



## Helminthiasis control in calves raised in a hot Semi-arid area



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### Abstract:

This study aimed to characterize the helminthiasis and anthelmintic effectiveness in calf herds raised in a hot semi-arid area. Sixty (60) cattle farms from the northern area of Minas Gerais, Brazilian sertão, were categorized by semi-structured questionnaires. It was also performed the fecal egg counts (FEC) reduction test to analyze the profile of anthelmintic resistance in eight herds. The study selected groups of at least 10 homogeneous calves with  $FEC \geq 150$  per treatment. After 12 h of fast, calf groups were treated with albendazole, levamisole, ivermectin, doramectin or abamectin, except the

control groups (untreated). It was collected feces before treatments and 14 d later larvae genera of nematodes were identified after coproculture. Extensive grazing was the predominant creation system for beef calves, deworming was employed every 6 mo in 64 % of the farms and macrocyclic lactones was the most frequently used anthelmintic group. The anthelmintic efficacy varied from 62 to 98.9 %. The resistance profile to ivermectin, levamisole, albendazole and (or) doramectin verified in this research is alarming as the genus *Haemonchus* was the most frequent one before and after the treatments. It was detected variations in the creation systems, in control practices and in anthelmintic susceptibility profiles between herds. Therefore, this work emphasize the importance of using strategic control with FEC reduction test for choice of anthelmintic and the encouragement of practices of alternative control.

**Key words:** Cattle, Anthelmintic resistance, Nematodes, Parasites, Strategic control.

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## Introduction

Cattle production represents an important economic activity in tropical and subtropical areas<sup>(1)</sup> and it is the main source of income for a large group of the rural population<sup>(2)</sup>. However, diseases such as gastrointestinal helminthiasis can influence the development of calves, increasing production costs<sup>(3,4)</sup>. Gastrointestinal nematodes (GN) are responsible for severe harm in young animals and in primiparous females, promoting reduction in development, low productivity, economic losses, and, in extreme cases, increasing the mortality rate in highly infected calves<sup>(3,5,6)</sup>.

The synthetic anthelmintics (AH) benzimidazoles (BZ), macrocyclic lactones (ML) and imidazothiazole (IMZ) have been intensively used for the control of bovine GN<sup>(7,8)</sup>. However, inappropriate usage, under dosing, wrong diagnosis, and the lack of knowledge about epidemiology have contributed to the selection of resistant GN<sup>(4,9)</sup>.

Therefore, efficacy tests of these products must be performed on the farms at least once a year, to replace AH classes with low efficiencies<sup>(4,10)</sup>. Compared to small ruminants,

only a few researches have investigated the occurrence of AH resistance of cattle GN in tropical areas, so the number of cases might be considerably underestimated<sup>(11)</sup>.

Reports of AH multi-resistance described bovine herds that were raised in different continents<sup>(4,12)</sup>. However, little is known about the susceptibility profile to AH, epidemiology and the control management of bovine helminthiasis in regions with hot semi-arid climate. This study characterized the control of gastrointestinal nematodes and AH effectiveness in calves raised in the northern of the Minas Gerais State, Brazil.

## **Material and methods**

### **Study area and cattle farms investigated**

It was applied questionnaires in 59 farms obtaining information about management, infrastructure, use of AH, and measures employed to GN control. It was conducted the study during dry seasons (April to September of 2013-2015) in farms located in the northern of Minas Gerais State, Brazilian sertão (Table 1 and Figure 1). During these periods the monthly average rainfall, humidity and temperature were respectively 17.14 mm, 57.57 % and 20.82 °C, respectively (5° Distrito, Instituto Nacional de Meteorologia, Brazil). This area's climate is characterized as hot semi-arid (BSh) according to the Köppen-Geiger climate classification, warm with a short rainy season (summer) and a long drought (winter)<sup>(13)</sup>.

