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Comparative persistent efficacy of doramectin, ivermectin and fenbendazole against natural nematode infections in cattle

C. Eddi [a], R.A. Muniz [b,1], J. Caracostantogolo [a], J.O. Errecalde [c], R.S. Rew [b,2], S.L. Michener [b], M.E. McKenzie [d,*]

[a] *Instituto de Patabiologia, CICV-INTA, C.C. 77-1708 Moron, Argentina*
[b] *Animal Health Group, Pfizer, New York, NY 10017, USA*
[c] *Facultad de Ciencias Veterinarias, Universidad La Plata, La Plata, Argentina*
[d] *Animal Health Group, 235 East, 42nd Street, New York, NY 10017, USA*

Abstract

A study was conducted in Argentina, to investigate the period of protection of a single injection of doramectin administered subcutaneously (SC) at 200 $\mu g\ kg^{-1}$ (1 ml/50 kg) compared with single treatments of ivermectin (200 $\mu g\ kg^{-1}$ SC) and fenbendazole (5 mg kg^{-1} PO), against field infections of gastrointestinal parasites of cattle. Eighty-three animals were selected and ranked on the basis of serial fecal egg counts (e.p.g.'s). From this group, three animals were slaughtered before treatment and their lungs, abomasum, small and large intestines, were processed for parasite counts and identification. The remaining 80 animals were allocated in ranked groups of four to a control or one of three treated groups. Animals of the four groups were grazed together in the same pasture for the duration of the study. Treatments were administered on Day 0. Individual fecal samples were collected at weekly intervals for the first 49 days post-treatment and twice a week from Day 52 to Day 84 (end of study). At each collection day fecal samples were pooled for coprocultures. On Day 28 and 56, two animals from each group, previously identified on Day 0, were killed and their parasite burdens determined. The duration of protection of a single injection of doramectin was longer than ivermectin or fenbendazole treatment. On Day 56, the total number of parasites found in doramectin-treated animals was significantly ($P < 0.05$) lower than parasite burdens found in either ivermectin- or fenbendazole-treated animals. The longer persistent activity of doramectin was expressed by the lower number of adults and L_4 stages of *Ostertagia ostertagi*. Data from this experiment demonstrated the

* Corresponding author.
[1] Deceased.
[2] Current address: Pfizer Animal Health, North American Region, Exton, PA 19341, USA.

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limitations of using fecal egg counts to evaluate the persistent efficacy of anthelmintics. The duration of activity of doramectin was demonstrated more accurately by parasite counts in cattle from each group since decreasing e.p.g.'s were seen in non-medicated animals without changes in total parasite burdens. © 1997 Elsevier Science B.V.

Keywords: Doramectin; Gastrointestinal nematodes; Persistent efficacy; Control methods; Cattle; nematoda

1. Introduction

The anthelmintic efficacy of doramectin (Dectomax™; Pfizer) has been established against a broad range of gastrointestinal (GI) and pulmonary nematodes in the Northern (Jones et al., 1993) and Southern Hemispheres (Eddi et al., 1993; Moreno et al., 1995; Lima et al., 1995). Working with a non-commercial, micelle formulation. Goudie et al. (1993) observed that a single injection of doramectin protected animals against induced infections of *Ostertagia ostertagi* and *Cooperia oncophora*, for a period of 12 days post-treatment. Additional studies were conducted in the Northern Hemisphere using the commercial formulation of doramectin in parasite naive calves. In the first study, Weatherley et al. (1993) used trickle infections during 14 to 28 days and found periods of protection of 28 days against *O. ostertagi* and 14 days against *C. oncophora.* Vercruysse et al. (1993), used naturally acquired larval challenges of *Ostertagia* spp. and *C. oncophora,* and observed periods of protection of 19 to 22 days when calves were grazed on contaminated pastures at mid-season.

Grazing patterns of cattle differ between temperate areas and the tropical and subtropical regions of the Southern Hemisphere. In temperate areas grazing is confined to periods of 6 months or shorter, whereas in tropical and subtropical zones, grazing is continuous throughout the year and the life of the cattle. These differences have a direct impact on parasite epidemiology, the development of immunity to parasites, and the nutritional/host/parasite balance required for optimal productivity. Under conditions of continuous grazing, the period of protection conferred by a single anthelmintic dose needs to be realistically determined, in order to design strategic programs for seasonal control of GI parasites. The objectives of the study reported here were twofold: first, to investigate the persistent efficacy of a single subcutaneous (SC) injection of doramectin at 200 μg kg^{-1} in comparison with single treatments of ivermectin SC (Ivomec™, Rahway, NJ, USA) at the same dose, and fenbendazole (Axilur™, Hoechst, Argentina) at a dose rate of 5 mg kg^{-1} orally, against field infections of gastrointestinal parasites, and second, to establish the duration of protection of doramectin treatment in cattle of approximately 1 yr of age under conditions of continuous grazing in Argentina.

