough to protect sheep against subsequent challenge with *H. contortus*. In our 1st field trial *T. axei* was dosed on 2 November, but in the present trial this was delayed either until 23 November on Day 0 (Groups 2, 3 and 4) or until 7 December on Day +14 (Groups 8, 9 and 12). This was too late, and sheep were not infested long enough with *T. axei* to withstand challenge with *H. contortus*.

Secondly, with 486,9 mm of rain in January and 131 mm in February and a total of 807,6 mm of rain (31,8 inches) from November–June this was the wettest summer ever recorded at UP. This wet summer, with 14,2 °C minimum − 32 °C maximum temperature was ideal for the pasture stages of *H. contortus* (Table 3).

Thirdly, we dosed 122 sheep with 5 000 infective larvae of *H. contortus* each, some of them (20 Dorpers) on 15 November 1976 and the last (23 Merinos) on 4 January 1977. The grazing was therefore heavily contaminated at the time when climatic conditions were ideal for the free living stages of *H. contortus*.

Fourthly, 5th stage and adult *H. contortus* were dominant in 63 out of the 114 animals necropsied in the present trial. Apart from Sheep 144, which died on 3 March, these animals died or were killed between 20 March and 9 May. Fourth stage larvae were dominant in these months in the previous year at UP (Reinecke *et al.*, 1984).

Those observations confirmed work previously done by Horak (1978a) on natural pastures in the Transvaal highveld. He showed that development of *H. contortus* is delayed in the 4th larval stage from March to October and reaches a peak in May and June. In the present trial retarded larval development was not a feature in the different groups, except that on some of the sheep that had been predosed with *T. axei* or *T. axei* combined with a chemoprophylactic injection of DNP showed retardation. What is the reason for this?

The weather in the highveld in normal years consistently experiences dry periods from 10–30 days, rarely longer. In December 1977 it was hot and dry until Christmas, when the rains started, and up to the end of April there were rarely periods of more than 7 days without rain. This was not a factor in trials in the previous summer, nor in those of Horak (1978a). Drought is probably a factor which retards development in *H. contortus* as it does in the case of the cattle parasites *Haemonchus placei*, *Cooperia pectinata* and *Cooperia punctata* (Horak, 1978; Fabiyi, Oluyede & Negedu, 1979) and *Ostertagia ostertagi* (Smeal, 1978). As the statistical analysis and all the comparisons between controls and other groups using either the NPM, Mann-Whitney U test or the Kruskal-Wallis test show, the number of deaths of sheep from March to May is unacceptably high.

The notable exception was Group 12 in which only Sheep 200 died in April from an unknown cause. There were no *H. contortus* in this sheep. We slaughtered another 4 animals in this group to compare the burden in Group 12 with the burdens of other groups. From 1 710–2 358 L_4 and only 0–460 5th stage and adult *H. contortus* were present in the latter 4 sheep slaughtered on 9 May. Two interesting conclusions can be drawn from the comparative results of Group 12. The best protection against massive challenge with *H. contortus* is predosing with a combination of *T. axei* and DNP, i.e. there is a synergistic action between *T. axei* and DNP. This is not seen either when sheep are predosed with *T. axei* only, because 8 sheep died or were killed *in extremis* in Group 8 (*T. axei* on Day +14), nor when DNP alone is injected, and thereafter Merinos are exposed experimentally to repeated doses of infective larvae of *H. contortus*. Reinecke, Brückner & De Villiers (1981) found no difference in the worm burdens of controls and sheep predosed with DNP and then given trickle doses of infective larvae of *H. contortus* for 5 months.

A more remote possibility to account for the poor protective effect is the strain of *T. axei* used. This had been passaged frequently through sheep, then cattle were dosed once and larvae from this single passage were used to protect the sheep in this trial. No apparent pattern could be discerned when massive or moderate *T. axei* burdens were compared with those of *H. contortus* in the same sheep. We concluded that the bovine strain of *T. axei* used in this trial was unable to protect sheep. Even if an ovine stain of *T. axei* had been used to predose these sheep, it is very doubtful whether better results would have been obtained if the sheep had been exposed to a similar massive challenge of *H. contortus*.

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