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Anthelmintic resistance in a dairy cattle farm in the State of Minas Gerais

Resistência anti-helmíntica em uma propriedade de bovinos leiteiros em Minas Gerais

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Abstract

Eighty-four half-blood Gir × Holstein (F1) calves aged six months who were naturally infected by gastrointestinal helminths and maintained in rotational grazing received different anthelmintic treatments. Group A received anthelmintics according to the usual management in the property (eight treatments, seven including a macrocyclic lactone agent). Group B received strategic treatment (ivermectin 3.15%) at the beginning and at the end of the rainy period. Eggs per gram of feces (EPG) counts and genus of larvae from fecal cultures were determined on a monthly basis from April 2002 to December 2003. There was no significant reduction ($p > 0.05$) in EPG counts in any group after anthelminthic treatment, and the larvae in fecal cultures observed were *Cooperia, Haemonchus, Oesophagostomum* and a few *Trichostrongylus. Cooperia* was the most prevalent genus in the first four months of the experiment and *Haemonchus* in the following months. In 2003, tracer calves were introduced onto the pastures monthly and they showed high nematode burden many times throughout the year, and *Cooperia punctata* and *Haemonchus contortus* were the main species identified. The results suggest that there is anthelminthic resistance in this farm, mainly to macrocyclic lactones, and the development of immunity by crossbred animals was vital to reduce nematode burden.

Keywords: Cattle, anthelmintic resistance, *Cooperia,* macrocyclic lactone, *Haemonchus*.

Resumo

Oitenta e quatro bezerras meio sangue Gir × holandês (F1) com seis meses de idade, naturalmente infectadas por helmintos gastrintestinais e mantidas em pastejo rotacionado receberam diferentes tratamentos anti-helmínticos. O grupo A recebeu anti-helmínticos segundo manejo empregado na propriedade (oito tratamentos, sete com produtos à base de lactonas macrocíclicas). O grupo B recebeu tratamento estratégico (ivermectina 3,15%) no inicio e final de período chuvoso. Mensalmente, no período de abril de 2002 a dezembro de 2003, foram realizadas contagens de ovos por grama de fezes (OPG) e coproculturas. Não houve redução significativa ($p > 0,05$) nas contagens de OPG em nenhum dos grupos após os tratamentos anti-helmínticos, e as larvas encontradas na coprocultura foram *Cooperia, Haemonchus, Oesophagostomum* e poucos *Trichostrongylus,* com predominância de *Cooperia* nos quatro meses iniciais e *Haemonchus* nos meses seguintes. No ano de 2003, bezerros traçadores foram alocados mensalmente nos pastos, apresentando altas cargas parasitárias na maioria dos meses do ano, sendo as principais espécies identificadas: *Cooperia punctata* e *Haemonchus contortus*. Os resultados indicam que os nematódeos da fazenda apresentam resistência anti-helmíntica, principalmente às lactonas macrocíclicas, e o desenvolvimento de imunidade foi primordial para reduzir a carga parasitária dos animais mestiços.

Palavras-chave: Bovinos, resistência anti-helmíntica, *Cooperia,* lactonas macrocíclicas, *Haemonchus*.

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Introduction

Brazil has the largest commercial cattle herd and is the second leading meat producer in the world, and meat production is a major national source of income (BARBOSA; MOLINA, 2006). Foreign market opening up together with a global growing interest in the production of pasture-fed animals, free of chemical products and residues in feed of animal origin, can make the external market more attractive to producers, since most cattle is extensively raised. In order to meet this potential demand it is important to increase effectiveness of production, which is closely related to appropriate conditions of animal sanitation, genetic selection and nutrition (VERCRUYSSE; DORNY, 1999).

Animal sanitation involves the control of worm parasitic infections with the use of anthelmintics based on strategic control programs associated with support management aiming to avoid reinfection and delay the emergence of resistance thus maintaining parasite burdens low enough to create protective immunity to the host and prevent economic losses (BORDIN, 2004). However, frequent use of anthelmintics has promoted the development of resistance to different agents used in several regions in Brazil (CONDI et al., 2009; SOUTELLO et al., 2007; SOUZA et al., 2008). Farmers usually fail to notice the growth of helminth populations resistant to anthelmintics and, sometimes even veterinarians fail to notice it as treatment failure is not clinically evident and can only be identified with proper investigation, as well as unawareness of emerging anthelmintic resistant intestinal nematodes.

