The efficacy of a controlled-release albendazole capsule in suppressing nematode burdens in sheep

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SUMMARY: An experiment was undertaken between July and November 1985 in East Gippsland, Victoria, to determine the efficacy of an intra-ruminal controlled-release albendazole capsule against naturally acquired worm burdens and larval challenge in Merino hoggets.

Two groups of 20 sheep, one group untreated, the other dosed with a capsule were grazed together; 5 sheep from each group were slaughtered for total worm counts 30 and 101 d after capsules were administered. Serum anthelmintic concentrations, faecal egg counts and body weights were monitored.

Most capsules were exhausted within 91 d of administration. During the estimated 80 d for which they remained active the capsules were highly effective against the benzimidazole-susceptible worm populations. Faecal egg counts were reduced to zero and total worm populations were reduced by over 97% 30 d after administration. By 101 d egg counts were increasing and worm counts indicated that sheep were becoming reinfected. Sheep treated with the capsules grew faster than those not treated.

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Introduction

Prolonged administration of anthelmintics has been shown to increase both their efficacy and their spectrum of activity (Prichard *et al* 1978). The intra-ruminal, controlled-release capsule (Laby 1978) has provided a practical method by which such administration can be achieved in the field. Experimental devices containing oxfendazole were effective against artificially administered nematode infections in the laboratory (Anderson *et al* 1980; Le Jambre *et al* 1981) and against naturally acquired infections in the field (Anderson *et al* 1980). This paper describes the efficacy of a commercial prototype capsule, designed to release albendazole in the rumen at a constant rate for up to 100 d, in removing naturally acquired infections and in preventing reinfection in grazing sheep.

Materials and Methods

Site

This experiment was undertaken on a site 10 km west of Bairnsdale in the East Gippsland region of Victoria. A 2.45 ha area, previously contaminated with nematode larvae by grazing with young sheep, was used for the experiment. Stocking rate during the experimental period was 17/ha for the first 30 d; this declined to 12/ha for the next 70 d after 10 sheep were removed at day 30: it returned to 17/ha between day 100 and day 120, after a further 10 sheep were removed at day 100 and the area was halved.

Pasture consisted predominantly of capeweed (*Arctotheca calendula*), with subterranean clover (*Trifolium subterraneum*) and annual grasses also present. Conditions for pasture growth during the experimental period (July to November 1985) were excellent due to adequate rainfall and mild temperatures.

Experimental Design

Forty Merino wether hoggets, 12 mo old at the start of the experiment, were used. They were ear tagged, weighed, and samples of faeces were collected 18 d before the capsules were administered. All sheep were grazed together. They were allocated by restricted randomisation to give 2 groups with similar means and distributions of egg counts and body weights. Sheep in each group were again stratified by egg count on the day capsules were administered to the "treated" group (day 0), and allocated to either a "retention" subgroup (10 sheep), or to subgroups of 5 destined for slaughter 30 or 101 d after administration of the capsules.

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Observations

Rectal faecal samples were collected from all sheep 18 d prior to administration of capsules, on day 0 (22 July 1985) and 10, 29, 60, 91, 100, 109 and 120 d after treatment. Subgroups of 5 sheep from the treated and untreated group were killed for total worm counts 30 and 101 d after treatment. Blood samples were collected by jugular venepuncture from all sheep on day 0 and 10, 29, 60, 91, 100, 109 and 120 d after treatment. Serum was stored at −18 °C until analysed for concentrations of albendazole metabolites. Sheep were weighed on all except the last two of the above dates. Pasture samples were collected on day 0 and day 100 and infective larvae separated and counted. *In vitro* tests for benzimidazole resistance were undertaken on a bulked sample of faeces collected from all sheep on day 0, and on a bulked sample from the 15 sheep in each group on day 100.

Techniques

Faecal egg counts were done using a McMaster technique with a sensitivity of 25 eggs per g. Total differential worm counts utilised standard parasitological techniques (Barton 1983) with both abomasum and small intestine digested in a pepsin/HCl mixture, and 10% aliquots of abomasum and small and large intestines counted. Concentrations of albendazole sulphone and sulphoxide in the serums of treated sheep were determined using the extraction and HPLC method described by Prichard *et al* (1985). With 2 ml serum used for the assays the limit of detectability was 0.005 μg/ml. Infective larvae were separated from pasture using the technique of Young and Trajstman (1980). *In vitro* tests for benzimidazole resistance were done using a test based on that of Whitlock *et al* (1980).