2. Materials and methods

2.1. Study sites and animals

The study was conducted from August to November in Venado Tuerto, Provincia de Santa Fe, Argentina. Experimental animals were 83 castrated Hereford yearlings from a single source, weighing between 203 to 247 kg at the beginning of the experiment.

2.2. Treatment

All animals that received injectable medication were treated by SC injection in the mid-cervical region. In doramectin- and ivermectin-treated groups, animals received 200 $\mu g\ kg^{-1}$ of body weight (1 mg/50 kg). Fenbendazole-treated cattle received 5 mg kg^{-1} orally, and saline-treated animals received 1 ml SC/50 kg of body weight.

2.3. Experimental design

Eighty-three animals were selected from a large herd on the basis of the average of two fecal egg counts (e.p.g.'s) at Day −7 and Day −3 before treatment. On Day 0, three animals were selected at random, slaughtered and their lungs, abomasum, small and large intestines processed for parasite counts and identification. The remaining 80 animals were identified by a numbered white ear tag and weighed. Animals were ranked in descending order on the basis of the average of 2 serial e.p.g. counts. The first four animals on the list were randomly allocated to either a control group or one of three treated groups. The procedure was repeated with the second four animals and thus successively, until all animals were allocated to the four treatments. After allocation, a second treatment-color-coded eartag was applied to animals of the four different groups.

On Day 0, treatments were administered and animals of the four groups were grazed together on the same improved pastures, at an approximate stocking rate of six to eight head per hectare for the duration of the experiment (84 days). From Day 0 to Day 49 (first 7 weeks), individual fecal samples were collected for e.p.g.'s at weekly intervals. From Day 52 to Day 84, fecal samples were collected twice a week, with the exception of Day 60, when no samples were obtained. At each collection day, pooled fecal samples were used for coprocultures and genus identification. On Days 28 and 56, two animals of each group (randomly selected on Day 0) were slaughtered and their lung and GI parasite burdens determined.

2.4. Parasitological techniques

Necropsies of cattle, collection of worms from specific gastrointestinal compartments including abomasal saline incubation at 37°C, aliquoting of samples and identification of species, were all done by standard parasitological techniques as described by Wood et al. (1995). Fecal egg counts were determined using the modified McMaster technique (MAFF, 1986). For coprocultures, feces from all animals in a treatment group were pooled, cultured and incubated at 28°C for 7 days before being placed on a Baermann apparatus. A maximum of 100 infective larvae were differentiated.

2.5. Statistical analysis

The percentage efficacy (PE) for each group was calculated at each necropsy day comparing the mean number of total GI parasites in the control group on Days 28 and

56, with the mean number of total GI parasites found in the treated groups on Days 28 and 56, using the following formula (Wood et al., 1995):

$$PE = \left\{ \frac{\text{Mean number of total GI parasites in Control Group} - \text{Mean number of total GI parasites in Treated Group}}{\text{Mean number of total GI parasites in Control Group at Days 28} - 56} \right\} \times 100$$

Mean number of total nematode counts by species and by stage were analyzed as natural logs + 1 for each necropsy day using a one-way analysis of variance (ANOVA) (SAS Institute, Cary, NC, USA). Fecal egg counts are presented as arithmetic means, but they were also analyzed at each observation day as natural logs of the counts + 1 using a one way ANOVA. The level of significance for both analyses was set at $P < 0.05$. The cumulative sum of e.p.g.'s are presented as the total sum of e.p.g. counts up to each observation day and were not analyzed for statistical differences. Up to 100 larvae were counted and differentiated from coprocultures at each observation day.

3. Results

3.1. Fecal egg counts

The mean e.p.g.'s are presented in Table 1. For the first 21 days, cattle treated with doramectin, ivermectin or fenbendazole, had significantly ($P < 0.05$) lower e.p.g.'s than

Table 1
Mean number of nematode eggs per gram (e.p.g.) of feces in fecal samples taken at weekly or bi-weekly intervals from untreated cattle or cattle treated with doramectin, ivermectin, or fenbendazole during an 84-day observation period