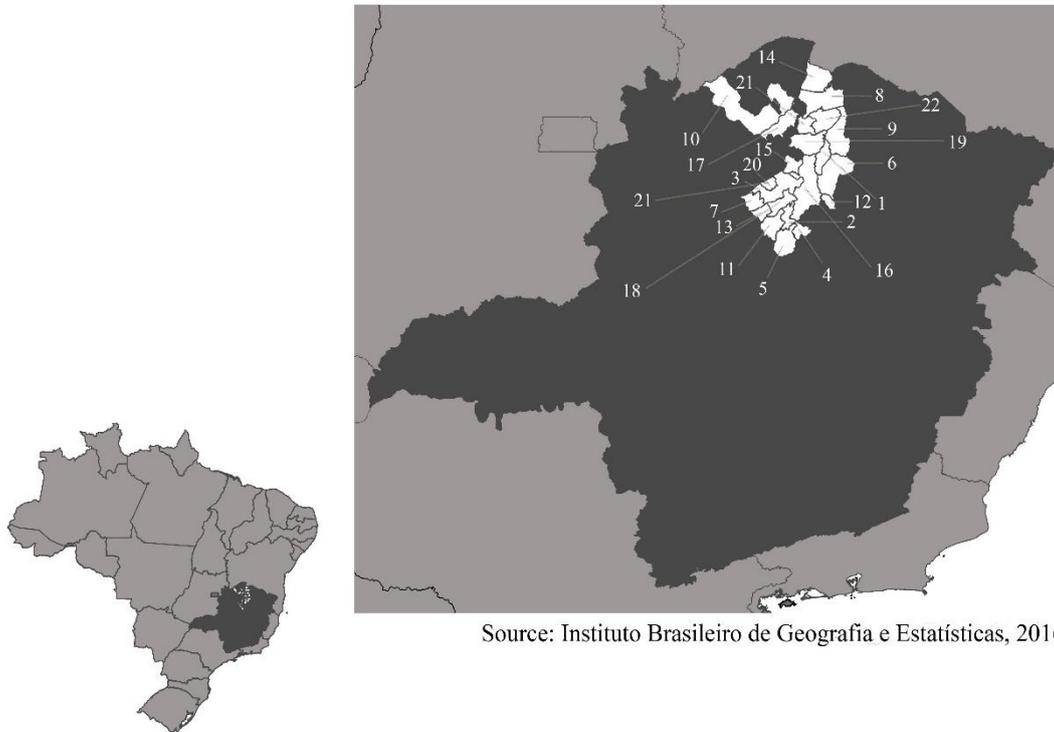
**Table 1:** Distribution and geographical characterization of cattle herds evaluated in the northern of Minas Gerais, Brazil

Cities	Number of animals	Number of farms	Latitude	Longitude
1. Capitão Enéas	54	4	-16°19'28"	-43°42'38"
2. Claro dos Poções	-	1	-17°04'47"	-44°12'31"
3. Coração de Jesus	-	4	-16°41'47"	-44°21'54"
4. Engenheiro Navarro	30	1	-17°16'47"	-43°57'00"
5. Francisco Dumont	-	3	-17°31'33"	-44°23'42"
6. Francisco Sá	-	6	-16°47'61"	-43°48'86"
7. Ibiaí	-	1	-16°51'40"	-44°54'52"
8. Jaíba	-	1	-15°20'18"	-43°40'28"
9. Janaúba	-	1	-15°48'09"	-43°18'32"
10. Januária	-	2	-15°29'17"	-44°21'42"
11. Jequitaiá	44	2	-17°14'08"	-44°26'44"
12. Juramento	-	1	-16°84'81"	-43°58'67"
13. Lagoa dos Patos	-	1	-16°59'00"	-44°34'56"
14. Matias Cardoso	-	1	-14°51'17"	-43°55'19"
15. Mirabela	-	1	-16°15'46"	-44°09'52"
16. Montes Claros	108	19	-16°73'50"	-43°86'22"
17. Pedras de Maria da Cruz	-	1	-15°60'58"	-44°39'19"
18. São João da Lagoa	59	3	-16°51'11"	-44°21'07"
19. São João da Ponte	-	3	-15°55'45"	-44°00'28"
20. São João do Pacuí	-	1	-15°32'31"	-44°30'58"
21. Varzelândia	-	1	-15°70'17"	-44°02'72"
22. Verdelândia	-	1	-15°35'21"	-43°36'10"
Total	295	59		

Latitude and Longitude of Brazilian cities, available at: <<http://www.apolo11.com/latlon.php?uf=mg>>.