Statistical Analysis

The distribution of the egg counts of the treated and control groups were non-normal and variances differed on most occasions. Hence, egg counts were compared using the 1-tailed version of the non-parametric Mann-Whitney "U" test. Total worm counts were transformed using a $\log(x + 5)$ function where 5 = 0.5 x minimum detectable worm burden. Where geometric mean burdens of untreated groups exceeded 100 worms percentage efficacy of treatment was determined. Provided the variences of transformed counts from control and treated groups were homogeneous, confidence limits for each reduction were calculated (Finney 1952). The significance of these reductions at P = .05 was determined using a 1-tailed Mann-Whitney test. Body weights and changes in body weight of treated and control groups were compared by Student's "t" test.

Results

Faecal Egg Counts

Arithmetic mean egg counts are depicted in Figure 1. Egg counts of 18 of the 20 sheep given the capsule had fallen to zero

TABLE 1

Geometric mean numbers (+ 5) of gastro-intestinal nematodes in groups of 5 untreated and 5 treated sheep killed at 30 and 101 days after administration of an intra-ruminal slow-release anthelmintic capsule

Nematode species	30 Days				101 Days			
	Untreated group mean	Capsule group mean	% reduction	Confidence limits	Untreated group mean	Capsule group mean	% reduction	Confidence limits
Ostertagia								
Adult/L5	1062	14*	98.7	96.8 - 99.4	972	263	73.0	0.0 - 96.6
L4/L3	17266	483*	97.2	93.6 - 98.8	8775	1031*	88.3	70.7 - 95.3
T. axei								
Adult/L5	150	6*	95.8	90.4 - 98.2	512	23*	95.6	29.7 - 99.7
L4/L3	443	68*	84.6	44.3 - 95.7	6	5	-	-
Nematodirus								
Adult/L5	12	5	-	-	14	12	-	-
L4	79	30	-	-	72	72	-	-
L3	1366	82*	94.0	---	4739	583*	87.7	76.5 - 93.6
Trichostrongylus sp.								
Male Adult/L5								
T. vitrinus	594	8*	98.7	---	37	17	-	-
T. rugatus	548	5*	99.1	---	207	12	94.3	17.5 - 99.6
T. colubriformis	20	10	-	-	31	8*	-	-
Female Adult/L5	1511	15*	99.0	---	513	58*	88.7	0.0 - 99.3
L4	82	6*	-	-	26	42	-	-
L3	3733	13*	99.6	99.4 - 99.8	4512	2440	45.9	8.1 - 68.2
Oes. venulosum	13	5*	-	-	11	5	-	-
Trichuris sp.	21	10	-	-	15	7	-	-
Total Adult/L5	5012	51*	99.0	---	3799	456*	88.0	15.0 - 98.3
Total L4/L3	24868	764*	96.9	94.3 - 98.3	19992	5167*	74.2	61.0 - 82.9
Total Worms	32886	816*	97.5	95.2 - 98.7	26181	6415*	75.5	54.2 - 86.9

* Difference significant ($P < .05$; Mann Whitney U, 1 tailed test)
\- Efficacies not determined because of low worm numbers in untreated sheep
--- Confidence limits not determined because variances were heterogeneous

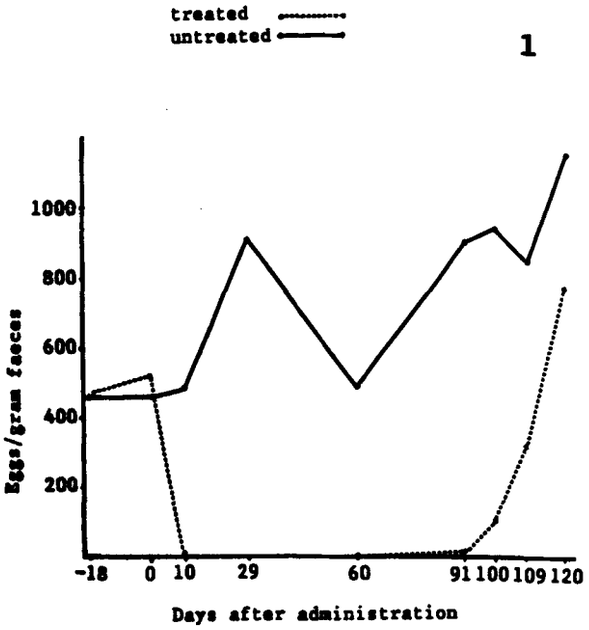

Figure 1. Arithmetic mean faecal egg counts of groups of sheep treated with a controlled-release intra-ruminal albendazole capsule or untreated.

