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Control of *Rhipicephalus microplus* on taurine cattle with fluralaner in a subtropical region

Tiago Gallina[1], Camila dos Santos Lagranha[1], Giancarlo Bilo[1], Cristiano Malavolta[1], Lorena Lopes Ferreira[2], Fernando de Almeida Borges[3], Daniel de Castro Rodrigues[4,5], Tom Strydom[5], Siddartha Torres[6], Emmanuel Arnhold[7] and Welber Daniel Zanetti Lopes[7*]

Abstract

Background In Rio Grande do Sul, Brazil, a region with a subtropical climate, *Rhipicephalus microplus* is present in taurine cattle raised for beef and milk. In addition, ticks resistant to multiple acaricides are present in this region. Recently, fluralaner (isoxazoline) was launched on the market. Thus, there is a need to evaluate the effects of fluralaner for the control of *R. microplus* on taurine cattle. In addition, occurrence of myiasis by *Cochliomyia hominivorax* larvae after tick parasitism and weight gain of cattle during the experimental period were evaluated.

Methods Thirty naturally infested cattle were divided into two experimental groups: T01, treated with fluralaner (2.5 mg/kg) pour-on; T02, control. T01 received fluralaner on Days 0 (early summer in January), 42 and 84 (early autumn), whereas T02, a control group, received palliative treatment with a spray formulation when the group mean was ≥ 30 ticks. Counts of *R. microplus* females and calculation of the efficacy of fluralaner were performed on Days 3, 7, 14, 28, 35, 42, 56, 70, 84, 98, 112 and 126. The occurrence of myiasis was assessed throughout the study period. In addition, the weight, weight gain and daily weight gain of the animals were evaluated.

Results In the 12 evaluations performed, the parasitic load of T01 was near zero. Fluralaner showed 99.5% efficacy on the 3rd day after the first treatment and 100% efficacy from Day 7 to Day 126. *Cochliomyia hominivorax* larvae ($n=6$; $p=0.0251$) were found only in the control group (T02). At the end of the study, the animals subjected to treatments with fluralaner gained 32.8 kg more than the animals in the control group.

Conclusions Application of fluralaner in summer and autumn, with 42-day intervals between treatments, was effective to control *R. microplus* on taurine cattle, which also gained more weight than control cattle. Additionally, no cases of myasis were documented in animals treated with fluralaner.

Keywords *Bos taurus*, Cattle tick, Isoxazoline, Multiresistant strain, Screwworm

*Correspondence:
Welber Daniel Zanetti Lopes
wdzlopes@hotmail.com
Full list of author information is available at the end of the article

Background

The tick *Rhipicephalus microplus* causes severe damage to cattle, especially taurine breeds, in tropical and subtropical areas of the world [1]. High parasitic loads increase the risk of transmission of hemoparasites, leather lesions, myiasis and decreased yield due to spoliation [2, 3]. Depending on the temperature and humidity conditions of the region, this tick can complete three to five generations per year [4–7].

In Rio Grande do Sul, southern Brazil, the first generation begins in late winter/early spring with low infestation levels, followed by a second generation in the summer with more ticks and another generation in the autumn (i.e. the third generation) with the highest infestation level [8, 9]. Further studies are needed in this region of Brazil, but some population dynamics studies have demonstrated four peaks of infestation in cattle by this ectoparasite throughout the year [9, 10]. In addition, in this region, both beef and dairy cattle are predominantly taurine [11], which further aggravates the level of cattle infestation by *R. microplus* during the months of the year when the climatic conditions for the development of this ectoparasite are favorable.

The use of acaricides has been the only way to control this tick. This has become another obstacle in the attempt to control this ectoparasite in several countries, including in this region, due to the resistance of *R. microplus* populations to acaricides. The number of chemical treatments required per year to control this tick in southern Brazil is sometimes higher than in other regions [11–14]. This fact has triggered reports of ticks resistant to multiple acaricides, especially in southern Brazil, where resistance to pyrethroids, organophosphates, amidines, phenylpyrazoles, macrocyclic lactones and benzophenylureas has been reported [15–23]. Therefore, before establishing control, it is necessary to consider the epidemiological aspects of this tick in each region and the history of local acaricide resistance [5, 13, 24, 25].

A recent compound belonging to the isoxazoline class, fluralaner, was developed for the control of ectoparasites of dogs, cats and birds as an alternative to other chemical classes on the market, with results showing high efficacy against parasites of these animals in in vivo tests [26–29]. Considering the difficulty of cattle tick control in Rio Grande do Sul, this study aimed to evaluate the control of *R. microplus* in taurine cattle in a region with a subtropical climate using fluralaner to examine the occurrence of myiasis by *Cochliomyia hominivorax* larvae after tick infestation and to evaluate the weight, weight gain and daily weight gain of cattle during the study period.