Accessed on: August 7th, 2014.

**Figure 1:** Geographical distribution of the cities of the calf herds evaluated in the northern of Minas Gerais, Brazil. The numbers represent the cities according to Table 1.



Source: Instituto Brasileiro de Geografia e Estatísticas, 2016.

Among the evaluated herds, it was selected five farms to perform the efficacy tests. Besides the geographic location, we chose cutting herds which had not received AH in the last 2 mo. The groups were homogeneous in weight, age and quantity of at least 30 calves.

### **Parasitological exams and anthelmintic resistance test**

It was evaluated Nellore or Girolando calves of 6-14 mo old, naturally infected by GN, sampling a minimum of 10 g of faces from the rectal ampulla. The samples were identified in plastic bags and kept refrigerated to determine fecal egg counts (FEC) and for obtainment of larvae in fecal cultures.

FEC was performed by the usage of saturated sodium chloride solution and a reading under a microscope by using the 10X objective into two McMaster chambers for each sample, obtaining a medium value per animal<sup>(14)</sup>. Fecal egg counts were determined via

the McMaster technique - 4 g of feces and a detection sensitivity of 25 (EPG)<sup>(15)</sup>. For identification of the main genera present in the herds, fecal culture<sup>(16)</sup> was performed before and after the treatments, in which approximately 100 third-stage larvae for each respective treatment group were identified<sup>(17)</sup>.

The animals were identified, weighed and grouped in homogeneous groups of breed, age, sex and body weight (bw) and, on day one, calves were distributed according to their parasite loads (balanced) into experimental groups, containing at least ten animals per treatment. The Ethics Committee on Animal Experiments of the Federal University of Minas Gerais approved all procedures adopted under the protocol 42/2008.

Fecal egg count reduction test (FECRT) was performed as it is recommended by the World Association for the Advancement of Veterinary Parasitology, to diagnose AH resistance<sup>(18)</sup>. The inclusion criteria for the selection of the test of AH efficacies were: (i) herds with a population of homogeneous calves, (ii) calves not dewormed during 60 days prior to the study and (iii) herds with calves excreting more than 150 eggs per gram (EPG) of feces. The major factor that limited the number of herds evaluated was the lack of homogeneous animals excreting more than 150 EPG.

The AH choice for each farm varied according to its historical control, and the number of products tested depended on the availability of animals with infections > 150 EPG. Prior to the treatment, animals were weighed individually for the correct administration of AH doses. Therefore, variability was avoided among the doses used for treatments.

The AHs evaluated were albendazole (10 mg/kg bw), levamisole chloridrate (7.5 mg/kg bw), ivermectin, doramectin or abamectin (0.2 mg/kg bw). The products were administered subcutaneously, according to the manufacturer recommendations.

Fourteen days (14) after the treatment other fecal samples were obtained for calculation of FEC and new coprocultures were performed per group, as mentioned previously, to identify the GN genera (third larval stage) involved with resistance. The AH effectiveness was estimated using the following equation<sup>(18)</sup>:

$$\text{Efficacy} = [1 - (\text{FEC average of treated group} / \text{FEC average of control group})] \times 100$$

After FEC reduction tests, cattle farmers were instructed about parasitism control with the distribution of technical reports and information on specific parasitological examinations of each herd.

Evaluation of the AH effectiveness was based on the determination proposed by Common Market Group (CMG), being highly effective when it reduces more than 98 % of the EPG, effective with 90 to 98 %, moderately effective from 80 to 89 %, insufficiently active with less than 80 % reduction, and, non-registrable<sup>(19)</sup>. Nematodes were considered

resistant when the FEC reduction percentage was less than 95 % and the lower limit of the confidence interval was smaller than 90 %<sup>(20)</sup>.

## Statistical analyses

FEC data was compared by nonparametric Kruskal-Wallis or Wilcoxon tests. To compare the frequencies of nematode genera and questionnaire information it was used chi-square test. Data were evaluated at 5% significance by SAEG 9.1 statistical package software<sup>(21)</sup>.

