Effect of levamisole on gastrointestinal nematodes and weight gain of grazing livestock in the municipality of San Jose Chiltepec, Oaxaca, Mexico

Ulises Libreros-Osorio

Universidad Realistica de Mexico

Daniela Donaji Vázquez-Arrieta

Universidad Realistica de Mexico

Ilhuicamina Daniel Limón

Benemerita Universidad Autonoma de Puebla

Aleidy Patricio-Martínez

Benemerita Universidad Autonoma de Puebla

Felipe Patricio-Martínez (✉ felipe.patmar@gmail.com)

Universidad Realística de México <https://orcid.org/0000-0003-0531-7229>

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Abstract

The aim of the present work was to study the effect of administration of levamisole on parasitic load of gastrointestinal nematodes (GIN) and the percentage of weight gain of grazing cattle. The animals were randomly distributed to form three experimental groups, levamisole, ivermectin and control. The collection of feces samples was carried out one day before and 15 days' post-treatment and subsequently it was quantifying the eggs number per gram of feces. The body weight of the animals was recorded at days 0, 15, 30 and 45 after treatments to evaluate its gain. The results observed up to the final day (45 days' post-treatment) showed the group treated with Ivermectin had a 10.1% increase in body weight, while the group under Levamisole treatment had an increase of 14.3%. The groups under ivermectin and levamisole showed a decrease in the number of eggs per grams of feces (EPG), which were measured 15 days after their treatment (22.5 EPG in ivermectin) and (55.0 EPG in levamisole) compared to the vehicle group (377.5 EPG) and pre-treatment (486.5 EPG). In the present work, it was possible to show that with two subcutaneous administrations of levamisole in grazing calves in the municipality of Chiltepec, Oaxaca, it's possible to increase the weight in cattle while decreasing its parasite load of GIN. However, it is necessary to determine the anthelmintic resistance in farms that report the use of levamisole with different treatment and repeated doses of levamisole.

Introduction

Gastrointestinal nematode (GIN) infections gravely affect animal health (Waller 2003), and productivity (Charlier et al. 2009), farm profitability (Morgan et al. 2013) and overall welfare in growing cattle all over the world (Vande Velde et al. 2018). In Mexico, particularly, it has been demonstrated that parasitic diseases in livestock can cause serious economic loss. In fact, the yearly economic loss due to the six major parasites of cattle in Mexico was estimated to be US\$ 1.41 billion (Rodríguez-Vivas et al. 2017).

The main effect of GIN infections in grazing beef cattle production is a reduction in weight gain (Mertz et al. 2005) so that, pharmacological control of GIN in grazing cattle could be necessary (Vande Velde et al. 2018). Up until now, GIN infection control in livestock cattle has been performed by administering drugs such as macrocyclic lactones, benzimidazoles and imidazothiazoles (McArthur and Reinemeyer 2014; Gasbarre 2014). However, the increase of resistant nematodes to anthelmintic macrocyclic lactones in the warm subhumid tropics of Mexico has presented a challenge (Canul-Ku et al. 2012).

The levamisole an imidazothiazole derivative drug have an anthelmintic efficacy and low resistance to GIN (Williams and Broussard 1995; Suarez and Cristel 2007; Cristel et al. 2017; Ali et al. 2018; Pivoto et al. 2020). Similarly, levamisole (alone or combined) has been shown to increase weight gain and economic profit (Williams 1991; Candy et al. 2018; Pivoto et al. 2020). Therefore, the aim of this work was evaluate the anthelmintic effect with a single treatment of levamisole as well as its impact on weight gain in grazing cattle within the municipality of Chiltepec, Oaxaca, Mexico.

Material And Methods

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Localization

This study was carried out in the town of San Jose Chiltepec, Oaxaca, Mexico. This municipality is located at 30 meters above sea level and has a warm-humid climate with rains all year round (50.8%), as well as warm-humid with abundant rains in summer (49.5%). The range of rainfall is 2 000–4 000 mm.

Animals and experimental design

Sixty calves (male and female) of the Beefmaster breed within the age range of ten to twelve months were used for this study. The animals were extensively grazed with palisade grass (*Brachiaria brizantha*) and stargrass (*Cynodon nlemfuensis*). No animal had any anthelmintic treatment before the collection of samples for the coprology test, even with counts of 450 eggs per gram of feces (epg) were obtained.

From November 26, 2019 to January 10, 2020, the experiment was carried out with the 60 calves, from which fecal samples were collected to measure the parasite load. This process was done a day before and 15 days after the administration of drugs. In the temporal line (Fig. 1), it was assigned day zero due to the formation of three experimental groups for the administration of Levamisole ($n = 20$), ivermectin ($n = 20$) or vehicle ($n = 20$). The drugs were administered on day zero and day 15 for the second doses of Levamisole 5 mg/kg (i.m.), and Ivermectin 0.2 mg/kg (s.c.). Ivermectin was used as a positive control and thus we have a comparative group. Individual coprology samples were carried out on days - 1 and 15, and the body weight record was made on days 0, 15, 30 and 45.