Day on test	Mean e.p.g.			
	Negative control	Doramectin	Ivermectin	Fenbendazole
0[d]	234.5	227.5	226.1	227.5
7	245.0[a]	0.0[b]	0.0[b]	0.0[b]
14	220.5[a]	0.0[b]	0.0[b]	1.5[b]
21	142.1[a]	1.4[b]	1.4[b]	4.9[b]
28	122.5[a]	4.2[b]	2.8[b]	37.1[c]
35	98.8[a]	2.3[b]	3.1[b]	53.7[c]
42	97.2[a]	3.1[b]	4.7[b]	77.0[a]
49	61.4[a]	3.1[b]	2.3[b]	67.7[a]
52	37.3[a]	0.8[b]	7.8[b]	37.3[a]
56	45.9[a]	0.0[b]	3.9[b,c]	24.9[a,c]
63	33.2	15.8	37.6	35.0
66	22.8	23.5	43.8	42.9
70	28.9	17.5	29.8	21.9
73	9.6	7.0	22.8	3.5
77	0.9	0.9	1.8	1.8
80	2.6	0.0	0.9	0.0
84	0.0	0.9	0.0	0.0

[a,b,c]Across treatments, means with different superscripts are different ($P < 0.05$).
[d]Fecal sample taken before treatment.

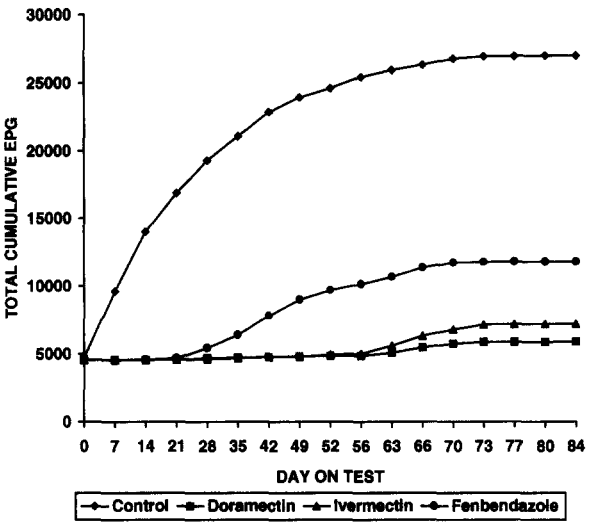

Fig. 1. Cumulative sum of e.p.g.'s at weekly or bi-weekly intervals.

the control group. From Days 28 to 52, e.p.g.'s for doramectin and ivermectin groups were similar and were significantly ($P < 0.05$) lower than either the controls or fenbendazole-medicated animals. From Day 63 to the end of the study (Day 84), differences in e.p.g.'s between groups were not statistically different.

The total cumulative sum of e.p.g.'s for the four groups is presented in Fig. 1. The areas under the curve were consistently smaller for doramectin- and ivermectin-treated groups, than for the saline or fenbendazole groups throughout the experiment.

3.2. Parasite counts

The mean number of total GI parasites are shown in Table 2. Mean worm burdens of 10 200 parasites/animal were found before treatment. At 28 days, total parasite burdens

Table 2
Mean total number of gastrointestinal parasites and percent efficacy in control-group cattle (saline treatment), and cattle treated with doramectin, ivermectin, or fenbendazole before[e], and 28 and 56 days after treatment

Treatment	Dosage	Day 28[f]		Day 56[f]	
		Number of parasites	% Efficacy	Number of parasites	% Efficacy
Controls (Saline)	1 ml/50 kg, SC	13 250[a]	N.A.[g]	12 750[a]	N.A.[g]
Doramectin	200 μg/kg, SC	50[a]	99.6	250[b]	98.0
Ivermectin	200 μg/kg, SC	100[a]	99.2	2900[c]	77.3
Fenbendazole	5 mg/kg, PO	2200[a]	83.4	5600[d]	56.1

[a,b,c,d]Across treatments, means with different superscripts on the same day were significantly ($P < 0.05$) different from each other.

[e]On Day 0 before treatment, the mean total number of gastrointestinal parasites for three animals from the herd was 10 200.

[f]Mean counts for 2 animals from each group.

[g]N.A.: not applicable.