## Results

### Characterization of the production systems and animals

Beef cattle business was considered the most important one for 96.2 % of the farms. 3.7 % of farms produced both, beef and dairy calves. The predominant production system was extensive, representing 83.9 % of the farms.

Among forage species, 49.5 % of farms cultivated *Urochloa* spp., 19.5 % *Panicum* sp., 17.53 % *Andropogon gayanus*, 5.84 % *Cynodon* sp., 3.59 % *Hyparrhenia rufa*, 3 % *Cechrus ciliaris* and 0.64 belong to *Penninsetum purpureum*. Rotational grazing system were used in 54.5 % of them and 68.5% of the herds parted the animals into age group. The pastures for the calves were in lower areas if compared to older animals in 83 % of investigated herds and only 55.5 % of the farms owned a maternity picket.

The greater frequency breed ( $P<0.05$ ) was Nellore, representing 56.1 % of beef herds. Nellore crossbred population was raised in 10.3 % of farms and Caracu, Sindhi, Guzera

or Red Angus were reported in 8.4 % of farms, respectively. In 146 dairy farms, Girolando represented the single breed in the evaluated herds.

### Control of helminthiasis

Macrocyclic lactones was the most frequent active principle of AH used to evaluate beef farms ( $P<0.01$ ), and ivermectin was its most common component (Table 2). In 78.9 % of the farms, the practice of weighing animals was not used before treatments and dosing was calculated by body score evaluation. Just 14.54 % of them used fast before the treatments.

**Table 2:** Anthelmintic used in beef herds in the North of Minas Gerais, Brazil

<b>Anthelmintics class</b>	<b>Observation</b>	<b>Frequency (%)</b>
Macrocyclic lactones	71*	86.4
Ivermectin	52	62.9
Abamectin	6	7.4
Doramectin	10	12.3
Moxidectin	3	3.7
Benzimidazoles (Albendazole)	4	4.9
Imidothiazoles (Levamisole)	4	4.9
Associations	2	2.5
Abamectin + Ivermectin	1	1.2
Fluazuron + Abamectin	1	1.2
Homeopathy	1	1.2
<b>Total</b>	<b>82</b>	<b>100.00</b>

\*Class of products used with higher frequency by chi-square test ( $P<0.05$ ).

\*Frequency= number of farms using the commercial product/total number of products reported.

\*Number of farms is different from the total number of observations due to the use of more than one product for control of helminthiasis in the same farm.

All cattle categories were treated in 72.7 % of herds and only the calves were treated in 26.3 % of herds. Females at peripartum were wormed in only 33.3 % of these farms. The frequency of AH treatments varied according to each farm, being that 60 % followed the vaccine schedule for control of foot-and-mouth disease virus in May and November. The use of strategic control with AH during dry season was only performed in 33.2 % of the farms and the alternation of active principles of AH products were occurring in 66.8 % of them.

### Occurrence of helminthiasis

The FEC averages were low for both beef ( $174.0 \pm 84.8$ ) and dairy ( $162.4 \pm 122$ ) calves raised in the North of Minas Gerais and no significant differences were observed between these two animal groups ( $P>0.05$ ) (Table 3). The herds 4 and 7 showed the lower FEC with beef and dairy calves, respectively ( $P<0.05$ ).

**Table 3:** Average of fecal egg count (FEC) in calves raised in the Northern Minas Gerais and percentage of nematode genus identified before worming

Farms	EPG (day 0)	Haem (%)*	Trich (%)	Oeso (%)	Coop (%)	Bunos (%)
Beef calves						
1	158.87 <sup>a</sup>	70	15	-	15	-
2	138.06 <sup>ab</sup>	97	-	2	-	-
3	190.00 <sup>a</sup>	89	1	10	-	-
4	11.80 <sup>c</sup>	92	-	4	-	4
5	50.00 <sup>b</sup>	70	11	12	5	2
Dairy calves						
2	248.50 <sup>a</sup>	95	-	2	3	-
6	80.00 <sup>bc</sup>	88	-	-	12	-
7	69.50 <sup>c</sup>	92	-	-	8	-
8	145.10 <sup>ab</sup>	93	1	1	4	1
CV (%)	82.2					

Haem= *Haemonchus* spp., Trich= *Trichostrongylus* spp., Oeso= *Oesophagostomum* spp.,  
Coop= *Cooperia* spp., Bunos= *Bunostomum* spp., (-)= off.