Parasitological analysis

Fecal samples were collected and transported in a cooler at approximately 4°C to be examined by the modified McMaster technique (Roberts and O'Sullivan 1949), where 1 egg represents 10 epg. Fecal egg count reduction (FECR) was calculated as follows:

$$FECR = \frac{[(AvgFECPre - treatment) - (AvgFECPost - treatment)]}{(AvgFECPre - treatment)} * 100$$

where Avg is average.

The standard of < 90% reduction (McKenna 1994; Leveck et al. 2012) post-treatment was used to define the effectiveness of a drug as anthelmintic. Since the objective of the study was to measure the effect of Levamisole on body weight gain, resistance to anthelmintic was not defined.

Statistical Analyses

All the results were expressed as mean $\pm$ standard error of the mean (SEM). Body weight control expressed in the table were subject to one-way ANOVA test, followed by a Tuckey post hoc analysis. On the other hand, the percentage body weight gains to the 45 days' post-treatment and the number of eggs

per gram of feces 15 days' post-treatment were done by the Kruskal-Wallis test, followed by a Dunns post hoc analysis. The criterion for significance was $p < 0.05$.

Results

Body weight was recorded (Table 1) and subsequently studied the percentage of body weight gain in all experimental groups (Fig. 2) on days 0, 15, 30 and 45. The ivermectin and levamisole groups showed increases in the body weight with significant differences ($p < 0.01$ and $p < 0.001$) at 30 days' post-treatment (246.4 ± 2.3 and 248.9 ± 3.5 , respectively) and 45 days' post-treatment (253.5 ± 2.3 and 260.1 ± 3.5 , respectively) compared with day 0 (230.2 ± 2.3 and 227.7 ± 3.6 , respectively). The vehicle group only had a significant difference at 45 days' post-treatment compared to the pre-treatment (Table 1). The comparison in the body weight gain of the calves treated with ivermectin, increased the registered weight against that of the vehicle group showed a statistically significant difference at days 15, 30 and 45 (7.3 ± 0.94 , 8.9 ± 0.22 and 8.9 ± 0.22 , respectively). On the other hand, the weight increase within the levamisole group against that of the vehicle group and the ivermectin group showed a statistical difference, at 15, 30 and 45 days (9.9 ± 0.49 , 11.2 ± 0.20 and 11.2 ± 0.6 , respectively). Likewise, the percentage of weight gain observed up to day 45 showed an increase of 10.1% in the ivermectin group compared to the vehicle group ($p < 0.001$), which had an increase of 7.0%. However, the levamisole group showed an increase of 14.3% compared to both the ivermectin ($p < 0.001$) and vehicle groups ($p < 0.01$) (Fig. 2).

Table 1

Body weight control in the experimental groups. The increase in weight in cattle is observed during pre-treatment and post-treatment. Values expressed in kilograms (kg)

Experimental Group	Pre-treatment	Post-treatment		
	0 D	15 D	30 D	45 D
Vehicle	245.1 ± 1.4	250.4 ± 1.4	256.5 ± 1.4	$262.3 \pm 1.5^{***}$
Ivermectin	230.2 ± 2.3	237.5 ± 2.4	$246.4 \pm 2.3^{**}$	$253.5 \pm 2.3^{***, ++}$
Levamisole	227.7 ± 3.6	237.6 ± 3.5	$248.9 \pm 3.5^{***}$	$260.1 \pm 3.5^{***, ++}$
The values show mean $\pm$ SEM, with a one-way ANOVA test, followed a Tuckey post hoc analysis: $**p < 0.01$, $***p < 0.001$ vs pre-treatment; $++p < 0.01$ vs 30 D., ($n = 20$ bovines per group) D = days				

To determine the decrease in the parasite load, the number of eggs per gram of feces (EPG) was measured 15 days after treatment. The ivermectin and levamisole groups showed a decrease in EPG (22.5 EPG and 55.0 EPG, respectively) compared to the vehicle group (377.5 EPG) $p < 0.001$, and all animals before treatment (without an assigned group) (486.5 EPG) with statistical difference ($p < 0.001$) (Fig. 3). On the other hand, the results of fecal egg count reduction (FECR) showed a value $> 90\%$ in the ivermectin group (95.4%). However, the vehicle and levamisole group had a value $< 90\%$ (24.3% and 84.1%, respectively) (Table 2).

Table 2
Fecal egg count reduction (FECR) after of treatment with Ivermectin or levamisole in bovines.

