Controlled Trials with Levamisole, Cambendazole, and Morantel Tartrate against Naturally Acquired Helminth Infections in Sheep

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ABSTRACT: Forty-eight ram lambs, similarly exposed to helminth infections on a contaminated pasture, were assigned to six equal groups for use in controlled anthelmintic trials. The treated lambs were given single therapeutic doses of the following anthelmintics: Group 1, levamisole (a reference drug), 8 mg/kg; Group 2, cambendazole, 20 mg/kg; Group 3, cambendazole, 30 mg/kg; Group 4, morantel tartrate, 8 mg/kg; Group 5, morantel tartrate, 12.5 mg/kg; Group 6, unmedicated controls. Drug efficacies were assayed by standard methods. Both mature and immature parasites of some species were recovered from the controls at necropsy; the most numerous species were *Haemonchus contortus*, *Strongyloides papillosus*, *Trichostrongylus axei*, and *T. colubriformis*. For groups 1 to 5, respectively, the calculated efficacies against all stages of parasites were: *H. contortus*, 99, 38, 73, 99, and 99%; *Ostertagia* spp., 96, 96, 98, 95, and 96%; *T. axei*, all 99%; *T. colubriformis*, 99, 99, 100, 94, and 98%; *S. papillosus*, 71, 100, 99, 36, and 0%; *Nematodirus* spp., all 100%; *Cooperia* spp., all 100%; *Oesophagostomum venulosum*, all 100%; *Trichuris* spp., 50, 37, 75, 0, and 37%; *Moniezia expansa*, 25, 75, 100, 25, and 0%. Only cambendazole was highly effective against *S. papillosus* and *M. expansa*, but this drug was relatively ineffective, particularly at 20 mg/kg, against *H. contortus*. There were no significant differences in efficacy among the three drugs against fourth- and fifth-stage *H. contortus* and *Nematodirus* spp.

Levamisole (Bullock, Hand, and Waletsky, 1968), the levo isomer of *dl*-tetramisole (Thienpont et al., 1966) has proved to be a highly effective livestock anthelmintic. The pertinent literature on this drug was recently summarized by Kates et al. (1971). Cambendazole, first described by Hoff et al. (1970), has been tested on a limited scale in ruminants by Baker and Walters (1971), Benz (1971a, b), Ciordia and McCampbell (1971), Egerton and Campbell (1970), Egerton et al. (1970), Restani (1971), and Restani and Borrelli (1971). The anthelmintic activity of morantel tartrate, a methyl analogue of pyrantel tartrate, was first reported by Howes (1968); details of its chemistry and preliminary studies of its anthelmintic activity were reported by McFarland et al. (1969). This drug has been tested alone on a limited scale in sheep by Cornwell and Jones (1970a, b, c), and in combination with diethylcarbamazine by Cornwell, Jones, and Pott (1971).

Earlier, we reported on the comparative activity of levamisole, thiabendazole, and parbendazole (Kates et al., 1971), as well as levamisole, parbendazole, and pyrantel tartrate (Colglazier et al., 1971b), against naturally acquired infections of sheep helminths. In both instances, the same pasture in successive years was contaminated with helminth eggs by grazing it with the same flock of infected breeding ewes. Thereafter, lightly infected lambs were confined on the pasture until they acquired heavy helminth infections for use in controlled anthelmintic trials. These studies contributed to our knowledge of the broad spectrum activity of thiabendazole, parbendazole, levamisole, and pyrantel tartrate against sheep helminths. They also indicated that the populations of *Haemonchus contortus* in these lambs were somewhat resistant to the two benzimidazoles (thiabendazole and parbendazole) but not to the two non-benzimidazoles (levamisole and pyrantel tartrate). The present trials were designed to compare the activity of two of the newer anthelmintics, cambendazole and morantel tartrate, against similar naturally acquired infections in lambs, using levamisole as a reference drug. In earlier trials (Colglazier, Kates, and Enzie, 1972) we reported on the activity of these drugs against

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experimental infections of *H. contortus* and *Trichostrongylus* spp. in lambs.