The purpose of the present study was to evaluate the performance and parasite infection of Gir calves after treatment with two anthelmintic regimens including endoparasiticides commonly used in dairy cattle to control intestinal nematodes.

Material and Methods

The study was carried out from April 2002 to December 2003 in a typical dairy cattle farm in the Vale do Mucuri located in the municipality of Teófilo Otoni (17° 51' 15" W and 41° 30' 23" S), in the State of Minas Gerais, southeastern Brazil.

Eighty-four weaned calves aged from five to eight months (mean 6.4 ± 0.8 months in the beginning of the experiment) were identified with ear tags and, during the experimental period, kept in rotational grazing in an area of 18 hectares covered with pasture grass *Braquiaria brizanta,* with one day of grazing and 30 days of rest with food supplements, mineral salt, and water. Two months prior to the beginning of treatment (April 2002), the animals were evaluated as for eggs per gram of feces (EPG) counts, and were categorized by weight and divided into two groups (A and B). Table 1 shows anthelmintic treatments applied.

After the beginning of treatments, calves were gathered and individually weighed on a monthly basis. Their feces were then collected directly from the rectal ampulla for EPG counts according to the modified Gordon and Whitlock (1939) method and stool cultures following the modified method proposed by Roberts and O'Sullivan (1950). Larvae present in stool cultures were identified using Keith parameters (1953).

Table 1. Anthelminthic treatments applied to calves in group A (according to the usual management in the farm) and B (in June and October 2002 and May and October 2003), Minas Gerais, Brazil, from April 2002 to December 2003.

Month	Group A	Group B
2002		
June	Ivermectin 1%[a]	Ivermectin 3.15%[a]+ Fibronil[b]
Aug.	Albendazole sulfoxide[a]	-
Sept.	Ivermectin 1%	-
Oct.	-	Ivermectin 3.15%
Nov.	Ivermectin 1%	-
2003		
May	Abamectin 1%[a]	Ivermectin 3.15%
June	Abamectin 1%	-
Aug.	Ivermectin 1%	-
Oct.	Doramectin 1%[a]	Ivermectin 3.15%

[a]Injectable, 1 mL/50 kg subcutaneously; [b]Pour on, 1 mL/10 kg, Merial Laboratórios.

From January to December 2003, helminthic infestation in pastures was monitored using tracer calves (Holstein × Zebu crossbred) aged 5 to 7 months, prepared according to Lima (1998). Tracers were introduced monthly into pastures together with calves on the first day and removed on the last day of the month, when they were kept in stalls made of brickwork with concrete-paving floors, and fed with hay and water ad libitum for 15 days. After this period, animals were sacrificed and then necropsied using the technique as described by Costa et al. (1970). Helminths recovered were identified following Douvres (1957) and Yamaguti (1961) procedures.

Information on rainfall index and maximum and minimum temperatures during the study period were collected from a weather station located in the farm.

Data on EPG counts and weight gain were analyzed using the Kolmogorov-Smirnov test to evaluate normality. The analysis of variance (ANOVA) with repeated measures and Bonferroni multiple comparison test were performed to assess differences between groups. In order to assess differences between the months studied, one-factor ANOVA was used followed by Tukey's test. Pearson's correlation coefficient was used to assess potential correlations among variables. The analyses were carried out using GraphPad Prism 5 (GraphPad Inc.) software. The level of significance adopted was $p < 0.05$.

Results and Discussion

During the study period, the average temperature ranged from 22 to 33 °C, except in June 2003 when it was lower, with an average minimum temperature of 13 °C. Two seasons were defined by rainfall with slightly varying duration in the years studied. In 2002, the driest period was from April to July, whereas in 2003 it was from May to October. The wet period was from September 2002 to April 2003, with a late start (in November) in 2003 (Figure 1).

As for EPG counts, there was no significant reduction of counts in any of the groups after the beginning of anthelmintic treatment (Figure 2). Except for January 2003, EPG counts in April and June were not statistically different ($p > 0.05$) from counts performed from July 2002 to May 2003. EPG counts were significantly lower ($p < 0.05$) from June to December 2003 when compared with pre-treatment months (April and June 2002). This reduction seen in the fifteenth month after the beginning of treatment cannot be explained by anthelmintic treatment and was not significantly correlated to rainfall. Pearson's correlation coefficient between EPG counts and rainfall was $r = 0.36$, showing low impact of rainfall on EPG counts.