Table 3
Mean number of gastrointestinal parasites (adults and L_4) found at necropsy in saline-treated cattle, and cattle treated with doramectin, ivermectin or fenbendazole on Day 0 before treatment and on Day 28 and Day 56

Parasite species/Stage	Mean number of parasites								
	Controls (Saline)			Doramectin		Ivermectin		Fenbendazole	
	Day 0	Day 28	Day 56	Day 28	Day 56	Day 28	Day 56	Day 28	Day 56
Ostertagia ostertagi									
L_4	1367	1800	4400[a]	50	200	50	2550[a]	400	4000[a]
Adult	2033	2600[a]	700[a]	0[c]	0[b]	0[c]	150[a]	600[b]	500[a]
Haemonchus placei									
L_4	0	0	100	0	0	0	0	0	0
Adult	833	850[a]	700[a]	0[b]	0[b]	0[b]	0[b]	450[a]	150[a]
Trichostrongylus axei									
L_4	267	400[a]	300	0[b]	0	0[b]	0	0[b]	0
Adult	2900	4000[a]	2600	0[b]	50	0[b]	200	50[b]	500
Cooperia spp.									
L_4	67	0	0	0	0	0	0	0	0
Adult	933	1700[a]	2000[a]	0[b]	0[c]	50[b,c]	0[c]	700[a,c]	450[b]
T. colubriformis									
L_4	67	150[a]	50	0[b]	0	0[b]	0	0[b]	0
Adults	1733	1750[a]	1900[a]	0[b]	0[b]	0[b]	0[b]	0[b]	0[b]

[a,b,c]Across treatments, on the same observation day, means with different superscripts are different ($P < 0.05$).

had increased in saline-cattle (13 250 parasites/animal). Doramectin- and ivermectin-treated animals had worm burdens that were 99% lower than those in the control group. Parasite counts in fenbendazole-treated cattle suggested that reinfection may have already occurred by Day 28. Total worm counts in animals slaughtered at Day 56, indicated that doramectin-treated cattle had significantly ($P < 0.05$) lower parasite burdens than either ivermectin- or fenbendazole-treated cattle. At 8 weeks after treatment, worm burdens were reduced by 98% in doramectin-treated cattle when compared to the controls, while reductions of 77% and 56% were found in ivermectin and fenbendazole groups, respectively.

The number of gastrointestinal parasites by species and developmental stage are presented in Table 3. Worm burdens present before treatment were predominantly adult stages of *O. ostertagi* (33%), *Trichostrongylus axei* (31%) and *T. colubriformis* (18%); *Cooperia* spp. (10%) and *Haemonchus placei* (8%) were present in smaller numbers. At Day 28, these proportions were similar, but on Day 56, there was an increase in the L_4 stages of *O. ostertagi* and adult forms of *Cooperia* spp. At Day 28, doramectin- and ivermectin-medicated groups had almost no parasites, whereas fenbendazole-treated animals had significantly ($P < 0.05$) higher populations of *O. ostertagi* and *H. placei*. On day 56, doramectin-treated cattle had significantly ($P < 0.05$) lower numbers of adult and L_4 stages of *O. ostertagi* than animals treated with ivermectin or fenbendazole or cattle in the control group.