<sup>abc</sup> Means followed by the same letter in the column are not different ( $P<0.05$ ).

CV= Coefficient of variation

On day 0 (zero), GN *Haemonchus*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum* and *Bunostomum* genera infections were found. The profile of nematodes genera was not different ( $P>0.05$ ) among the herds, and the most frequent GN for both calf groups and all evaluated farms was the *Haemonchus* spp. ( $P<0.01$ ) (Table 3).

### Anthelmintic efficacies

It was observed FEC reduction in all deworming calf groups if compared with untreated groups of all evaluated herds ( $P<0.05$ ). Nevertheless, ivermectin and doramectin were not efficient, showing only 24.28 % at 81.63 % of FEC reduction (Table 4). High AH efficacies ( $>98$  %) were observed to albendazole or levamisole treatments in beef calves of farm 2, but the levamisole administered to dairy calves showed lower efficacy than with beef calves ( $P<0.05$ ) (Table 5).

**Table 4:** Average of fecal egg count per gram of in beef calves after worming and anthelmintic efficacy (%)

Herds	Control	Albendazole	%	Levamisole	%	Ivermectin	%	CV%
1	490.0 <sup>a</sup>	77.5 <sup>b</sup>	84.18	-	-	90.00 <sup>b</sup>	81.63	91.3
2	233.3 <sup>a</sup>	2.77 <sup>c</sup>	98.81	3.57 <sup>c</sup>	98.47	118.75 <sup>b</sup>	49.09	88.2
3	175.0 <sup>a</sup>	22.5 <sup>b</sup>	87.14	47.90 <sup>b</sup>	72.62	42.50 <sup>b</sup>	24.28	85.3

CV%= coefficient of variation.

<sup>abc</sup> Averages followed of different letters on line differs ( $P<0.05$ ).

**Table 5:** Average of fecal egg count per gram of in dairy calves after worming and efficacy of synthetic anthelmintics

Herds	Untreated	Albendazole	%	Levamisole	%	Doramectin	%	CV%
2	289.5 a	-	-	57.1B	80.27	-	-	87.3
8	150.83 a	32.2 b	78.65	-	-	54.2 b	64.06	90.4

CV%= coefficient of variation.

<sup>ab</sup> Averages followed of different letters on line differs ( $P<0.05$ ).

## Genus of nematodes identified post-treatment

The most frequent post-treatment nematode for both treated and untreated calves was the *Haemonchus* genus ( $P<0.01$ ). For the herd 1, the genus *Trichostrongylus* represents 13 % of the L3 identified from coproculture of calves treated with ivermectin (Table 6). For the herds 2 and 8, the *Haemonchus* spp. was also more frequent (87-93 %); despite that, L3 numbers retrieved from treated groups were insufficient for statistical analysis.

**Table 6:** Profile of nematode genera (%) from beef calves after anthelmintic treatment

Genera	Herd number 1			Herd number 3			
	Control	Ivermec	Albend	Control	Ivermec	Albend	Levam
<i>Haemonchus</i>	93*	80*	93*	83*	96*	97*	97*
<i>Trichostrongylus</i>	4	13	2	4	0	0	0
<i>Cooperia</i>	3	1	0	7	0	2	0
<i>Oesophagostomum</i>	0	6	5	4	2	1	3
<i>Bunostomum</i>	0	0	0	2	2	0	0

\*Genus with greater frequency in the Chi-square test ( $P<0.01$ ).

Ivermec= ivermectin, Albend= albendazole, Levam= levamisole.

## Discussion

### Characterization of the creation systems and animals

In Brazilian livestock, there is a predominance of the extensive system with continuous grazing. Although pasture is the main food source, it also represents the main source of L3 infection of GN<sup>(22,23)</sup>.