The variable with the strongest correlation with EPG counts was age. In June 2003, animals were about 18.4 months old, which is the age when cattle usually develop immune resistance to intestinal nematodes and are able to fight infection leading to lower egg production by females and thus lower EPG counts (GASBARRE et al., 2001; LIMA, 1998). The Pearson's correlation coefficient between EPG counts and age was 0.58, but only when the last 11 months of the experimental period was considered, which corresponds to 14.4 to 24.4 months of age and consequently to the main period of immunity development by the animals, when the correlation coefficient was very high ($r = 0.93$). These results showed that nematodes in the farm studied were resistant

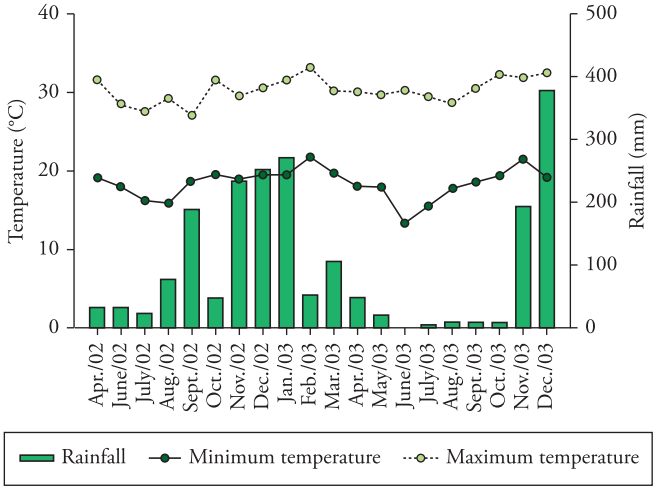

Figure 1. Mean temperature and rainfall during the period of April/02 to December/03.

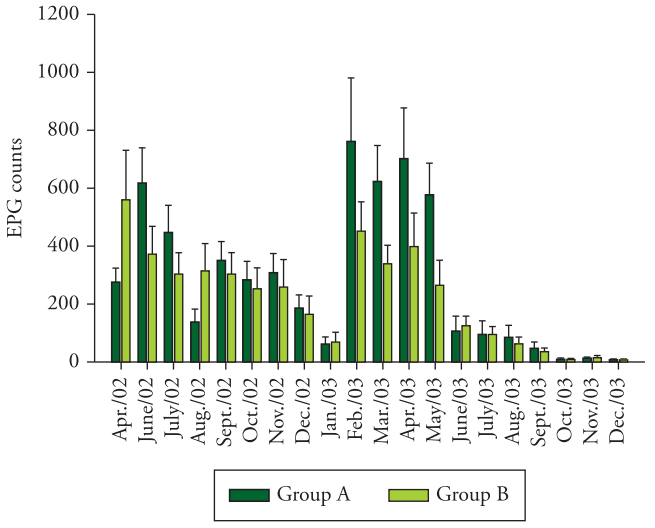

Figure 2. Mean EPG counts of groups A and B during the period of April/02 to December/03.

Table 2. Monthly means of nematode larvae recovered from stool cultures in groups A and B, Minas Gerais, Brazil, from April 2002 to December 2003.

Month	*Cooperia*		*Haemonchus*		*Oesophagostomum*		*Trichostrongylus*	
	A	B	A	B	A	B	A	B
2002								
Apr.	83.9	89.8	15.8	10.0	0.2	0.1	0.0	0.1
June	68.2	63.2	30.9	34.9	0.9	1.8	0.0	0.0
July	78.5	79.9	21.5	20.1	0.0	0.0	0.0	0.0
Aug.	93.7	53.5	6.3	46.5	0.0	0.0	0.0	0.0
Sept.	36.3	29.4	62.8	70.6	0.9	0.0	0.0	0.0
Oct.	29.5	24.8	69.8	75.2	0.7	0.0	0.0	0.0
Nov.	16.5	18.8	83.5	81.2	0.0	0.0	0.0	0.0
2003								
Jan.	25.3	35.4	74.0	64.6	0.7	0.0	0.0	0.0
Feb.	5.3	5.8	93.3	93.9	1.4	0.2	0.0	0.0
Mar.	2.9	4.4	91.2	91.1	5.9	4.5	0.0	0.0
Apr.	1.7	1.4	84.4	84.6	13.8	13.9	0.0	0.0
May	1.5	3.8	89.7	77.3	8.8	18.9	0.0	0.0
June	14.6	4.5	85.4	95.5	0.0	0.0	0.0	0.0
Aug.	20.3	4.1	60.7	74.4	17.8	21.3	1.1	0.2
Oct.	3.8	19.9	93.9	19.7	2.2	60.3	0.0	0.0
Nov.	62.7	93.0	36.1	0.0	0.0	0.0	1.2	7.0
Dec.	26.5	60.3	71.2	36.6	2.3	3.1	0.0	0.0