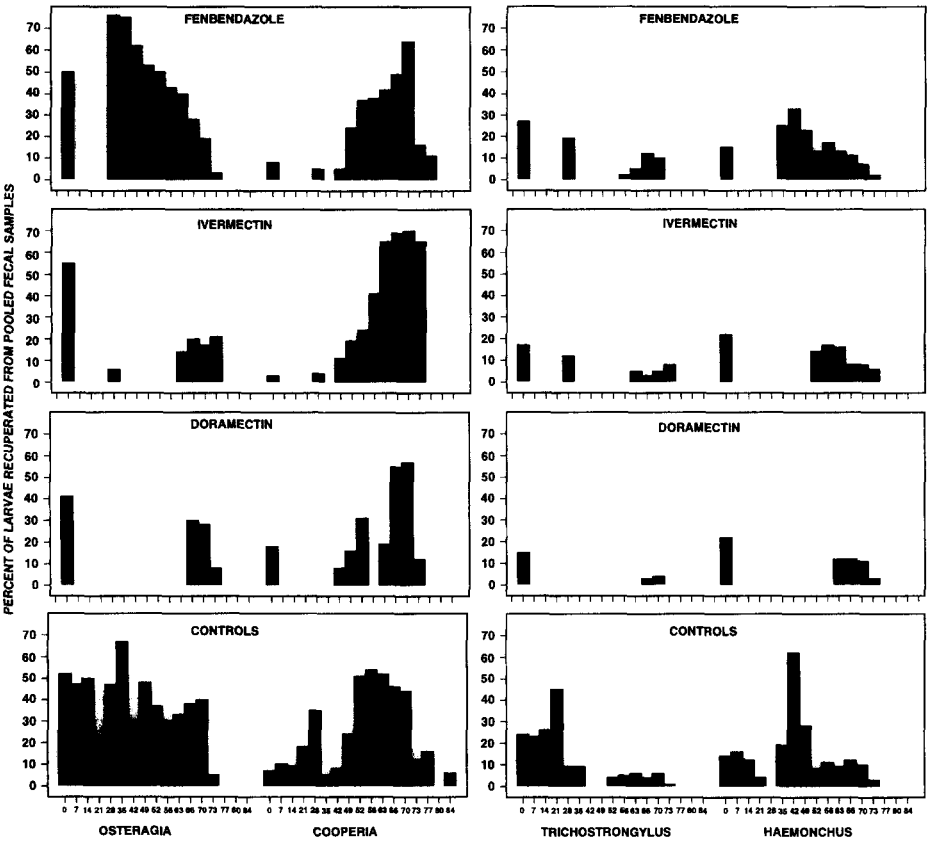

Fig. 2. Percent of parasite larvae recovered from coprocultures.

3.3. Larval differentiation

The nematode genera found in coprocultures of pooled fecal samples of animals of the four groups are presented in Fig. 2. Results from the control group indicated that the predominant parasite genus for the first 49 days of the experiment was *Ostertagia*; numbers were smaller (30% to 40%) from Days 52 to 70. The majority of *Trichostrongylus* and *Haemonchus* larvae were found at Days 35 and 49, respectively. *Cooperia* was minimal during the first 49 days, but was predominant from Day 52 to day 70. Only a few *Oesophagostomum* were recovered during the first 28 days.

4. Discussion

Results of this study indicated that the duration of protection of a single injection of doramectin at a dose rate of 200 $\mu g\ kg^{-1}$ of body weight was longer than ivermectin or fenbendazole at their recommended use level. At Day 56, the total number of GI parasites found in doramectin-treated animals was significantly ($P < 0.05$) lower than parasite burdens found in either ivermectin- or fenbendazole-treated cattle. The longer

persistent efficacy of doramectin resulted in smaller numbers of adults and L_4 stages of *O. ostertagi* recovered at Day 56. These results, obtained under field conditions of continuous grazing in the Southern Hemisphere, were consistent with results of experiments conducted in temperate areas of the Northern Hemisphere in which cattle were given induced infections (Weatherley et al., 1993) or naturally infected (Vercruysse et al., 1993).

Fecal egg output of non-medicated controls, which had decreased (from Day 0 counts) by 58% on Day 52 and by 86% on Day 63, approached zero in all four groups from Day 73 to the end of the study. This reduction appeared to be related to two factors: (a) the expected immune response against GI parasites by yearling cattle, especially against *O. ostertagi* which is characterized by reduction in fecundity of female worms (Michel, 1963) and (b) the arrested development of ingested *O. ostertagi* larvae in the mucosa of the abomasum. *Ostertagia* spp. inhibition has been previously described in the Humid Pampa of Argentina (Suarez, 1990; Muñoz-Cobeñas et al., 1993) during the spring and early summer, which coincides with the time when parasite oviposition ceased in this study. In the control group, parasite populations were not being totally replaced by incoming larvae as those animals were not initially cleared of their existing burdens as were the treated groups.

These data demonstrated the shortcomings of using e.p.g.'s to assess the persistent efficacy of anthelmintics in cattle exposed to continuous grazing conditions. In this experiment average e.p.g.'s on Day 56 were either zero (doramectin) or very low in controls (46), ivermectin (4) or fenbendazole (25) groups. At the same time however, mean total parasite counts, of which *O. ostertagi* was the predominant species, were: 250; 12 750; 2900; and 5600 parasites/animal, for the same groups, respectively.

In conclusion, doramectin administered at 200 $\mu g\ kg^{-1}$ by subcutaneous injection demonstrated a longer period of protection against reinfection with *O. ostertagi,* the most pathogenic parasite of cattle (Williams, 1986), than ivermectin or fenbendazole at their recommended dose rates. This persistent efficacy, which lasted nearly 2 months, was obtained in yearling cattle under conditions of continuous grazing and constant exposure to naturally acquired field infections of GI parasites in Argentina. Because all groups were grazed together, untreated animals or animals treated with an anthelmintic of limited persistent efficacy, contributed to pasture contamination during the first 60 days of the experiment, which increased the challenge by pasture larvae to the ivermectin- and doramectin-treated cattle. Under field conditions of use, treatment with doramectin of all animals of the herd at the beginning of the parasite season or at pasture turnout is likely to result in longer duration of effective control. The use of a two dose program of doramectin given approximately at a 60-day interval has been shown to be an effective anthelmintic control program in Europe (Lestang et al., 1995; Vercruysse et al., 1995). Such a two dose program could potentially be of great assistance in controlling nematode parasites of cattle under continuous grazing conditions.

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