

DURATION OF THE PROTECTION PERIOD OF DORAMECTIN AGAINST FIELD INFECTIONS OF GASTROINTESTINAL NEMATODES IN CATTLE IN SOUTHERN BRAZIL.

A.C. PINHEIRO¹; F. ECHEVARRIA¹; L. CAPRONI JR.²; O. UMEHARA² & L.C. BOTTINO GONÇALVES²

¹ EMBRAPA Bagé, CP 242, Cep 96.400-970, Bagé, RS, Brazil.

² Laboratórios Pfizer Ltda - Av. Pres. Tancredo de Almeida Neves, 1111, Cep 07.190-916, Guarulhos, SP, Brazil.

SUMMARY: A study was conducted in southern Brazil from October 1995 to January 1996 to evaluate the duration of protection of a single subcutaneous injection of doramectin (200 mcg/kg) for controlling naturally acquired mixed field infections of gastrointestinal nematodes in grazing cattle. Ninety-one, 4- to 12-month-old crossbred Hereford male and female calves were selected for the study. Three animals, which were selected at random on day 0 before treatment, were slaughtered and their lungs, abomasum, and small and large intestines were processed for determination of the number and types of worms. The remaining 88 calves were randomly allocated to 2 groups of 44 animals each, based on the mean number of nematode eggs determined on 3 and 1 days before treatment. Calves were grazed together on a common pasture at a stocking rate of 2 animals per hectare for the duration of the study. On day 0, all calves were treated by subcutaneous injection of saline solution (1 ml/50 kg) or doramectin (200 mcg/kg). On days 25, 50 and 75 after treatment, 4 calves from each group were slaughtered and their parasite burdens determined. At weekly intervals from day 0 to day 49 after treatment and twice weekly from day 52 to day 84 (end of the study), individual faecal samples were collected for determination of egg counts. At each observation day, faecal samples were pooled for larval culturing and species identification. Treatment with doramectin significantly ($p \leq 0.05$) reduced parasite burdens and faecal egg counts, compared to those for the control group at all points throughout the study. Efficacy of doramectin was 99.4%, 97.2% and 96.1% on days 25, 50 and 75, respectively. Results of larval culture revealed the predominant nematodes to be *Cooperia* spp., *Haemonchus* sp., *Oesophagostomum* sp. and *Ostertagia* spp., with moderate numbers of *Trichostrongylus* spp.

KEY WORDS: Doramectin, Endectocide; Persistent efficacy; Nematoda, Cattle, Control.

INTRODUCTION

Gastrointestinal parasitism in cattle is an important cause of economic losses in southern regions of Brazil. The most prevalent nematodes are *Cooperia* spp., *Trichostrongylus axei*, *Ostertagia ostertagi* and *Haemonchus* sp. These parasites may be responsible for reductions in body weight of approximately 40 kg/animal/year and mortality as high as 30% in untreated animals (PINHEIRO, 1970). Epidemiological studies have shown two main peaks for these nematodes: one

in the spring and another in late autumn. Hypobiosis in *O. ostertagi* occurs from mid winter (July) to the beginning of summer (December), and clinical signs of parasitism usually develop on the following autumn if animals are not treated (PINHEIRO *et alii*, 1987). Strategic control programmes have been designed to avoid this situation and are based on the use of broad-spectrum anthelmintics.

Doramectin is an endectocide that has been shown to be highly efficient in controlling established infections as well as in preventing reinfection under both laboratory and field

conditions (EDDI *et alii*, 1993; VERCRUYSSSE *et alii*, 1993; WEATHERLEY *et alii*, 1993; LIMA *et alii*, 1995; EDDI *et alii*, 1997). The purpose of this study was to evaluate the duration of the protection period of a single subcutaneous (SC) injection of doramectin (200 mcg/kg) against naturally acquired mixed field infections of gastrointestinal nematodes in grazing cattle in southern Brazil.

MATERIALS AND METHODS

The study was conducted in the facilities of EMBRAPA, Bagé, Rio Grande do Sul, Brazil from October 1995 to January 1996.

Ninety-one crossbred Hereford male and female calves, weighing 48 to 192 kg, were obtained from a common source. Calves were grazed together on a nematode-contaminated pasture at a stocking rate of 2 animals per hectare for the duration of the study.