by day 10 and all were negative on day 29. Except for one eg (25 epg) in one sheep on day 61, counts remained zero until day 91 when 5 of 15 sheep had positive egg counts. Both the number of sheep positive, and the mean egg count, continued to increase until the end of the experiment. Egg count was significantly suppressed ($p < .01$) on all occasions from day 10 to day 100 but was not significantly affected thereafter.

Total Worm Counts

Geometric mean (worm counts + 5) of treated and control sheep on day 30 and day 101 are recorded in Table 1. By day 30, adult worm burdens were reduced by an average of 99% and immature worm burdens by an average of 96.9%; an overall reduction in the mean total worm burden of 97.5%. All significant components of the worm population except *Nematodirus* spp adults and L_4 larvae and *Trichostrongylus colubriformis* males were significantly reduced: only one untreated, and no treated animals were infected with *Nematodirus* spp adults. Female *Trichostrongylus* spp were not distinguished.

By day 101 most nematode populations appeared to be re-establishing in the treated group, although the lower efficacy recorded largely reflects the worm burden in one animal. Despite this, the calculated mean reductions of 88% in adult worms, 74.2% in larvae and 75.5% in total worms found, were all significant. The major components of the larval burden, L_4 *Ostertagia* (very few L_3 *Ostertagia* were recovered) and L_3 *Nematodirus* were significantly reduced, while the reduction in the L_3 *Trichostrongylus* spp burden just failed to reach significance by the non-parametric test. Reductions in the burdens of most adult *Trichostrongylus* spp were significant.

Concentrations of Anthelmintic in Serum

Concentrations of albendazole sulphone and sulphoxide in sheep given the capsules are displayed in Figure 2. Initial concentrations of both metabolites were 0.08 to 0.09 μg/ml at day 10; these were maintained at a slightly lower concentration 29 and 60 d after treatment, but were not detectable in 11 of 15 sheep at day 91. No metabolites were detected in any sheep beyond this date.

Capsules

The prototype commercial capsules, with a nominal payload of 2.18 g of albendazole, and a nominal duration of release of

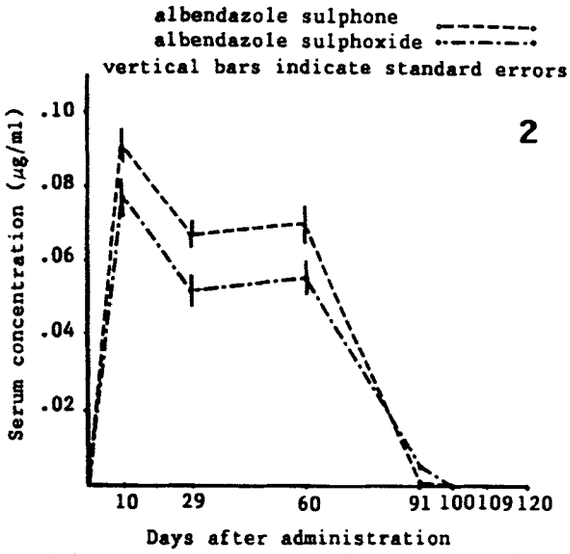

Figure 2. Mean serum concentrations of albendazole sulphone and albendazole sulphoxide in sheep following administration of a controlled-release intra-ruminal albendazole capsule.

100 d, were retrieved from the treated sheep slaughtered 30 and 101 d after treatment. Mean capsule expiry, estimated by extrapolation from the length of the matrix core expelled at day 30, was 74 d (range 69 to 80 d). Similarly, extrapolation of the weight of material expelled gave a mean expiry rate of 78 d (range 73 to 83 d). All capsules were empty at day 101. The actual dose rate ranged from 0.50 to 1.01 mg albendazole/kg d^{-1} for the heaviest (54.5 kg) and lightest (27 kg) animal respectively.

