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Anthelmintic resistance against gastrointestinal nematodes in beef cattle: effect on liveweight gain of grazing livestock in Mexico

[*Resistência anti-helmíntica em nematódeos gastrointestinais de bovinos de corte em uma região tropical: efeito no ganho de peso vivo do gado de pasto no México]*

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ABSTRACT

This study aimed to determine the efficacy and parasite resistance of levamisole (LV) and ivermectin (IVM) in beef cattle naturally infected with gastrointestinal nematodes, as well as the effect on the liveweight gain in a tropical wet region of Oaxaca, Mexico. From November 2019 to January 2020, sixty-six grazing calves were randomly allocated into three groups of twenty-two animals each, treated with LV or IVM or an untreated control group (day 0). Feces were collected 1 day before treatment and 15 days after treatment. The liveweight gain from each animal was recorded at days 0, 15, 30 and 45 post treatment. The LV group presented the highest reduction of eggs per gram (EPG) of feces, followed by the IVM group. Resistance to IVM was detected, although LV resistance was also suspected. The IVM group had significantly higher effective treatment at 93.5%, resulting in an increase (P<0.05) of liveweight gain of 16.1kg, followed by the LV group (92.4%) with 17.1kg, compared to the untreated control group. A significant (P < 0.05) negative correlation was observed between EPG and weight gain for the LV (r = -0.46) and IVM groups (r = -0.32). LV and IVM showed a lack of efficacy against gastrointestinal nematodes, as well as an adequate capacity for EPG reduction but with IVM resistance and detrimental effects on growth performance in grazing beef cattle.

Keywords: gastrointestinal parasites, beef cattle, anthelmintic resistance, levamisole, ivermectin, weight gain, grazing livestock

RESUMO

Os nematódeos gastrointestinais do gado de pastoreio causam perdas econômicas substanciais em todo o mundo. Este estudo teve como objetivo determinar a eficácia e a resistência parasitária do levamisol (LV) e da ivermectina (IVM) em bovinos de corte naturalmente infectados com nematódeos gastrointestinais, bem como o efeito no ganho de peso vivo, em uma região tropical úmida de Oaxaca, México. De novembro de 2019 a janeiro de 2020, 66 bezerros de pasto foram distribuídos aleatoriamente em três grupos de 22 animais cada um, tratados com LV ou IVM, ou em um grupo controle sem tratamento (dia 0). As fezes foram coletadas 1 dia antes do tratamento e 15 dias após o tratamento. O ganho de peso vivo de cada animal foi registrado nos dias 0, 15, 30 e 45 pós-tratamento. O grupo do LV apresentou a maior redução de ovos por grama de fezes (EPG), seguido pelo grupo IVM. A resistência à IVM foi detectada, embora também se suspeitasse de resistência ao LV. O grupo IVM teve um tratamento eficaz significativamente maior, com 93,5%, resultando em um aumento (P < 0,05) do ganho de peso vivo de 16,1kg, seguido pelo grupo LV (92,4%), com 17,1kg, em comparação com o grupo controle sem

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tratamento. Foi observada uma correlação negativa (P < 0,05) entre o EPG e o ganho de peso para os grupos LV (r = -0,46) e IVM (r = -0,32). LV e IVM mostraram falta de eficácia contra nematódeos gastrointestinais, assim como uma capacidade adequada de redução de EPG, mas com resistência IVM e efeitos prejudiciais no desempenho de crescimento em gado de corte em pastagem.

Palavras-chave: parasitas gastrointestinais, gado bovino, resistência anti-helmíntica, levamisol e ivermectina, ganho de peso

INTRODUCTION

Gastrointestinal nematodes (GINs) of grazing cattle cause huge economic losses to the livestock industry worldwide and constitute an important constraint on productivity, causing decreased reproductive performance, delayed growth, liveweight gain and milk yield, even where infection levels are subclinical (Borges *et al.,* 2013; Charlier *et al.,* 2009; Candy *et al.,* 2018). The economic impact of GINs in Mexico has been estimated at US $1.41 billion per year, and milk production losses of 0.542kg/cow/day have been reported (Villa-Mancera *et al.,* 2018; Rodríguez-Vivas *et al.,* 2017). Previous studies have reported significant differences in liveweight gains between cattle treated with low-efficacy drugs compared with highly effective drugs for GINs (Fazzio *et al.,* 2014; Edmonds *et al.,* 2018).