Methods

Study site, animals and experimental groups

The study was conducted from January to May 2021 (summer to autumn, 2nd and 3rd tick generations, respectively, according to Evans [8]) on a commercial farm located in the municipality of Uruguaiana (29°50′09″S, 57°06′35″W), Rio Grande do Sul, southern Brazil. According to the temperature and rainfall criteria for the Köppen climate classification, the city of Uruguaiana can be classified as "Cfa": (C) humid subtropical zone; (f) oceanic climate, without a dry season and (a) with a hot summer [30].

Thirty male Aberdeen angus cattle that were non-castrated, naturally infested by *R. microplus* and approximately 20 months old, with an average body weight of 315 kg ± 75, were used. These cattle had not received an antiparasitic drug for 70 days prior to the start of the study and were in good health at the beginning of the study. A 20-hectare paddock, also naturally infested by *R. microplus*, was divided into two equal parts with equivalent shady areas, and the cattle were kept separately in the paddocks throughout the experimental period (126 days). During the study, the animals received native pasture as forage and ad libitum mineral supplementation.

The cattle were allocated to the experimental groups on Day 0 and were randomly assigned to the treatments according to a block design. The formation of blocks was performed based on the arithmetic mean of the number of female ticks (measuring 4.5–8.0 mm in length) counted on 3 consecutive days (days -3, -2 and -1), as recommended by Wharton and Utech [31]. The animals were distributed into 15 blocks containing two cattle each and then randomly allocated into one of the groups (T01 and T02) within each block.

Treatments

Following the premise that tick control should be performed when few ticks are seen on the animals [32, 33], at the beginning of the present study, the animals had an average parasite load of 4.3 ticks/animal. Cattle in group T01 were subjected to three treatments with fluralaner (2.5 mg/kg) pour-on (Exzolt® 5%, MSD Animal Health) on Days 0, 42 and 84 (early summer to early autumn), based on previous results [34]. The interval of 42 days between treatments was determined according to the efficacy results obtained in the registration studies. For animal welfare reasons, animals belonging to T01 would receive palliative treatment if the mean tick counts at any time during the study were ≥ 30 [12]; the palliative treatment consisted of a spray formulation of 125 ppm alphacypermethrin + 400 ppm ethion + 212 ppm chlorpyrifos (Potenty®, MSD, Animal Health). Cattle belonging

to T02 were kept as controls. Also for animal welfare reasons, the cattle belonging to this group received this same spray formulation with a combination of pyrethroid and organophosphates whenever the mean tick count of the group was ≥ 30 [12]. All treatments were performed according to the manufacturer's recommendations.

Tick counts and efficacy

After dividing the groups, counts of *R. microplus* females measuring between 4.5 and 8.0 mm in length on the left side of each animal and without multiplying by two were performed by the same person and at the same time, according to the methodology proposed by Wharton and Utech [31] on Days 3, 7, 14, 28, 35, 42, 56, 70, 84, 98, 112 and 126.

The acaricidal efficacy was calculated by arithmetic means using the formula recommended by Roulston et al. [35] and adopted by the Brazilian Ministry of Agriculture and Livestock (Ministry of Agriculture, Livestock and Food Supply—MAPA) [36] and Holdsworth et al. [37]:

$$\text{Efficacy percentage} = \left[1 - \frac{\text{Ta} \times \text{Cb}}{\text{Tb} \times \text{Ca}}\right] \times 100$$

where "**Ta**" is the mean number of female ticks (4.5–8 mm) that were counted on treated animals after the treatment; "**Tb**" is the mean number of female ticks (4.5–8 mm) that were counted on treated animals during the 3 days preceding the treatment date; "**Ca**" is the mean number of female ticks (4.5–8 mm) that were counted on untreated control animals after the treatment date; "**Cb**" is the mean number of female ticks (4.5–8 mm) that were counted on untreated control animals during the 3 days preceding the treatment date.

Detection of myiasis

The occurrence of myiasis caused by *C. hominivorax* larvae was observed in the body of the animals, after infestation by *R. microplus*. The *C. hominivorax* larvae were classified as active (at least one live *C. hominivorax* larva/lesion), which was identified by the number one (1), or nonactive (zero *C. hominivorax* larvae/lesion), which was identified by the number zero (0), as described by Lopes et al. [38].

Animals affected by screwworms received treatment with an oil solution containing 30% dichlorfenthion (Mata Bicheira Coopers®, MSD Animal Health, Brazil) at the wound site. In addition, the larvae were removed from the wound site to confirm the identification of *C. hominivorax* according to the taxonomic criteria described by Spradbery [39].

Weighing of animals

The animals were weighed individually without previous fasting on D0, D70 and D126. In addition, the animals were also weighed on the dates corresponding to the treatments with fluralaner to adjust the dose of the product used. Weight gain and daily weight gain were calculated for each animal considering differences in body weight during the study (D0, D70 and D126). The scales used for weighing the animals had been tested using a previously known weight.

