

Resistance of *Haemonchus*, *Cooperia*, *Trichostrongylus*, and *Oesophagostomum* to ivermectin in dairy cattle in Paraná

Resistência de *Haemonchus*, *Cooperia*, *Trichostrongylus* e *Oesophagostomum* à ivermectina em bovinos de leite no Paraná

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Abstract

The objective of this study was to analyze the effect of ivermectin (3.15%) on gastrointestinal nematodes found in calves through the fecal egg count reduction test (FECRT), and larval culture in 16 animals from a school farm at the Universidade Estadual do Norte do Paraná. The animals were divided into two experimental groups: animals treated with ivermectin 3.15% (G1 group) and control animals (G2 group). Stool samples were collected from the animals twice i.e., on days 0 and 10 for fecal examinations. There was no significant change in the egg count per gram of feces (EPG) in both the groups. Helminths were found to be resistant to ivermectin (3.15%), due to the observed 5.62% FECRT rate. *Cooperia* was found to be the dominant nematode larvae present (53% on day 10 and 8% in day 0), while *Trichostrongylus* was detected in small numbers (0% on day 0 to 1% on day 10). The drug was found to be effective only in *Trichuris*, while the same was inefficient against *Haemonchus* and *Cooperia*, although *Trichostrongylus* and *Oesophagostomum* were found to be 100% resistant. Therefore, the findings of this study suggest that ivermectin 3.15% should not be used for the control of nematodes in calves, since most of the parasites demonstrated drug resistance. This is the first report on the resistance of *Haemonchus*, *Cooperia*, *Trichostrongylus*, and *Oesophagostomum* in cattle to ivermectin 3.15% in the state of Paraná.

Key words: Anthelmintic resistance, bovine, macrocyclic lactone

Resumo

Objetivou-se, neste estudo, analisar o efeito da ivermectina (3,15%) sobre nematódeos gastrointestinais de bezerras com aptidão leiteira por meio do Teste de Redução na Contagem de Ovos nas Fezes (RCOF) e da cultura de larvas em 16 animais da Fazenda Escola da Universidade Estadual do Norte do Paraná. Para tanto, dois grupos experimentais foram formados: animais tratados com ivermectina 3,15% (grupo G1) e grupo controle (Grupo G2). Foram coletadas amostras de fezes dos animais em dois momentos, ou seja, nos dias 0 e 10 para realização dos exames coproparasitológicos. Não houve alteração significativa na contagem de ovos nas fezes (OPG) em ambos os grupos. Os helmintos foram considerados resistentes à ivermectina 3,15%, já que a taxa de RCOF foi de 5,62%. O gênero de nematódeo mais observado na cultura de larvas foi *Cooperia* (53% no dia 10 a 88% no dia zero) e o menos foi *Trichostrongylus* (0% no dia zero a 1% no dia 10). Observou-se eficiência apenas sobre o gênero *Trichuris* e ineficiência sobre os gêneros *Haemonchus* e *Cooperia* e ainda 100% de resistência dos gêneros *Oesophagostomum* e *Trichostrongylus*. Concluiu-se neste estudo que a ivermectina 3,15% não deve ser indicada no controle de

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nematódeos dos bezerros da propriedade, pois a maioria dos parasitas apresenta resistência à droga. Este foi o primeiro relato de resistência de *Haemonchus*, *Cooperia*, *Trichostrongylus* e *Oesophagostomum* de bovinos a ivermectina 3,15% no estado do Paraná.

Palavras-chave: Resistência anti-helmíntica, bovino, lactona macrocíclica

Gastrointestinal helminths in cattle cause economic losses to livestock breeders due to decreased milk production and decreased weight gain (TODD et al., 1978). Ivermectin is an anthelmintic widely used because of its broad spectrum of action (SPINOSA et al., 2006). However, with the indiscriminate use of lower or higher doses than the recommended dosage and lack of drug turnover, resistance to parasites result in multiple active ingredients, including ivermectin (FIEL et al., 2001). Based on various reports that detail the parasite to this drug and estimated drug resistance due to routine use of this drug, this study aimed to estimate the effectiveness of ivermectin on nematodes in calves reared on the school farm at the Universidade Estadual do Norte do Paraná (UENP) in Bandeirantes city.