The main strategy to control GIN infection in cattle remains the use of anthelmintic drugs such as macrocyclic lactones, benzimidazoles and imidazothiazoles (McArthur and Reinemeyer, 2014; Gasbarre, 2014). However, the increase of anthelminthic-resistant nematode populations has become a significant problem for bovine practitioners and managers (Baiak *et al.,* 2018; Ramos *et al.,* 2016). Anthelmintic resistance of GINs in cattle has been observed in several regions worldwide (reviewed by Baiak *et al.,* 2018) with widespread resistance reported in Mexico (Alonso-Díaz *et al.,* 2015; Becerra-Nava *et al.,* 2014; Encalada Mena *et al.,* 2008; Muniz-Lagunes *et al.,* 2015). The distribution of GINs in grazing livestock varies in the different regions of Mexico, which have dramatically different climatic conditions; tropical conditions are even more favorable for the development of GINs. To our knowledge, no previous studies exist on parasite resistance in tropical wet regions in the Mexican state of Oaxaca. Thus, this study aimed to determine the resistance to levamisole (LV) and ivermectin (IVM) and evaluate their effect on the liveweight gain of beef cattle grazing in Mexico.

MATERIAL AND METHODS

This study was approved by the Animal Care and Ethics Committee of Realistic University of Mexico, and all procedures complied with national legislation pertaining to animal health research. The experiment was performed between November 2019 and January 2020 on a farm in the municipality of San José Chiltepec, state of Oaxaca, Mexico. According to the Köppen climate classification modified by Garcia (1981), the climate of the area is tropical wet (Am), with a mean annual temperature range of between 24°C and 26°C and annual precipitation ranging from 2,000 to 4,000mm, and 30m elevation above sea level.

A total of sixty-six 10–12-month-old Beefmaster calves (male and female) were extensively grazed with palisade grass (*Brachiaria brizantha*) and stargrass (*Cynodon nlemfuensis*) and had free access to water. The cattle did not receive any anthelmintic treatment during the 8 weeks before the experimental procedure. On day -1, all calves were checked for eggs per gram (EPG) of feces count using the McMaster modified technique with a sensitivity of 50 EPG. Cattle with an EPG count ≥200 were included in the study (day -1). All parasitized animals (n = 66) were randomly assigned to one of three experimental groups. Before treatment (day 0), the calves were weighed individually to dose them correctly with each anthelmintic compound.

Two commercially available anthelmintic drugs were tested and administered by a veterinarian following the manufacturers' recommendations. On day 0, the experimental animals received either IVM (0.2mg/kg subcutaneous; Ivomec®, Boehringer Ingelheim) or LV (5mg/kg intramuscular; L-Vermizol®, Aranda), and the untreated group was maintained as a control to

determine efficacy. Fecal samples were collected directly from the rectum of each calf 15 days after anthelmintic treatment, and another EPG count was performed. The anthelmintic efficacy of the different treatments was measured by fecal egg count reduction test (FECRT), calculated according to the following formula: FECR (%) = 100 × (1 – [mean EPG count post-treatment at 15 days/mean EPG count pre-treatment at -1 days]. The upper and lower 95% confidence intervals (CIs) were calculated as reported by Coles *et al*. (1992). Resistance was considered present if both the FECR (%) was less than 95% and the lower limit of the 95% CI was less than 90%, suspected if only one criterion was met, and absent (susceptible) when neither criterion were fulfilled (Coles *et al*., 1992). In addition, all calves were weighed individually on days 15, 30, and 45 post treatment.

Values for EPG means and bodyweight data are reported as mean ± standard deviation (SD). The results were analyzed using the IBM SPSS 25 software package (SPSS Inc., Chicago, USA) for Windows. The Shapiro–Wilk test was employed to analyze the distribution of the variables (EPG and body weight). The non-parametric Kruskal–Wallis test was used to compare the results of the EPG means. The difference between weight and EPG means were compared with analysis of variance using Tukey's post hoc test. The correlation between EPG and weight was evaluated by the Spearman correlation test for nonparametric distributions. All statistical analyses were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

This study is the first to determine the anthelmintic resistance of GINs and the effect of treatments on the liveweight gain of Mexican grazing calves in a tropical wet region. The overall EPG obtained for all experimental groups on the day before treatment (day -1) and 15 days after treatment are shown in Table 1 and Supplementary Table 1. Cattle treated with LV and IVM showed a reduction in EPG of 92.4% and 93.5%, respectively, on day 15 post treatment, compared to the day of treatment (day 0). Treatment with IVM and LV showed <95% effectiveness, and resistance was confirmed in cattle treated with IVM, whereas calves had GIN populations considered suspect (possibly resistant) to LV. Statistically significant differences ($P < 0.001$) were found between both the LV and IVM groups and the untreated control group on day 15 post treatment. However, in the EPG counts on day 15 post treatment, no significant differences were observed between LV and IVM ($P > 0.05$). From these findings, the anthelmintic efficacy against GIN infections may be associated with its excessive use and may have led to resistance.