Materials and Methods

Origin and infection of experimental lambs

These aspects were similar to those previously described (Kates et al., 1971; Colglazier et al., 1971b), except that the lambs were purchased in West Texas and shipped to Beltsville, Md., arriving 27 May 1970. Upon arrival, the lambs averaged 32 kg in weight, and fecal examinations indicated that they were lightly infected with nematodes of the genera *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum*, and *Nematodirus*. On 1 June 55 lambs were confined on the 4.05-hectare helminth-contaminated pasture used in previous trials (loc. cit.). The lambs were kept under parasitological surveillance until they had acquired substantial helminth infections.

Test procedures and anthelmintic treatment*

On 20 July the lambs were removed from pasture to a clean concrete-floored pen. Forty-eight lambs were randomly separated into six groups of eight lambs each, and anthelmintic treatment was given as follows on 22 July:

Group 1: Levamisole (levo-tetramisole) [*l*-2,3,5,6-tetrahydro-6-phenylimidazo (2,1-*b*) thiazole hydrochloride]: 8 mg/kg (pure chemical for experimental use; American Cyanamid Co., Princeton, New Jersey).

Group 2: Cambendazole [2-(4-thiazolyl)-5-isopropoxycarbonylaminobenzimidazole]: 20 mg/kg (7.58% suspension for experimental use; Merck & Co., Rahway, New Jersey).

Group 3: Cambendazole: 30 mg/kg.

Group 4: Morantel tartrate [trans-2[2-(3-methyl-2-thienyl) vinyl]-1-methyl-1,4,5,6-tetrahydropyrimidine tartrate]: 8 mg/kg (pure chemical for experimental use; Charles Pfizer & Co., Inc., Groton, Conn.).

Group 5: Morantel tartrate: 12.5 mg/kg.

Group 6: Unmedicated controls.

Levamisole was used only at one dose level as a reference drug. The two newer anthelmintics were used at two dose levels within the range likely to be used under normal field conditions. Each dose was given as an aqueous drench with a Whitlock syringe. At 5 to 7 days posttreatment, all lambs were necropsied for residual worm counts. Drug efficacy calculations were done by the standard method and statistical analysis was done as previously described (Kates et al., 1971).

Results and Discussion

This trial is the third in a series to compare the efficacy of modern, broad spectrum anthelmintics against naturally acquired gastrointestinal helminths of sheep under similar conditions. The two earlier trials involved levamisole, thiabendazole, parbendazole, and pyrantel tartrate (Kates et al., 1971; Colglazier et al., 1971b). Generally, these four anthelmintics showed the broad-spectrum activity expected. It was noted, however, that the populations of *H. contortus* were somewhat resistant to the benzimidazole drugs, thiabendazole and parbendazole, but not to the other two anthelmintics, levamisole and pyrantel tartrate. The present trials extend these studies to the newer anthelmintics, cambendazole and morantel tartrate, using levamisole primarily as a reference drug. Because the test lambs acquired most of their helminth infections from the same pasture used in previous trials, their *H. contortus* populations were considered to be resistant to at least some benzimidazole anthelmintics, a group to which cambendazole belongs but morantel tartrate does not.

The data and the statistical analysis are summarized in Table 1. In these trials, the most numerous parasites in the control lambs were *H. contortus*, *Trichostrongylus* spp., and *S. papillosus*, but lesser numbers of *Ostertagia* spp., *Nematodirus* spp., *Oesophagostomum venulosum*, *Cooperia* spp., *Trichuris* spp., and *Moniezia expansa* were present also. Furthermore, substantial numbers of larvae (fourth stage) of *H. contortus* and *Nematodirus* spp. were recovered from the controls. When animals are removed from a contaminated pasture, as in these trials, and then treated with an

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Table 1. Average number of helminths recovered from unmedicated control and treated lamb groups, drug efficacies, and summary of statistical analysis of differences between groups (eight lambs per group).

