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Comparative efficacy of levamisole, thiabendazole and fenbendazole against cattle gastrointestinal nematodes

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Abstract

Efficacy of two older anthelmintics, levamisole and thiabendazole, was compared with a newer benzimidazole, fenbendazole, against naturally acquired gastrointestinal nematode infections in cattle superimposed with experimental infections of *Bunostomum phlebotomum* and *Dictyocaulus viviparus*. Twenty-four crossbred beef heifers of 7–9 months of age and 152 kg in average weight were randomly allocated to four groups of six calves. The cattle grazed on pastures contaminated with larvae of gastrointestinal nematodes and the lungworm for 2 months prior to Day 0. Treatment groups were as follows: Group 1—levamisole, topical at 10 mg kg^{-1}; Group 2—thiabendazole paste at 110 mg kg^{-1}; Group 3—fenbendazole paste at 10 mg kg^{-1}; Group 4—untreated controls. All calves were necropsied for worm recovery between 8 and 10 days after treatment. Fecal egg/larval per gram counts at 18 and 42 h post-treatment indicated greatest reductions in Groups 1 and 2. By 7 days post-treatment, reduction in counts for all treated groups ranged from 99.1 to 100%, except for the 66.7% reduction of *B. phlebotomum* in Group 2. Seven nematode species were present in a sufficient number of untreated controls for valid efficacy assessment at necropsy. Efficacy of fenbendazole was 100% against all species, including *Cooperia* spp. L_4 and immature (E5) *D. viviparus*. The overall efficacy of levamisole and thiabendazole was generally high (93.0–100% against *Haemonchus placei* adults, *Cooperia punctata* and *C. spatulata* adult males, *Cooperia* spp. adult females, *Oesophogastomum radiatum*, *B. phlebotomum*, and *D. viviparus* adults). Efficacy of levamisole was slightly better than that of thiabendazole, although group mean differences for *Ostertagia ostertagi* adults, *Cooperia* spp. L_4, *B. phlebotomum* adults and *D. viviparus* E5 were not significant ($P<0.05$).

Keywords: Levamisole; Thiabendazole; Fenbendazole; Control methods-Nematoda; Cattle-Nematoda

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1. Introduction

The anthelmintic compounds levamisole (LEV) and thiabendazole (TBZ) have been used extensively for control of gastrointestinal nematodes of cattle for approximately 30 years. During development and licensing of such products and initial years of marketing, large numbers of published reports provide readily available scrutiny of favorable or unfavorable efficacy characteristics. Considering the extended use-life of LEV and TBZ, which has generally diminished, particularly for TBZ, numbers of published reports of experimental studies expectedly have decreased to the level of only occasional papers. The latter are usually associated with some failure of the product, e.g. reduced efficacy or suspicion of drug resistance.

Both compounds have been extensively implicated in drug resistance in gastrointestinal nematodes of sheep and goats (Prichard, 1990). However, as with all other anthelmintics used for nematode parasitism of cattle, neither LEV nor TBZ has been definitively associated with drug resistance. When compared with newer benzimidazole anthelmintics, TBZ is described as having a reduced spectrum of activity (Prichard and Ranjan, 1993), although no recently published works have been reported. In the case of LEV, however, a number of reports have indicated poor activity against *Ostertagia ostertagi* adults as well as suspicions of drug resistance (Anderson, 1977; Anderson and Lord, 1979; Lyons et al., 1983; Geerts et al., 1987; Williams, 1991; Williams et al., 1991b).

The objective of the experiment reported here was to investigate current efficacy of the two long-used anthelmintics in comparison with efficacy of a more recently developed benzimidazole (fenbendazole) against naturally acquired gastrointestinal nematode infections superimposed with experimental *Bunostomum phlebotomum* and *Dictyocaulus viviparus* infections.

2. Materials and methods

2.1. Cattle and experimental pastures

Twenty-four crossbred beef heifers of 152 kg in average weight and 7–9 months of age were acquired from a local livestock auction barn in early December. Existing gastrointestinal nematode infections were not removed by anthelmintic. The cattle were grazed during December and January consecutively on three 1.2 ha winter ryegrass pastures which had been occupied in the two previous months by other cattle infected with gastrointestinal nematodes and *D. viviparus*. While on pasture the cattle were supplemented with a grain ration at a rate of 2.0 kg per head per day and grass hay was also available.