Table 1. Means (± SD) of eggs per gram of feces (EPG), reduction percentages of faecal egg counts (FECRT) with 95% lower and upper confidence intervals, and status of resistance to levamisole and ivermectin of beef cattle in a tropical region of Oaxaca, Mexico

Experimental group	Pre-treatment EPG mean	SD	Post-treatment EPG mean	SD	95% CI	FECRT % Reduction	Status
Levamisole (LV)	755.0	84.0	57.4*	10.7	53.0–61.9	92.4	Suspect
Ivermectin (IVM)	435.0	65.2	28.3*	10.2	24.1–32.6	93.5	Resistant
Control	317.5	66.7	377.5	74.3	346.5–408.6	–	–

Pre-treatment, day -1; Treatment, day 0; Post-treatment, day 15; CI, confidence interval; SD, standard deviation. *Significant differences versus control group ($P < 0.001$).

The data demonstrate a similar LV efficacy (98.7%) and lower IVM efficacy (80.6%) in grazing beef cattle for the tropical climate compared to the equatorial savannah (Aw) in Mexico, and similar reported findings for LV resistance ranged from 42% to 91% in cattle farms in the Mexican humid tropics (Becerra-Nava *et al.*, 2014; Muniz-Lagunes *et al.*, 2015). Although the findings of this study require confirmation on a larger scale, they support previous data reporting anthelminthic resistance. Additionally, of the GIN populations, *Cooperia oncophora* exhibited IVM resistance, and *Haemonchus placei* and *Cooperia spp.* were

identified after treatment with LV (Muniz-Lagunes *et al*., 2015; Encalada-Mena *et al*., 2008). The easy administration, relatively low cost, effectiveness, and commercial availability of LV and IVM encourage frequent use, resulting in anthelminthic resistance.

The mean liveweight gains of grazing cattle are shown in Table 2. The initial mean liveweights recorded were 200.5kg for the LV group, 199.2kg for the IVM group and 198.7kg for the untreated control group. At the end of the experimental period (day 45), the mean liveweights were 228.7kg for the LV group (weight gain 28.2kg), 226.4kg for the IVM group (weight gain 27.2kg), and 203.6kg for the untreated control group (weight gain 11.1kg). Our tests revealed significant differences (P <0.05) in weight gain between anthelmintic drugs (LV and IVM) and the untreated control group at each post-treatment experimental time point, although no significant differences were observed between LV and IVM (P>0.05). Reports on weight gain using highly effective drugs versus control groups have shown liveweight gains of 12.9kg in a year in New Zealand grazing cattle, 14kg after 126 days of fattening calves in Argentina, and 17.06kg for a treatment period beyond one year in growing heifers in Brazil (Candy *et al*., 2018; Fazzio *et al*., 2014). However, in Nellore calves treated with low-efficacy IVM over an experimental period of 112 days, no significant differences were observed compared to the control group (Borges *et al.*, 2013). Thus, the impact of anthelmintic resistance in beef cattle on production performance may be much more severe than previously reported.

Table 2. Cumulative mean liveweight gain (kg) on each experimental day of beef calves treated with levamisole and ivermectin in a tropical region in the Mexican state of Oaxaca

Day	Treatment Levamisole (LV)	SD	Ivermectin (IVM)	SD	Control	SD
0	200.5	56.4	199.2	55.2	198.7	54.8
15	209.2[a]	55.5	207.3[a]	54.1	200.4[b]	54.6
30	218.9[a]	57.3	217.6[a]	56.8	206.2[b]	54.2
45	228.7[a]	59.1	226.4[a]	58.2	209.8[b]	53.7

Different letters in each line indicate significant differences (P < 0.05) between the experimental groups at each experimental time point by ANOVA post hoc Tukey's test; SD, standard deviation.

In this study, the statistical analysis showed a moderate negative correlation between EPG and bodyweight for the LV group (r = -0.46; P = 0.038) and a weak association for the IVM group (r = -0.32; P = 0.041). The significant negative correlation with EPG decreased as weight gain increased. A similar significant negative correlation was found in calves from three farms treated with LV and extensive breeding systems in Brazil (Ramos *et al.*, 2018). Further, a statistically significant but weak correlation (-0.22) was observed in Nellore calves treated with anthelmintics in a tropical climate (Borges *et al*., 2013).

CONCLUSIONS

This study demonstrated the lack of efficacy of LV and IVM against GINs, exhibiting an adequate capacity for EPG reduction but with IVM resistance and detrimental effects on growth performance, in grazing beef cattle in a tropical wet region of Oaxaca, Mexico. Potential indicators to treat grazing ruminants include fecal egg count or liveweight gains (Hodgkinson *et al.*, 2019). The routine use of diagnostic methods is needed to evaluate anthelmintic efficacy to develop regionally adapted control programs and minimize anthelmintic resistance.

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