Prior to formation of the experimental group, egg count per gram of feces (EPG) was estimated in 30 calves, and out of these, 16 were selected; these selected animals were naturally infected by gastrointestinal nematodes. The animals were bred in the cattle industry of the Farm School UENP on

the same paddock. The animals were divided into two randomized groups according to their EPG values as follows: group 1 (G1), which comprised of 8 crossbred (6 females and 2 males) weaned medium 665 EPG treated with 3.15% ivermectin (0, 63 mg/kg body weight [BW]) via a single subcutaneous route and group 2 (G2; control group), which comprised of 8 crossbred (5 females and 3 males) weaned animals with an average of 500 EPG and were untreated. The animals were bred in the cattle sector of the farm school UENP on the same picket fence.

Fecal samples were collected before treatment (day 0) and 10 days after treatment in order to estimate the fecal egg counts (FEC) second description of Ueno and Gonçalves and perform a larval culture by Roberts and O'Sullivan. Furthermore, the rate of reduction in the fecal egg count (RFEC) according to Coles et al. (1992) was evaluated. Larvae (L3) were identified according to the criteria put forward by Keith (1953), and the effectiveness of the identified treatment for each nematode genus was calculated using the following formula:

$$\text{Effectiveness of genus X} = \frac{(\text{LPG mean of X from GC} - \text{LPG mean of X from GT}) \times 100}{\text{LPG mean of X from GC}}$$

where:

LPG: larvae per gram of feces, calculated by multiplying the value of EPG by the percentage of each sex of larvae found in the culture

X: genus of larvae identified

GC: control group

GT: treated group.

The mean values were log-transformed and were then compared using the paired t tests and the

unpaired t test via a statistical GraphPadPrism 5.0 program.

Despite the small decrease in the mean EPG (21%) in the group treated with ivermectin (G1), this decrease was not significant ($p = 0.446$). In the control group, the 11% increase in mean EPG was also not significant ($p = 0.55$) (Table 1). A similar result was reported by Costa et al. (2011) in the State of Minas Gerais in Girolanda in crossbreds treated with ivermectin 3.15%.

Table 1. The mean counts of strongyle eggs and eggs of the genera *Strongyloides* and *Trichuris* (OPG \pm standard deviation, $\log x + 1$) in the feces of calves in the group treated with ivermectin 3.15% (n = 8) and the control group, untreated (n = 8) before (day 0) and 10 days after treatment.

Nematodes	Treated group		Control group	
	Day 0	Day 10	Day 0	Day 10
Strongyle	2.43 (\pm 0.66)	2.42 (\pm 0.56)	2.45 (\pm 0.51)	2.62 (\pm 0.35)
<i>Strongyloides</i>	0.43 (\pm 0.80)	0.00 (\pm 0.00)	0.39 (\pm 0.72)	0.00 (\pm 0.00)
<i>Trichuris</i>	0.47 (\pm 0.87)	0.00 (\pm 0.00)	0.51 (\pm 0.99)	0.24 (\pm 0.66)
Nematodes mean value	2.50 (\pm 0.60)	2.42 (\pm 0.56)	2.50 (\pm 0.54)	2.64 (\pm 0.32)

There were no significant differences between the means in the same group and between groups.

According to Coles et al. (1992) and with the recommendations from the World Association for the Advancement of Veterinary Parasitology (WAAVP), helminths are resistant to ivermectin 3.15%, since the RFEC rate was only 5.62%. Ivermectin is routinely used in calves; these calves were housed in the same picket fence for 10 years. The efficiency may have decreased by the time ivermectin was assessed in flocks, because the indiscriminate treatment of animals in the herd and exhaustive use of the same active ingredient accelerate the resistance rate (ECHEVARRIA et al., 1996). Once the parasite develops resistance to 1% macrocyclic lactones, administration of high concentrations of the same drug or an overdose may not provide the expected efficacy (CEZAR et al., 2010).