Table 3. Species and number of nematodes recovered from necropsies of tracer animals, Minas Gerais, Brazil, from January to December 2003.

Species \ month	Jan.	Feb.	Mar.	Apr.	May	June	Aug.	Oct.	Nov.	Dec.
Cooperia punctata										
Adults	6	6	583	1	2195	6638	13	838	11	943
Immature*	0	0	26	1	147	743	3	330	1	100
Total	6	6	609	2	2342	7381	16	1168	12	1043
Haemonchus contortus										
Adults	716	519	200	368	529	734	67	166	6	973
Immature	226	45	62	554	349	64	120	12	6	336
Total	942	564	262	922	878	798	187	178	12	1309
Oesophagostomum radiatum										
Adults	2	5	2	0	9	23	1	3	5	3
Immature	1	0	0	0	0	0	1	0	1	8
Total	3	5	2	0	9	23	2	3	6	11
Trichuris discolor										
Adults	3	4	0	0	0	3	4	7	7	8
Immature	0	0	0	0	4	0	0	0	1	3
Total	3	4	0	0	4	3	4	7	8	11
Total	954	579	873	924	3233	8205	209	1356	38	2374

*4th or 5th stage larvae

to albendazole and macrocyclic lactones, even in formulations with higher concentration (such as ivermectin 3.15%). EPG count reduction lower than 90% indicates anthelmintic resistance (MOLENTO, 2004; SOUTELLO et al., 2007). In the study area, the results show the occurrence of resistance; however, it is not possible to infer about the level of resistance as more specific protocols are needed to evaluate it (MOLENTO, 2004).

Overall, EPG counts in group B were slightly lower than those in group A, however, there was not significant difference ($p > 0.05$). EPG counts were never negative in any monthly analysis, probably due to anthelmintic resistance acquired by nematodes against the agents used together to treat reinfection of the animals by infective larvae present in pastures. These results showed that the study treatments were not effective in reducing parasite burden, and the difference between the groups was masked by the high level of resistance to macrocyclic lactones by the nematodes in this farm.

Nematode larvae recovered in stool cultures were basically classified in four genera as follows: *Cooperia, Haemonchus, Oesophagostomum* and *Trichostrongylus* (Table 2).*Cooperia* and *Haemonchus* larvae were obtained from stool cultures of animals throughout the experimental period and were more predominant than other genera. *Cooperia* was the most predominant genus in the beginning of the experimental period (April to August 2002), however its prevalence was reduced two months after the beginning of treatment (September 2002) in detriment of or due to increase in the prevalence of *Haemonchus* larvae, which had higher rates when compared to other genera from September 2002. *Oesophagostomum* larvae were infrequently observed, however, as well as *Haemonchus* larvae, they showed growing rates after the beginning of treatment and were more abundant from January to October 2003. Larvae of *Trichostrongylus* were sporadically recovered, seen only in stool cultures from April 2002 and August and November 2003 (Table 2). A *Bunostomum* larvae was found in the stool culture of an animal in group A in March 2003. These variations observed in genus prevalence suggest that *Cooperia*, despite its resistance to the anthelmintics used, was the genus with the most significant reduction after the beginning of treatment. The genera *Haemonchus* and *Oesophagostomum* showed the highest levels of resistance to macrocyclic lactones and were more frequently seen only after the beginning of the anthelmintic treatment. Another fact that may have influenced genus prevalence is acquired immunity by the animals. Anti-*Cooperia* immunity is acquired at an earlier age (starting when the animals are four months old), while for the genera *Haemonchus* and *Trichostrongylus* the reduction of parasite burden is more slowly and occurs at an older age (ARMOUR, 1989). These variations were also observed by Araujo and Lima (2005) in animals without anthelmintic treatment raised in other regions of the State of Minas Gerais.