The wide distribution of cultivated pastures of the genus *Urochloa* sp. (*Brachiaria* sp.) in the evaluated tropical region can be justified by its adaptation in acid and low fertility soils in addition to considerable drought tolerance<sup>(24)</sup>. Pasture management strategies are essential to the control of GN when reducing the contamination and ingestion of L3 by animals<sup>(25)</sup>. Environmental conditions are important for the development and survival of free-living stages and for L3 migration along forage grasses. The morphological differences among forage species influence the development and survival of eggs and larvae due to the different microclimates that were provided by plants<sup>(26)</sup>.

The reserving areas for *Brachiaria* spp. (*Urochloa* spp.) grazing in late summer and grazing deferment, is a seasonal strategy to enable the excess of forage produced in late summer to be used during the dry season<sup>(27)</sup>. This strategy has been widely used by cattle farmers in the North of Minas Gerais and probably it could drastically reduce the survival of NG larvae in pastures, which could have contributed to the low FEC observed in the present study.

A study in São Paulo, Brazil, indicated a significant higher overall recovery rate of *Haemonchus* sp. larvae from feces after depositing fecal samples on *Panicum* sp. If compared to *Urochloa* sp. and *Cynodon* grasses in August, February, and May<sup>(28)</sup>. A research on the retrieval of *Trichostrongylus colubriformis* infective larvae from contaminated grass in winter and in spring compared *Urochloa*, Coast-cross and Aruana forage grasses. *Urochloa* (*Brachiaria*) spp. showed to be the densest forage and the effect of the higher density was dilution of L3, leading it to present the lowest concentrations of L3/kg of dry matter<sup>(26)</sup>.

In this study, the use of rotational grazing was found in 54.5 % of the farms, so farmers' attention to the number of animal units introduced for grazing is mandatory. The period to rotate the pickets should be greater than that one, which allows inactivation of eggs and larvae, reducing L3 infection<sup>(29)</sup>. Age separation of animals was used in 68.5% of the herds in this study, being this strategy crucial, as young individuals are more susceptible than adult ones<sup>(5)</sup>.

Racial composition influences the intensity of parasitism; zebu breeds are more resistant than European breeds<sup>(2,9)</sup>. In the northern region of Minas Gerais, herds with Zebu and Nellore were predominant, justifying the low FEC observed in beef herds. Studies of progeny resulting from crosses between taurine and zebu breeds have intermediate levels of susceptibility to GN<sup>(2,9)</sup>.

Genetic selection for resistant cattle constitutes a relevant alternative to GN control. It was observed that within each herd, few calves (5-8 %) presented higher FEC, indicating greater susceptibility to it and should not be selected to breeding programs. Bovine

selection can increase the frequency of resistant animals to these parasites and should be included in strategic programs of GNs control<sup>(30,31)</sup>.

## **Control of helminthiasis**

In this investigation, the predominant the use of macrocyclic lactone group can confer higher selection pressure for resistant GN. Resistance to ivermectin was described in different regions such as Northern California, United States<sup>(32)</sup>, Buenos Aires, Argentina<sup>(33)</sup> and in Brazil, more precisely, in São Paulo and Minas Gerais<sup>(30,34)</sup>. Thus the evaluation of AH susceptibility profile in each region or herd is important to ensure effective GN control<sup>(33)</sup>.

AH efficacy depends on chemical class alternation at proper periods<sup>(35)</sup>. In this study, only 66.8 % of the farms performed rotation practices, which could favor the selection of resistant GN. Therefore, change frequency of these products should be highlighted, since it may favor the selection of multi-resistant GN<sup>(35,36)</sup>. The AH must be replaced immediately by other classes when it presents effectiveness that are smaller than 80% in order to avoid the establishment of resistant populations of GN<sup>(37)</sup>.

All bovine categories were treated in most herds (72.7 %) of this study and it can favor the selection of resistant GN. The categories of cattle that should be prioritized for this control represent calves up to 24 mo old and females at peripartum. These young animals are significantly more susceptible to helminthes, up to 2 yr old<sup>(38,39,40)</sup>.