Experimental Group	Day	Mean $\pm$ SEM	Reduction % vs day 0
Vehicle	0	499.2 $\pm$ 93.0	24.3
	15	377.5 $\pm$ 51.5	
Ivermectine	0	499.2 $\pm$ 93.0	95.4
	15	22.5 $\pm$ 7.6	
Levamisole	0	499.2 $\pm$ 93.0	84.1
	15	55.0 $\pm$ 30.9	

Discussion

In the present work we study the effect of levamisole as an anthelmintic and its effect on the weight gain of grazing cattle in the municipality of Chiltepec, Oaxaca, Mexico. The efficacy of levamisole on bovine parasitosis has been demonstrated for several decades (Ciordia and McCampbell 1971; Pouplard et al. 1986; Vanparijs and Quick 1991), particularly against gastrointestinal nematodes (GIN) (Williams 1991; Anziani et al. 2004; de Soutello et al. 2010; Idika et al. 2012). However, in this study we show that levamisole maintains the anthelmintic effect and increases weight gain in grazing cattle with two administrations in a period of 15 days, with an effect that last until day 45 (Fig. 2; Table 2) in the Chiltepec region, Oaxaca. To determine that levamisole has such efficacy, we used ivermectin as a control that has also shown to have anthelmintic resistance in cattle from Mexico (Muñiz-Lagunes et al. 2015; Alonso-Díaz et al. 2015; Alegría-López et al. 2015). We observed that levamisole is more effective than ivermectin in increasing both the gain and the percentage of weight of the animals. It is likely that parasitic control with levamisole from an early age in calves can interfere both in animal welfare, as well as in the stimulation of innate immunity (Pekmezci and Cakiroglu 2009). Reports have shown that levamisole can enhance the immunology responses and therefore improve the performance of production animals (Mohri et al. 2005). However, it has not been shown whether levamisole treatment can have a direct correlation between weight gain and its immunomodulatory effects in both rearing and adulthood in beef producing cattle.

On the other hand, a great impact of resistance to anthelmintic has been manifested in cattle both in our country and around the world (Canul-Ku et al. 2012; Muñiz-Lagunes et al. 2015; Pivoto et al. 2020). In fact, the main factors for the development of resistance to anthelmintic drugs is underdosing, frequent treatments and low sheltering. That is why it is necessary to regulate the use of anthelmintic only with prescription in cattle, as is the case of Denmark, where since 2000 it is required to register all production animal prescriptions at the “VetSat”, the danish system monitoring the use of veterinary drugs (Stege et al. 2003). The standard for the determination of anthelmintic resistance in cattle is carried out by the

Fecal Egg Count Reduction Test (FECR) (Levecke et al. 2012). Resistance is inferred when there is a < 95% reduction in FECR, 7–10 days after treatment (Waghorn et al. 2006; Levecke et al. 2012). In the present work, it was inferred 15 days after treatment, and a decrease of 95.4% and 84.1% are shown in FECR for ivermectin and levamisole, respectively. Although our objective did not contemplate evaluating anthelmintic resistance, it highlights the need to follow up the use of levamisole for a longer time and with the study of fecal samples in a longer time.

In conclusion, the present work demonstrated that two subcutaneous administrations of levamisole in grazing calves in the municipality of Chiltepec, Oaxaca, are able to increase the body weight gain of animals, as well as decrease their parasite load of GIN. However, it is necessary to determine the anthelmintic resistance in farms that report the use of levamisole with different treatment and repeated doses of levamisole.

Declarations

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Authors' contributions

The conceived, designed the experiment and data collection was performed by Libreros-Osorio U., and Patricio F. Data analyses were performed by Vázquez-Arrieta DD. The manuscript preparation was performed by Limon ID., Patricio-Martínez A., and Patricio F. All of the authors read and approved the final manuscript.

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Ethics approval

The experimental procedures with grazing livestock followed all applicable international and national guidelines for the care and use of animals.

Consent to participate

Not applicable.

Consent for publication

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Not applicable.

Availability of data and material

The data that support the findings of the present study are available with the corresponding author upon reasonable request.

Code availability

Not applicable.

Conflict of interest

The authors declare no conflict of interest with any aspect concerning the writing and publication of this article.

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Figures

EXPERIMENTAL DESIGN

Figure 1

Experimental design. Three groups were obtained: Vehicle, Ivermectin and Levamisole. The administration of anthelmintic drugs was on day zero and day 15. The sampling feces was performed one day before deworming and on day 30 post-deworming. On the other hand, the weight of the animals was taken every 15 days for the determination of the weight gain of the animals.

Figure 2

The two doses administration of levamisole increase the percentage body weight gain to the 45 days' post-treatment. It is show the effective result of levamisole regarding to ivermectin. The values show mean $\pm$ SEM, with a Kruskal-Wallis test, followed a Dunns post hoc analysis: *** $p < 0.001$ vs vehicle group; ++ $p < 0.01$ vs ivermectin group. (n= 20 bovines per group).

Figure 3

The levamisole and ivermectin administration decreases the eggs per gram of feces 15 days' post-treatment. It is show reduction of the EPG regarding to Pre-treatment (all groups) and vehicle group. The values show mean $\pm$ SEM, with a Kruskal-Wallis test, followed a Dunns post hoc analysis: ***p<0.001 vs vehicle group; ++p<0.01 vs ivermectin group. (n= 20 bovines per group).