Among the helminths recovered from the necropsies of tracer calves (Table 3), *Cooperia punctata* was the species found in the largest number, peaking in months of low rainfall (May, June and October 2003). This may be explained by high resistance of infective larvae in the environment as this genus thrives with lower rainfall (5 to 7 mm) than larvae from other genera, and has a higher migration capacity (DURIE, 1962; ROBERTS et al., 1951). This genus frequently occurs in cattle from other regions and shows high levels of recovery in tracer cattle from other studies (ARAUJO; LIMA, 2005; FURLONG et al., 1985; GUIMARÃES et al., 1975).

The second most recovered species from tracer animals during the study period was *Haemonchus contortus*. It showed the highest frequency during the months with the highest average rainfall – January and December 2003, but relatively higher frequencies from February to June 2003. *Oesophagostomum radiatum* was recovered from tracer animals nearly every month, but always at lower rates, not exceeding 23 helminths.

A significant number of immature larvae were recovered during necropsy in all months of 2003, accounting for up to

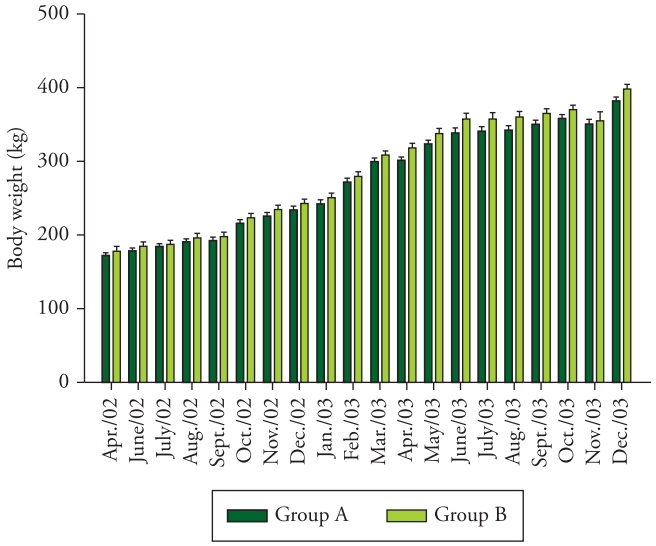

Figure 3. Mean body weight of animals from groups A and B during the period of April/02 to December/03.

50 and 64% for *Cooperia* and *Haemonchus*, respectively. These findings, together with the total number of nematodes recovered from tracer animals, which was higher than 500 in eight out of the ten months analyzed, indicate that infective larvae were available in pastures throughout the experiment period, even in months with lower rainfall and temperature and the use of rotational grazing and anthelmintic treatment. These findings reinforce the evidence that animal acquired immunity was the main factor contributing to EPG count reduction and it indicates that heifers managed to control infection even when repeatedly challenged with infective larvae.

The mean weight of calves was 171 and 177 kg in groups A and B at the beginning of the experiment, and they were 211 and 221 kg, respectively, by the end of the experiment (Figure 3), even with intestinal nematode parasitism that was not controlled by anthelmintic treatment. The use of animals genetically resistant to intestinal nematodes is extremely important in areas where anthelmintic resistance is established (GASBARRE et al., 2001). As expected, animals gained more weight after the rainy months (October 2002 to May 2003). There was no statistically significant difference ($p > 0.05$) regarding the weight of animals in groups A and B in any of the months studied, probably because no treatment was effective to control nematodes.

Conclusions

Nematodes showed drug resistance in the farm studied, mainly to the most commonly used agents in the property, such as macrocyclic lactones, and *Haemonchus* and *Oesophagostomum* genera were more resistant than *Cooperia*. The main factor contributing to the reduction of parasite burden was animal acquired immunity, which occurred when they were about 18 months old. The study results show the advantages of raising crossbred animals, such as Gir, to minimize damages caused by intestinal nematodes in areas of anthelmintic resistance. The effectiveness of anthelmintics should be evaluated to identify the most effective agents to control intestinal nematodes in the farm.

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