2.2. Experimental infections

Because of only minimal contamination of pasture with lungworm larvae and because transmission of the hookworm seldom occurs during winter, all cattle were given experimental infections with these species. All cattle were infected with 2900 *B. phlebotomum* infective L_3 on 13 December or 59 days prior to Day 0 and with 390 L_3 again on 13 January or 28 days prior to Day 0. The skin penetration method of infection with hookworm L_3 was according to the description of Williams et al. (1983). All cattle were additionally given a single experimental infection of 900 *D. viviparus* L_3 on 13 January by oral administration.

2.3. Treatment allocation

All cattle were taken off pasture on 29 January and placed in concrete-floored pens that were cleaned twice weekly. This was done to allow both natural and experimental infections to mature in the absence of reinfection prior to allocation and treatment on Day 0. On 10 February (Day 0), the 24 cattle were weighed and fecal samples were collected for nematode egg counts. The cattle were ranked by weight in a descending order of magnitude. The four heaviest cattle formed Block 1, the next heaviest four Block 2, and so on until six blocks of four were formed. The four cattle within each block were then assigned by a table of random numbers to each of four treatment groups. Treatments administered were as follows:

Group 1: levamisole (LEV) pour-on (Totalon®, topical) at 10 mg kg^{-1} (Lot No. BIE345, expiration November 1992, Pittman–Moore, Mundelein, IL), six cattle;

Group 2: thiabendazole (TBZ) paste (TBZ®, 43% cattle wormer paste) at 110 mg kg^{-1} (Lot No. T0580, expiration February 1994, Merck & Co., Inc., (Merck/AGVET) Rahway, NJ), six cattle;

Group 3: fenbendazole (FBZ) paste (Safeguard®, 10% paste cattle dewormer) at 10 mg kg^{-1} (Lot No. 0980390, expiration August 1992, Hoechst–Roussel Agri-Vet Co., Sommerville, NJ), six cattle;

Group 4: nontreated controls, six cattle.

Levamisole was administered topically from the tail head to withers along the mid backline by use of the packaged delivery bottle and dose reservoir. Both thiabendazole and fenbendazole paste were administered orally with appropriate dosage-measured guns. All three compounds were used at what are considered maximal therapeutic dosages for cattle, although fenbendazole is licensed only at 5 mg kg^{-1} for use in cattle. The cattle were returned to concrete-floored pens, but after treatment, all groups were maintained in separate pens.

2.4. Necropsy and parasitological procedures

Fecal egg/larval counts were run on Day 0 and at 18 h, 42 h, and 7 days after treatment. The method was a centrifugation flotation procedure with sucrose so-

lution for flotation medium (Cox and Todd, 1962). Pooled fecal samples from each group were cultured in vermiculite at the three post-treatment sampling dates for determination of larval (L_3) generic composition.

Eight cattle per day, representing all groups, were slaughtered in a commercial abattoir at 8, 9, and 10 days after treatment. All necropsy, worm recovery and identification procedures were as described previously (Williams et al., 1979, 1981; Eddi et al., 1989).

2.5. Statistical methods

Separate split-plot analysis of variance was determined for fecal egg/larval counts, worm counts, and liveweight changes. Before analysis, all counts were transformed to the natural logarithm of (counts plus one); actual values were analyzed for animal weights. Means resulting from all analyses were compared by Duncan's multiple-range test. Actual values were used in tables, but statistical comparisons were based on analysis of transformed variables. All computations were done with the General Linear Models procedure of the Statistical Analysis Systems Institute (1985).

3. Results

3.1. Fecal egg/larval counts

Group mean egg/larval counts on Day 0 varied considerably among treatment groups, but group allocation was based on animal bodyweight rather than parasite counts (Table 1). There were no significant differences for general strongyle eggs and *B. phlebotomum* eggs among groups, but *D. viviparus* L_1 were lowest ($P<0.05$) in Groups 1 and 2. All six control calves were infected with strongyle eggs and three of six and four of six had patent *B. phlebotomum* and *D. viviparus* infections, respectively. In all subsequent fecal examinations after Day 0, all calves were positive for stongyle eggs and at least four of six calves were positive for hookworm and lungworm infections.