On day 0 before treatment, 3 calves that had been selected at random were slaughtered and their lungs, abomasum, and small and large intestines were processed for identification and enumeration of nematodes. The remaining 88 animals were identified by a numbered white ear tag and weighed. Calves were ranked in descending order on the basis of the mean number of nematode eggs per gram of faeces (e.p.g.), which had been determined 3 and 1 days before treatment. Ranked pairs of animals were then randomly allocated to either the control group or the doramectin-treated group until all had been assigned to 1 of 2 groups containing 44 calves. On day 0, calves in the control group received saline (0.9% NaCl) solution subcutaneously (SC) at dose rate of 1 ml/50 kg, and those in the medicated group received doramectin at a dose rate of 200 mcg/kg of live weight (1 ml/50 kg) by SC injection in the lateral midline of the neck. Calves were then observed for 6 hours for adverse signs. During the first seven weeks of the study (days 0 to 49), individual faecal samples were collected for determination of egg counts at weekly intervals. From day 52 to the end of the study (day 84), faecal samples were collected twice weekly. At each observation day, pooled faecal samples for each group were used for coprocultures and larval species identification. On days 25, 50 and 75 after treatment, 4 calves from each group (randomly selected on day 1) were slaughtered for determination of parasite burdens.

Parasitological techniques

Determination of the number of nematode eggs per gram of faeces was done by using a modified McMaster method (MAFF, 1986), with larval cultures of pooled faecal samples from each group being incubated at 27 °C for 7 days before being placed in a Baerman apparatus. A maximum of 50 larvae were counted. Necropsy procedures followed those in WOOD *et alii* (1995). Nematodes were counted and identified according to genus, species and stage of development (SOULSBY, 1982; MAFF, 1986).

Statistical analysis

The percent efficacy for the doramectin-treated group was calculated for each necropsy day by comparing the mean number of gastrointestinal (GI) parasites counted in the control group on days 25, 50 and 75 with those found in animals of the doramectin-treated group on the same days, using the following formula:

$$\% \text{ Efficacy} = \frac{\left(\begin{array}{c} \text{Mean Number of} \\ \text{Total GI Parasites} \\ \downarrow \\ \text{in Control Group} \\ \text{on Days 25,50,75} \end{array} \right) - \left(\begin{array}{c} \text{Mean Number of} \\ \text{Total GI Parasites} \\ \downarrow \\ \text{in Treated Group} \\ \text{on Days 25,50,75} \end{array} \right)}{\left(\begin{array}{c} \text{Mean Number of Total GI Parasites} \\ \text{in Control Group on Days 25,50,75} \end{array} \right)} \times 100$$

The total number of GI parasites in the control group before treatment is reported to verify the progression of infection in the absence of treatment. Statistical analyses were conducted to evaluate the duration of protection of doramectin against naturally acquired mixed infections of gastrointestinal nematodes in grazing cattle. A one-way analysis of variance was conducted on the geometric means of LN (count +1) for eggs per gram of faeces and for each specie of nematodes for each day of the study. The level of rejection of the null hypothesis was set at $\alpha=0.05$.

RESULTS

The arithmetic mean number of GI nematodes and percent efficacy of doramectin on days 25, 50 and 75 after treatment are presented in Table 1. The geometric mean number of GI parasites organised by species and stages for both the control and doramectin-treated groups on days 0, 25, 50 and 75 are shown in Table 2. The number of parasite larvae recovered from larval cultures appear in Table 3. Faecal samples from 44 animals/group were available for determination of egg counts on days 0, 7, 14 and 21 after treatment; samples from 40 animals/group were available on days 28, 35, 42 and 49; 36 animals/group on days 52, 56, 59, 63, 66, 70 and 73; and 32 animals/group on days 77, 80 and 84. The geometric mean egg count in faecal samples obtained at weekly or bi-weekly intervals during the 84-day observation period are presented in Figure 1.

Treatment with doramectin was highly effective in removing naturally acquired field infections of gastrointestinal parasites for 75 days after treatment. On the basis of worm counts, efficacy was determined to be 99.4%, 97.2% and 96.1% on days 25, 50 and 75, respectively. (Table 1)

Numbers of adult *Ostertagia ostertagi*, adult and L₄ *Ostertagia spp.*, adult *Haemonchus sp.*, adult *Trichostrongylus axei* and adult *Oesophagostomum radiatum* in the doramectin-treated group were significantly lower ($p < 0.05$) than those in the control group on days 25, 50 and 75. The group treated with doramectin had significantly fewer adult *Cooperia*

Table 1 - Arithmetic mean number of GI nematodes and percent efficacy of doramectin (200 mcg/kg), compared to the saline control, on days 25, 50 and 75 after treatment.

Treatments	Day 0*	Day 25**		Day 50**		Day 75**	
	No. of Parasites	No. of Parasites	% Efficacy	No. of Parasites	% Efficacy	No. of Parasites	% Efficacy
Saline	12,894	18,560	-	9,023	-	20,361	-
Doramectin	-	111	99.4	253	97.2	799	96.1

* Mean counts of 3 animals before treatment.

** Mean counts of 4 animals from each group.

Table 2 - Geometric mean number of GI parasites organised by species and stages in the saline-control and doramectin-treated groups on days 0, 25, 50 and 75 after treatment.