In this study, only 33.3 % of farms treated cows at peripartum. The practice is relevant for heifers in development and they have compromised immunity, making them more susceptible to endoparasitoses in pre and post-partum. Multiparous beef Zebu cows did not require deworming; these animals have showed natural resistance to GN and low potential for contamination when well managed<sup>(5,41)</sup>. A different GN control should be advocated<sup>(38)</sup> according to the bovine categories and should follow climate and regional criteria that consider the profile of resistant GN populations<sup>(37)</sup>.

The criteria adopted for the worming period of the herds varied in this study. Most (60 %) of the properties treated all animals at the beginning (May) and at the end of the dry season (November) simultaneously with vaccination against foot-and-mouth disease.

For the northern region of Minas Gerais, the treatment should also be performed in September to cover the whole period of the season.

Another study in Central Brazil observed that the treatment could increase weight gain in Nellore calves during the growth phase. AH protocol in May, August and November, using AHs of long action, increased weight gains up to 34.1 kg (31.9 %) compared with animals that were not treated. Treatment during the vaccination periods against foot-and-mouth disease in May and November has not increased weight gains<sup>(6)</sup>. The climatic conditions of this area is the closest to the northern of Minas Gerais; despite presenting more rainfall, the strategic control proposed by<sup>(6)</sup> can also be applied to hot semi-arid areas, increasing weight gain of calves in the rearing.

According to the literature, climate changes and the intensive management of farms have influenced risks of infections and transmission<sup>(42)</sup>. Thus, the probability of alteration in the epidemiological of GN infections by climatic alterations, together with high frequencies of AH resistance, required adjustments to the practice of the current controls<sup>(42)</sup>. Future studies should also consider these climatic changes for the definitions of GN control practices in cattle herds raised in areas with hot semi-arid climates.

The study of homeopathic products is not focal to this study. This control alternative should be performed carefully and scientific studies should monitor it with discussions of applicability, as well as circumscription of the correct doses<sup>(43)</sup>.

### **Occurrence of helminthiasis in cattle herds**

Cattle herds in the Northern of Minas Gerais showed lower FEC, even though this kind of contamination differed between farms. The low averages observed to farms 4, 6 and 7 can be attributed to management conditions of calves. In the beef herd 4, the calves were weighed before applying AH, annual AH change; separation by age group could be a better GN control. The calves of dairy herds 6 and 7 were raised confined in pickets without pastures; the feces were weekly collected and send for composting, and the calves were fed with silage. Thus, the survival of L3 larvae was impaired, contributing to the lowest FEC observed.

The beef herds 1, 2, 3 and 5 presented similarities in GN control such as the epochs of annual deworming or during periods of higher infestation of flies and ticks. AH was used,

being ivermectin the most common anthelmintic in farms with rotation of AH class and lack of strategic control. The dairy farms 6 and 7 in Montes Claros showed herds composed of Girolando crossbred with higher FEC averages if compared to 1, 2, 3 and 5. The low value detected could be related to the confinement system of calves in pickets of land without vegetation.

The most frequent GN for beef and dairy herds in the area was *Haemonchus* spp. Frequently this genus was reported with higher prevalence in small ruminants, while the genus *Cooperia* sp. tends to be the most frequent with the Brazilian cattle<sup>(35,40)</sup>.

*Haemonchus* spp. represented the most common pathogenic nematode to cattle in tropical regions. In calves, it promotes reduction in the mean hematocrit values and reduced weight. The (L4) of *Haemonchus* is a bloodsucker in the abomasum and therefore animals infected with large numbers of larvae may present anaemia before FEC is detected in feces. The genera *Trichostrongylus*, *Cooperia*, *Oesophagostomum* and *Bunostomum* were also identified in coproculture before treatment. Infections with GNs frequently involve several different species, which can have an additive pathogenic effect on the calves<sup>(42)</sup>.

### **Anthelmintic efficacies**

Albendazole and levamisole were the most effective AH to GN from beef calves in the farm number 2, but resistant nematodes to levamisole were detected in feces of dairy calves of this same farm. The profile of resistance to ivermectin, levamisole, albendazole and or doramectin displayed by this study is worrisome. Multi resistant GN were present in herds 1, 3 and 8, what shows that no class of AH tested was effective for FEC reduction.