At 18 h after treatment the only group means substantially reduced by treatment were for strongyle eggs in Groups 1 (LEV) and 2 (TBZ); efficacy of FBZ was lowest. Mean counts for all treated groups for all parasites were significantly lower ($P<0.05$) than in controls at 42 h, except for the low efficacy observed for FBZ against *D. viviparus*. In the final fecal examination at 7 days after treatment, efficacy in all groups ranged from 99.1 to 100%, except for the 66.7% reduction of *B. phlebotomum* in Group 2 (TBZ).

3.2. Necropsy worm counts

Although worm counts were generally low, seven nematode species were present in a sufficient number of untreated control calves to allow for valid efficacy

Table 1
Fecal egg counts and lungworm larval counts

Treatment group	Sampling time											
	Day 0			18 h			42 h			7 days		
	GEN	BP	DV	GEN	BP	DV	GEN	BP	DV	GEN	BP	DV
1—Levamisole ($n=6$)												
Mean[1]	181^{a}	8^{a}	5^{b}	2^{c}	$<1^{b}$	2^{a}	$<1^{b}$	0^{b}	1^{b}	1^{bc}	0^{b}	0^{b}
Range	152–213	0–28	0–20	0–3	0–1	0–8	0–1	0–0	0–6	0–2	0–0	0–0
% Reduction	–	–	–	99.5	75.0	60.0	99.8	100.0	94.4	99.7	100.0	100.0
2—Thiabendazole ($n=6$)												
Mean	636^{a}	15^{a}	4^{b}	39^{b}	2^{ab}	1^{a}	3^{b}	$<1^{b}$	1^{b}	3^{b}	1^{b}	0^{b}
Range	277–1446	0–46	0–14	8–123	0–3	0–6	0–11	0–1	0–1	1–6	0–2	0–0
% Reduction	–	–	–	89.7	50.0	80.0	99.4	75.0	94.4	99.1	66.7	100.0
3—Fenbendazole ($n=6$)												
Mean	1072^{a}	38^{a}	12^{a}	353^{a}	9^{a}	4^{a}	4^{b}	$<1^{b}$	13^{a}	$<1^{c}$	0^{b}	0^{b}
Range	56–2723	0–194	2–21	26–1328	0–27	0–11	0–14	0–1	0–25	0–1	0–0	0–0
% Reduction	–	–	–	7.1	0	20.0	99.2	75.0	27.8	99.7	100.0	100.0
4—Nontreated ($n=6$)												
Mean	501^{a}	2^{a}	7^{ab}	380^{a}	4^{ab}	5^{a}	518^{a}	4^{a}	18^{a}	350^{a}	3^{a}	41^{a}
Range	173–1535	0–5	0–17	142–933	0–10	0–9	211–1140	0–12	0–56	90–763	0–10	0–162

GEN, general strongyle eggs; BP, Bunostomum phlebotomum eggs; DV, Dictyocaulus viviparus L_1.
[1]Means in columns (vertical) with differing superscripts are significantly different ($P<0.05$).

Table 2
Recovery of nematodes from the gastrointestinal tract of treated and control cattle at necropsy