GI Nematodes	Saline				Doramectin		
	Day 0	Day 25	Day 50	Day 75	Day 25	Day 50	Day 75
<i>Ostertagia ostertagi</i>							
L ₄ 's	0	0	0 ^a	0	0	1.14 ^a	0
Adults	1,212	594 ^a	101 ^a	1,632 ^a	0 ^b	0 ^b	0 ^b
<i>Ostertagia</i> spp.							
L ₄ 's	1,465	1,870 ^a	84.9 ^a	6,020 ^a	16.7 ^b	0 ^b	2.48 ^b
Adults	169	393 ^a	74.1 ^a	230 ^a	0 ^b	0 ^b	8.17 ^b
<i>Ostertagia lyrata</i>							
Adults	5.39	9.77 ^a	1.53 ^a	71.0 ^a	0 ^a	0 ^a	0 ^b
<i>Haemonchus</i> sp.							
Adults	2.45	232 ^a	218 ^a	491 ^a	0 ^b	0 ^b	4.98 ^b
<i>Trichostrongylus axei</i>							
L ₄ 's	0	0	0	0 ^a	0	0	4.10 ^a
Adults	1,961	1,055 ^a	801 ^a	5,874 ^a	0 ^b	6.80 ^b	51.4 ^b
<i>Cooperia oncophora</i>							
Adults	1,506	109 ^a	475 ^a	2,571 ^a	3.39 ^a	49.3 ^a	347 ^a
<i>Cooperia punctata</i>							
Adults	3,046	311 ^a	898 ^a	1,079 ^a	0 ^b	7.11 ^b	26.1 ^a
<i>Cooperia macmasteri</i>							
Adults	5.05	5.70 ^a	0	0	0 ^a	0	0
<i>Cooperia</i> sp.							
Adults	0	0	0 ^a	0	0	1.79 ^a	0
<i>T. colubriformis</i>							
Adults	0	1.14 ^a	0.82 ^a	4.05 ^a	0 ^a	0 ^a	0 ^a
<i>Oesophagostomum radiatum</i>							
Adults	2.00	55.0 ^a	214 ^a	195 ^a	0 ^b	0 ^b	4.23 ^b
<i>Trichuris</i> sp.							
Adults	0.26	25.3 ^a	6.83 ^a	5.70 ^a	0 ^c	1.99 ^a	5.09 ^a

^{a,b} Across rows, at each necropsy day, means with different superscripts are significantly different ($p < 0.05$).

punctata ($p < 0.05$) on days 25 and 50 after treatment, compared to that for the control group (Table 2).

Mean faecal egg counts for doramectin-treated calves were significantly lower ($p < 0.05$) than those for saline-treated calves from days 7 to 84 after treatment (Figure 1).

Larval culture results from animals in the control group indicated that the predominant nematodes throughout the study were *Cooperia* spp., *Haemonchus* sp., *Oesophagostomum* sp. and *Ostertagia* spp., with moderate numbers of *Trichostrongylus* spp. (Table 3).

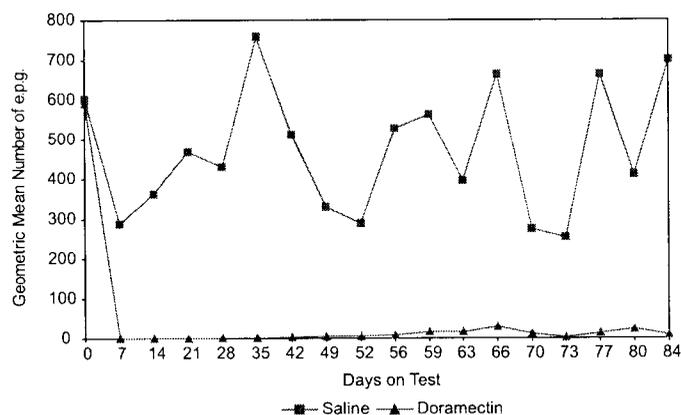


Figure 1 - Geometric mean faecal egg counts determined at weekly or bi-weekly intervals for doramectin-treated and saline-treated calves during a 84-day observation period.

Table 3 - Number of parasite larvae recovered per gram of faeces on larval culturing.

Days on test	Saline					Doramectin				
	Ost.	Hae.	Trich.	Coop.	Oes.	Ost.	Hae.	Trich.	Coop.	Oes.
7	6	8	0	9	27	0	0	0	0	0
14	7	25	0	10	8	0	0	0	0	0
21	8	12	4	22	5	0	0	0	50	0
28	6	8	3	31	5	2	2	0	29	0
35	7	3	5	27	8	4	0	0	25	0
42	5	9	0	25	11	8	17	0	8	0
49	4	11	0	18	15	0	22	0	10	0
52	7	12	0	18	13	12	10	0	30	0
56	12	24	2	18	5	8	14	0	28	0
59	6	8	0	27	8	3	6	0	41	0
63	6	12	3	7	10	1	6	0	12	0
66	5	8	3	26	10	11	4	0	35	0
70	6	13	0	19	12	11	10	0	26	4
73	10	9	5	24	12	7	6	0	28	0
77	5	15	0	21	8	0	6	0	43	2
80	3	6	2	27	11	2	8	1	22	1
84	3	11	1	26	7	1	5	3	31	4

Ost. = *Ostertagia* spp.; Hae. = *Haemonchus* sp.; Trich. = *Trichostrongylus* spp.; Coop. = *Cooperia* spp.; Oes. = *Oesophagostomum* sp.