Ivermectin, doramectin and abamectin presented the lowest effectiveness to FEC reduction. Low efficacy observed by the macrocyclic lactones could be associated with historic use of these AHs in this region, which favored the selection of resistant GN populations.

In this study, most of the farms (72.7 %) treated all cattle of herds, not favoring refuge to sensible nematode population. The larvae on pasture, the percentage of animals left untreated and the arrested larval stages were not affected by treatment of the host

determined by the GN in refuge. The proportion of these nematodes in refuge needs to be optimal in order to dilute out the resistant genes in the pool of susceptible genes<sup>(44)</sup>.

Data reported in this study corroborate with Gasbarre *et al*<sup>(45)</sup> who observed macrocyclic lactone resistance in GN from cattle in the United States. The indiscriminate use of these in arid and semiarid regions for infection control were compelled for higher efficacy and prolonged anthelmintic activity, resulting in nematode resistance due to higher usage drugs. However, it promoted high selection pressure of GN resistant ones<sup>(46)</sup>.

In Santa Catarina, in Brazil, efficacies >95 % for ivermectin were verified in seven beef cattle farms. Two farms detected efficacies <14 % showing evident resistance to ivermectin. Levamisole and albendazole were effective to GN control in accordance with the CMG, with efficiencies above 95 %<sup>(35)</sup>.

The genus *Haemonchus* sp. was the most frequent nematode in treated beef calves of herds number 1 and 3 (80-97 % of identified L3 larvae) and it was characterized as multiresistant to benzimidazoles, imidothiazoles and macrocyclic lactones. *Trichostrongylus*, *Oesophagostomum*, *Cooperia* and *Bunostomum* were also detected from fecal culture and post-treatments indicated an initial selection of resistant strains of these GNs.

The greater pathogenicity and higher biotic potential of *Haemonchus* sp. have led to a higher frequency of AH treatments and higher selection pressure of resistant strains of this nematode<sup>(35)</sup>. In Betim, Minas Gerais, Brazil, resistance to ivermectin and doramectin was also observed for the genera *Haemonchus* (72 %) and *Cooperia* (85 %), respectively<sup>(46)</sup>. Macrocyclic lactones resistance to genera *Haemonchus* and *Oesophagostomum* was reported, in Teófilo Otoni, Minas Gerais<sup>(40)</sup>. The authors reported that macrocyclic lactones were also the most common ones for the control in the farms of this area.

This research confirms the study performed in the state of Santa Catarina, when assessing resistance to ivermectin, phosphate of levamisole and dimethyl sulfoxide albendazole for cattle herds. There, *Haemonchus* spp. was predominant after deworming, showing evident multi-resistance<sup>(35)</sup>.

In the United States, records of resistance to macrocyclic lactones are frequent in commercial herds for *Cooperia* and *Haemonchus* genera, for ivermectin and doramectin specifically. However, the *Cooperia* genus was sensitive to benzimidazoles<sup>(46)</sup>. In Veracruz, México, a high frequency of farms with GN population that is resistant to ivermectin was also observed and these nematodes genera were the most frequent ones<sup>(4)</sup>.

In Europe, a study involving Germany, France, Italy and the United Kingdom farms has shown low efficacy for ivermectin and moxidectin, and confirmed cases of resistance in

12 % of 40 herds. Thus, the most frequent genera among treatments were *Cooperia* and *Ostertagia*, mainly in the United Kingdom and Germany farms<sup>(47)</sup>.

## **Conclusions and implications**

The most assessed farms did not practice strategic or tactical controls, promoted inappropriate and indiscriminate use of synthetic anthelmintics and macrocyclic lactones was common. All evaluated herds showed at least one anthelmintics with low efficacy, two beef farms presented multi-resistant nematodes and *Haemonchus* genus was the most frequent one. The applicability of strategic control in calves and tactics in heifers at peripartum, the alternation of AH classes, as well as the implementation of alternative measures such as the selection of resistant animals, the use of fungi for biological control and plant extracts to reduce resistant populations of these nematodes is essential for more sustainable control.

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### **Conflict of interest statement**

The authors of this manuscript have no financial or personal relationship with people or organizations that could inappropriately influence or bias the content of the paper.

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