Treatment group	O. ostertagi, adult	H. placei, adult	*Cooperia* spp., adult ♂				O. radiatum adult	B. phlebot. adult	D. viviparus	
			C. punct.	C. spat.	Adult ♀	L_4			Adult	E5
1—Levamisole ($n=6$)										
Mean[1]	129[b]	37[b]	0[c]	0[b]	0[c]	0[b]	0[b]	0[b]	1[b]	11[a]
Range	0–407	0–110	0–0	0–0	0–0	0–0	0–0	0–0	0–5	0–37
% Reduction	77.2	93.0	100.0	100.0	100.0	100.0	100.0	100.0	99.6	62.1
2—Thiabendazole ($n=6$)										
Mean	106[b]	18[b]	37[b]	0[b]	184[b]	18[a,b]	0[b]	30[b]	<1[b]	9[a]
Range	0–307	0–110	0–110	0–0	0–550	0–110	0–0	0–110	0–2	0–17
% Reduction	81.3	96.6	98.3	100.0	95.0	76.0	100.0	61.0	99.6	68.9
3—Fenbendazole ($n=6$)										
Mean	0[c]	0[b]	0[c]	0[b]	0[c]	0[b]	0[b]	0[b]	0[b]	0[b]
Range	0–0	0–0	0–0	0–0	0–0	0–0	0–0	0–0	0–0	0–0
% Reduction	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
4—Nontreated ($n=6$)										
Mean	566[a]	529[a]	2182[a]	349[a]	3666[a]	75[a]	143[a]	77[a]	283[a]	29[a]
Range	129–1364	110–1320	1318–3740	0–991	1210–8025	0–226	20–410	0–160	0–728	5–132
No. positive	6/6	6/6	6/6	4/6	6/6	3/6	6/6	5/6	4/6	6/6

[1]Means in columns with differing superscripts are significantly different ($P<0.05$).

assessment (Table 2). Efficacy of FBZ (Group 3) was 100% against all species present, including *Cooperia* spp. L_4 and immature (E5) *D. viviparus*. In regard to LEV and TBZ, general efficacy was high, but that of LEV (Group 1) was slightly better than efficacy of TBZ (Group 2), although differences between group means for *O. ostertagi*, *Cooperia* spp. L_4, *B. phlebotomum* and *D. viviparus* E5 were not significant ($P<0.05$). Liveweight changes between Day 0 and Day 7 post-treatment were minimal (Group 1 gained 7 kg, Group 2, 6 kg, Group 3, 9 kg, and Group 4, 4 kg) and differences were not significant.

4. Discussion

The results of this experiment indicate that a relatively high overall level of efficacy remains for both LEV and TBZ against gastrointestinal species in cattle when compared with efficacy of FBZ. Levamisole was the first modern anthelmintic to have a label claim for all developing stages of *D. viviparus*, but efficacy against adult lungworms was low in the present work. On the other hand, TBZ has no label claim for *D. viviparus*, but it has long been known that higher dosages yield moderate efficacy against adult lungworms (Arundel, 1985). Efficacy similar to that of LEV (99.6%) was demonstrated against adult lungworms as well as slightly higher efficacy against immature (E5) parasites. The senior author of this paper (J.C. Williams, unpublished data, 1980) treated three calves with mixed gastrointestinal nematode and lungworm infections with TBZ (110 mg kg^{-1}) in an attempt to remove the gastrointestinal nematodes and then prepare monospecific donor infections of the lungworm; all infections, including lungworm, were terminated by the treatment. A particular difference of note between LEV and TBZ was the complete removal of *Cooperia* spp. adults and L_4 by LEV, compared with small (adults) or substantially reduced (L_4) values for TBZ. Although larval inhibition of *O. ostertagi* was not a factor in the present work because it occurs during spring and summer in Louisiana, efficacy of both compounds against adult worms was low in comparison with FBZ, oxfendazole (Williams et al., 1991a), or albendazole (Williams, 1991). Even lower efficacy values for LEV against adult *O. ostertagi* were observed when tested during maturation of inhibited larvae in September and at lower dosages (6 and 8 mg kg^{-1}) against a predominant adult population in December (Williams et al., 1991b). Results of the present work and previous investigations strongly suggest that some degree of LEV resistance exists in *O. ostertagi*, perhaps even in all development stages.

The present results suggest that at dosage levels and formulations used, LEV and TBZ can have continued practical application in controlling gastrointestinal nematodes in cattle. Use of the two products in combination during a strategic treatment program in Louisiana during 1982–1983 yielded excellent parasite control and cattle gains (Williams et al., 1988). However, in those cases when LEV or TBZ is administered as single or sporadic therapeutic treatments, consideration must be given to known variability or poor efficacy of lower recom-

mended dosages against particular worm species adults and developmental or inhibited stages.

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