Low efficiency at a lowest dosage of ivermectin in this study was reported by Fazzio et al. (2012) in Argentina, who observed an RFEC rate of 28.4%, 22 days after the treatment of calves, while Soutello et al. (2010) reported that the highest RFEC (72.2%) was observed 3 days after treatment and for 28 days thereafter, which was evaluated after administration of 1% ivermectin. On the Santa Catarina plateau, 18% of the cattle had an efficacy greater than 95% and 5% and less than 14% (SOUZA et al., 2008).

In Minas Gerais state, Rangel et al. (2005) tested the efficiency of conventional ivermectin 1% and 1% long action and determined the efficiency via the

RFEC test, which were 0% and 18.9% respectively. Indiscriminate and persistent use of this molecule may have resulted in the inefficiency within a short period of time; this was evident ten years before in the study performed by Arantes et al. (1995), also in Minas Gerais, who also demonstrated that the efficiency of this same molecule in calves of the same age was 100%.

In Paraná, studies on the effectiveness of ivermectin are restricted to sheep breeding. Cunha Filho et al. (1998) reported ineffectiveness of orally administered ivermectin (0.2 mg/kg) in 80% of properties evaluated in Londrina city, and in 50%, the RFEC was 0%. In Arapongas, a municipal near Londrina, Cunha Filho et al. (1998) also found 0% of RFEC in naturally infected sheep 14 days after treatment. Holsback et al. (2013) orally administered this molecule in combination with levamisole and albendazole and demonstrated an effectiveness of 100% and 99.5%, 7 and 14 days after treatment, respectively.

The nematode genus most prevalent in the larval culture was *Cooperia* (53% on 10-88% at day zero), while *Trichostrongylus* was found sparingly (0% on day 0 to 1% on day 10). Similar results were reported in the southern region (HONER; VIEIRA-BRESSAN, 1992) and in the Southeast (ARAÚJO; LIMA, 2005; COSTA et al., 2011). Ivermectin 3.15% was found to be effective only for the genus *Trichuris* and was ineffective for *Haemonchus* and *Cooperia*. *Oesophagostomum* and *Trichostrongylus*

demonstrated 100% resistance. It was not possible to assess the effectiveness of ivermectin in the genus *Strongyloides*, since this genus was not observed in the control group (Table 2).

Table 2. Larvae per gram of feces (LPG) *Haemonchus*, *Oesophagostomum*, *Cooperia*, and *Trichostrongylus* found in the larval culture, beyond the genus *Strongyloides* and *Trichuris* obtained from EPG and efficiency the anthelmintic ivermectin 3.15% for each genus nematode found.

	Treated group		Control group		Efficiency %
	Day 0	Day 10	Day 0	Day 10	Day 10
<i>Haemonchus</i>	63	32	43	60	47%
<i>Oesophagostomum</i>	13	210	4	33	0%
<i>Cooperia</i>	555	278	379	449	38%
<i>Trichostrongylus</i>	0	5	4	5	0%
<i>Strongyloides</i>	16	0	9	0	-
<i>Trichuris</i>	19	0	59	9	100%

$$\text{Efficiency \%} = \frac{(\text{LPG mean of X from control group in 10 day} - \text{LPG mean of X from treated group in 10 day}) \times 100}{\text{LPG mean of X from control group}}$$

Similarly, Souza et al. (2008) and Soutello et al. (2010) described the inefficiency of ivermectin in *Cooperia* and *Haemonchus*, while Rangel et al. (2005) showed the same in the genus *Oesophagostomum*. Cezar et al. (2010) reported 100% efficacy of ivermectin 3.15% in *Oesophagostomum*. In Uberlândia city, Minas Gerais, Arantes et al. (1995) demonstrated 100% efficiency of ivermectin in adults of the species *Haemonchus placei*, *Trichostrongylus axei*, *Cooperia punctata* and *Oesophagostomum radiatum*.

Therefore, the findings of this study suggest that ivermectin 3.15% should not be indicated for the control of nematodes in calves, because most of the parasites were drug resistant. This was the first report of resistance in the genera *Cooperia*, *Trichostrongylus*, *Haemonchus*, and *Oesophagostomum* isolated from cattle to ivermectin 3.15% in the state of Paraná.

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