Helminth species	Unmedicated controls (avg worms)	Levamisole 8 mg/kg		Cambendazole				Morantel tartrate			
				20 mg/kg		30 mg/kg		8 mg/kg		12.5 mg/kg	
		Avg worms	% Eff.	Avg worms	% Eff.	Avg worms	% Eff.	Avg worms	% Eff.	Avg worms	% Eff.
H. contortus											
4th-stage	5,015[a]*	33[c]	99	3,380[ab]	33	1,427[b]	72	15[c]	99	2[c]	99
5th-stage	10,103[a]	65[bc]	99	5,980[a]	41	2,660[a]	74	155[b]	98	33[c]	99
Total	15,118[a]	98[bc]	99	9,360[a]	38	4,087[a]	73	170[b]	99	35[c]	99
Ostertagia spp.											
4th-stage	25[a]	2[b]	92	0[b]	100	0[b]	100	0[b]	100	0[b]	100
5th-stage	260[a]	8[b]	97	10[b]	96	5[b]	98	15[b]	94	10[b]	96
Total	285[a]	10[b]	96	10[b]	96	5[b]	98	15[b]	95	10[b]	96
T. axei											
4th-stage	40[a]	0[b]	100	0[b]	100	0[b]	100	0[b]	100	0[b]	100
5th-stage	1,730[a]	18[b]	99	15[b]	99	2[b]	99	32[b]	98	5[b]	99
Total	1,770[a]	18[b]	99	15[b]	99	2[b]	99	32[b]	99	5[b]	99
T. colubriformis											
4th-stage	5[a]	0[b]	100	0[b]	100	0[b]	100	0[b]	100	0[b]	100
5th-stage	3,065[a]	2[b]	99	7[b]	99	0[b]	100	187[c]	94	67[c]	98
Total	3,070[a]	2[b]	99	7[b]	99	0[b]	100	187[c]	94	67[c]	98
Nematodirus spp.											
4th-stage	207[a]										
5th-stage	168[a]	all 0[b]	100	0[b]	100	0[b]	100	0[b]	100	0[b]	100
Total	375[a]										
S. papillosus†	3,218[a]	943[b]	71	0[c]	100	2[c]	99	2,072[a]	36	3,912[a]	0
Cooperia spp.†	30[a]	0[b]	100	0[b]	100	0[b]	100	0[b]	100	0[b]	100
O. venulosum†	21[a]	0[b]	100	0[a]	100	0[b]	100	0[b]	100	0[b]	100
Trichuris spp.†	8[a]	4[bc]	50	5[bc]	37	2[c]	75	11[a]	0	5[b]	37
M. expansa											
Scolices	4[a]	3[a]	25	1[b]	75	0[b]	100	3[a]	25	4[a]	0
Strobilar vol. ml	85[a]	63[a]	26	17[bc]	80	0[c]	100	35[ab]	59	101[a]	0

* Any means not followed by same superscript letter (read horizontally) are significantly different ($P < 0.05$).
† Only 5th-stage parasites recovered.

anthelmintic shortly thereafter, significant but unknown proportions of the worm populations at the time of treatment are usually immature. Therefore, when high efficacies ensue under these circumstances against immature and mature worm populations combined, they are more meaningful than high efficacies obtained only against mature worms when applied to helminth control in animals on the farm.

Levamisole, given at the recommended dosage of 8 mg/kg of body weight, was highly effective against all nematode species except *S. papillosus* (71%) and *Trichuris* spp. (50%). Interestingly, it had better activity against all stages of *Ostertagia* spp. (96%) than it had in the earlier tests, 76 and 82% (loc. cit.). Morantel tartrate at both dose levels was highly effective against many of the same nematode species as was levamisole, but it was less effective against *S. papillosus* (36 and 0%). The efficacy of cambendazole at both dose levels against the various nematode species generally paralleled that of the other two drugs, except that the drug was significantly less effective against *H. contortus* (38 and 73%) and significantly more effective against *S. papillosus* (100 and 99%). Also, only cambendazole showed marked action against *M. expansa*; the data, however, were limited.

There appeared to be no marked differences in efficacy among the three drugs with regard to the developmental status of the worms at the time of treatment.