DISCUSSION

The gastrointestinal nematodes found in this study were representative of the prevalent parasites affecting cattle in the tropical and subtropical regions of Latin America.

Doramectin administered as a single subcutaneous injection at a dose rate of 200 mcg/kg reduced these parasite burdens and faecal egg counts significantly ($p < 0.05$), compared to the saline control injection. Doramectin's overall efficacy against the main gastrointestinal nematodes of cattle was 99.4%, 97.2% and 96.1% on days 25, 50 and 75, respectively. These high nematode reductions are in accordance with other results obtained in tropical and subtropical areas of South America (EDDI *et alii*, 1993; 1997; LIMA *et alii*, 1995).

Cooperia sp. has been found to be the dose-limiting species for the macrocyclic lactone endectocides (YAZWINSKI *et alii*, 1994; WILLIAMS and BROUSSARD, 1995; WILLIAMS *et alii*, 1997). In this study, doramectin had a high therapeutic efficacy (96.9%) against *C. oncophora* on day 25 after treatment, but by days 50 and 75, the efficacy had decreased to 89.6% and 86.5%, respectively. In comparison, doramectin's efficacy against adult and immature *Ostertagia* spp., an important parasite in the region where this study was performed, was greater than 99% up to 75 days after treatment. Since this study was conducted during the spring and early summer when the prevalence of immature *O. ostertagi* is high, doramectin should fit well in the strategic control programmes recommended for the region (PINHEIRO *et alii*, 1987).

It should be noted that the protective results obtained in study with doramectin were obtained from treated calves grazing together with nonmedicated calves. In a typical farming system, all animals on a given pasture would be treated at the same time, likely extending the protection period over that obtained here since there would be no contaminating animals.

The results obtained in this study demonstrated that doramectin has an excellent and prolonged efficacy against the primary gastrointestinal nematodes of cattle under the extensive grazing conditions prevailing in the southern regions of Latin America.

SUMÁRIO

Um estudo foi conduzido no Rio Grande do Sul, Brasil, no período de outubro de 1995 a janeiro de 1996, para avaliar a duração do efeito de proteção de uma única injeção subcutânea (sc) de doramectin (200 mcg/kg) no controle de infecções por nematódeos gastrintestinais naturalmente adquiridas em bovinos. Noventa e um bovinos Hereford de 4 a 12 meses de idade incluindo machos e fêmeas foram selecionados para o estudo. Tres animais, selecionados ao acaso, foram necropsiados no dia 0 antes dos tratamentos e coletados os materiais dos pulmões, abomaso e intestinos delgado e grosso para determinação da carga de nematódeos. Os 88 animais restantes foram distribuidos aleatoriamente em dois grupos de 44 animais cada, baseado na média de ovos de nematódeos por grama de

fezes (o.p.g.) contados nos dias 3 e 1 antes dos tratamentos. Os animais foram mantidos juntos em um pasto comum em lotação de 2 animais por hectare. No dia 0, todos os animais foram tratados com injeção sc de solução salina (1 mL/50 kg) ou doramectin (200 mcg/kg). Nos dias 25, 50 e 75 pós-tratamentos (p.t.), 4 animais de cada grupo foram sacrificados para determinação da carga de vermes. Foram coletadas amostras fecais em intervalos semanais do dia 0 ao dia 49 p.t. e duas vezes por semana do dia 52 ao dia 84 (término do estudo) para determinação do número de o.p.g. A cada dia de observação, foram feitas coproculturas em pool de amostras fecais para identificação das espécies. O tratamento com doramectin reduziu significativamente ($p < 0,05$) a carga de nematódeos e também as contagens de o.p.g. comparadas com as cargas parasitárias dos animais controle em todos os dias de observação. A eficácia de doramectin foi de 99,4%, 97,2% e 96,1% nos dias 25, 50 e 75 p.t. respectivamente. Os resultados das coproculturas revelaram a predominância de *Cooperia spp.*, *Haemonchus spp.*, *Oesophagostomum spp.* e *Ostertagia spp.*, com número moderado de *Trichostrongylus spp.*

PALAVRAS-CHAVE: Doramectin, Endectocida, Eficácia persistente, Nematoda, Bovino, Controle.

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