Body Weights

Mean body weights of all treated and control sheep in the experiment are depicted in Figure 3. The treated sheep grew faster ($p < .05$) than the controls throughout the first 91 d after capsule administration and were significantly heavier ($p < .05$) at day 91. They maintained this weight advantage at day 100, by then having gained 8.2 kg compared with 4.2 kg by the controls.

Larval Contamination of Pasture

Counts of larvae on pasture indicted that 400 and 80 infective larvae/kg green herbage were present on day 0 and day 100 respectively.

Resistance to Benzimidazoles

In vitro testing of a bulk faecal sample collected just prior to administration of the capsules indicated that the dominant *Ostertagia/Trichostrongylus* population, with an LC_{50} of 0.1 parts per million (ppm) thiabendazole and 1% hatch at 0.4 ppm, was susceptible to benzimidazoles. A similar result was obtained from bulk samples from the control (LC_{50} 0.07 ppm, zero hatch at 0.4 ppm) and capsule groups (LC_{50} 0.09, 5% hatch at 0.4 ppm) at day 100.

Discussion

The capsules were highly effective, both in removing the existing burdens of mature and immature worms in the treated sheep, and in preventing egg excretion during the period in which the capsules were releasing albendazole.

The increased growth rate of the treated sheep, in the presence of a similar larval challenge to that experienced by the untreated group, does indicate potential production benefits. The ability of the capsules to reduce egg counts to close to zero for about 3 months after administration indicates their obvious potential for strategic use, with long-term production benefits (Anderson *et al* 1980; Anderson 1985).

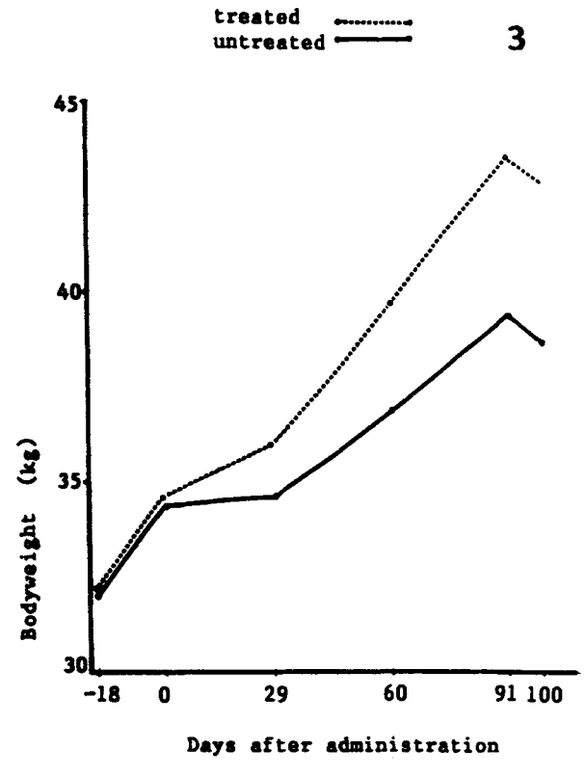

Figure 3. Mean body weights of groups of sheep treated with a controlled-release intra-ruminal albendazole capsule or untreated.

It has been expected during the design of the experiment that the second worm counts at 100 d after treatment would coincide with the exhaustion of these prototype capsules. Extrapolation of rate of usage of the capsules recovered at day 30 indicated that most capsules would have expired prior to day 80. This was consistent with the pattern of plasma metabolites, and the rapidly rising egg counts of the capsule group sheep from day 100 onwards. Thus, the worm burden of the treated sheep at day 100 would have been increased by the addition of many larvae ingested after the capsule finished releasing albendazole. Given that the total worm burden was still lower than that of the controls, it is evident that the capsules, while active, were able to prevent most ingested larvae from establishing. The rise in egg counts of the control groups during the experiment, and the rapidly rising egg counts of the treated groups following exhaustion of the capsule, both indicate that the larval challenge was substantial: this was confirmed by the recovery of larvae from pasture.

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