In the only other published studies on the anthelmintic activity of cambendazole in sheep, Hoff et al. (1970) and Egerton and Campbell (1970) reported that dosages of 5 to 20 mg/kg were highly effective against *H. contortus*, and that the 20-mg dose removed more than 99% of these worms. Similar results against *H. placei* in cattle were reported by Benz (1971a, b), Ciordia and McCampbell (1971), Egerton et al. (1970), and Restani (1971). In our trials, however, the efficacy of cambendazole at 20 and 30 mg/kg was only 38 and 73%, respectively, against all stages of *H. contortus*, indicating that the population was somewhat resistant to this drug. Previously, we reported

(Kates et al., 1971; Colglazier et al., 1971b) that populations of *H. contortus* acquired by lambs on the same pasture were not removed effectively by doses of 50 and 100 mg/kg of thiabendazole and 15 and 20 mg/kg of parbendazole. It appears, therefore, that these populations of *H. contortus* were cross-resistant to the three benzimidazole anthelmintics. However, these benzimidazole-resistant populations of *H. contortus* were highly susceptible to therapeutic doses of such non-benzimidazole anthelmintics as levamisole, pyrantel tartrate, and morantel tartrate. Rafoxanide, another non-benzimidazole anthelmintic, has also proved effective in controlling some benzimidazole-resistant strains of *H. contortus* (Colglazier et al., 1971a).

The only data previously available on the activity of cambendazole against *S. papillosus* in ruminants are those reported by Egerton and Campbell (1970), a dosage of 5 mg/kg was 99% effective in a few sheep, and by Restani and Borrelli (1971) who reported high efficacy with somewhat higher doses in calves. In our present trials, substantial infections of this nematode were almost completely removed with dosages of 20 and 30 mg/kg. It appears, therefore, that cambendazole, in addition to thiabendazole and parbendazole, is another drug that may prove useful for the control of this parasite. Levamisole at 8 mg/kg was only partially effective (71%) against *S. papillosus*, and morantel tartrate was ineffective at both dose levels used.

Although the data for tapeworms were limited, cambendazole at the 30-mg/kg level showed promise of effective action against this parasite.

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Acanthobothrium urolophi sp. n., a Tetraphyllidean Cestode (Oncobothriidae) from an Australian Stingaree

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ABSTRACT: *Acanthobothrium urolophi* sp. n. is described from a common stingaree, *Urolophus testaceus*, from South Australia. It differs from all other species in being apolytic and acraspedote, in having hooks 105–115 μ long, one accessory sucker 80–90 wide on each bothridium, and 40–72 testes in two longitudinal rows.

This report is based upon four specimens recovered from the spiral valve of a common stingaree, *Urolophus testaceus*, which I speared at Glenelg Beach near Adelaide, South Australia, in January 1970. They represent a new species and are the basis of the following measurements and description. All measurements are in microns unless otherwise stated.

Acanthobothrium urolophi sp. n. (Figs. 1–5)

Description

Scolex (Fig. 1) broad, clearly set off from peduncle, 360 to 440 long, 440 to 640 greatest width. Four bothridia present, each 400 to 410 long, 280 to 320 greatest width. Each bothridium with three loculi of the following lengths: anterior 150 to 200, middle 100 to 130, posterior 80 to 90. Muscular pad present on anterior end of each bothridium, each with single accessory sucker 80 to 90 wide. Two bifurcated hooks (Fig. 2) present at base of each muscular pad. Handle of hook 25 to 30 long, inner prong 75 to 80 long, outer prong 75 to 90 long. Total hook length (from tip of outer prong to tip of handle) 105 to 115. Tips of prongs very delicate. Peduncle 560 to 720 long (measured from base of scolex to first discernible segmentation), covered with minute spines.

Strobila (Fig. 3) thin, delicate, apolytic, acraspedote, 8.0 to 9.5 mm long, with 23 to 26 proglottids. Last three proglottids mature, 1.3 to 1.5 mm long, 275 to 300 greatest width. Proglottids continue growth after detaching (Figs. 4A, 4B); when gravid they measure 2.7 to 3.8 mm long, 400 to 560 greatest width. Reproductive systems (Fig. 5) protandrous. Genital pores about equatorial in mature seg-