Statistical analysis

Data obtained from the counts of *R. microplus* females were log transformed using the equation ln (x + 1) and analyzed in an entirely randomized design within each counting date, as the sphericity and orthogonality tests of the data did not allow a split-plot in-time analysis. The data complied with the assumptions of normality and homogeneity of variances and residuals. Treatment means for tick counts were compared using the F test.

The presence of viable *C. hominivorax* larvae in wounds was analyzed using Fisher's exact test, and differences were considered significant when $P \leq 0.05$. Regarding body weight, body weight gain and daily body weight gain, the values were compared between the treatments for all evaluation data using the F test. All data were analyzed with a significance level of 5% ($P \leq 0.05$) using SAS software [40].

Results

Tick counts and efficacy

From Day 3 to Day 126 of the study, in the 12 tick counts, the treated group had a lower parasite load (ANOVA, $F_{(1,\ 28)} = 2905.57$, $P < 0.001$) than the control group. The efficacy of fluralaner was 99.5% on the 3rd day after the beginning of the study and remained at 100% from the 7th to 126th day after the beginning of the study (Table 1), with no need for palliative treatment in animals belonging to this group. On the other hand, it was necessary to perform palliative treatment with the spray solution for the animals in T02 on the 28th, 56th and 98th days of the study, since the cattle in this group had a high parasite load according to the criteria adopted.

Myiasis

Active myiasis was found in 6 of 15 animals of the control group (Fisher's exact test, $P = 0.01686$), which may be attributed to the high parasitic load of *R. microplus* detected in cattle of this group on D98. One animal showed lesions with *C. hominivorax* larvae on D98, and myiasis was observed in the other five animals on D112 of the study (Fig. 1). *Cochliomyia hominivorax* larvae

Table 1 Counts of *Rhipicephalus microplus* females (4.5 to 8 mm in length) present in naturally infested cattle belonging to groups submitted to different control schemes and percentage of effectiveness

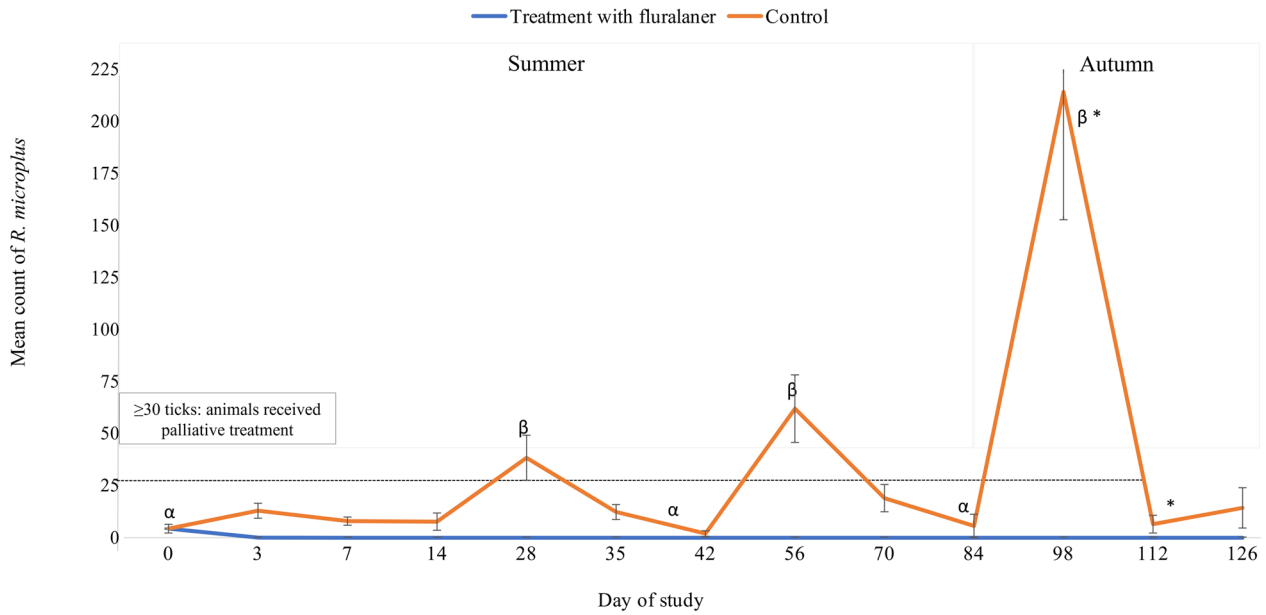
Days of the study	Experimental groups/Mean [1] number of *R. microplus* females (4.5 to 8 mm)				*P* value	Efficacy (%)
	T01: Treatments with fluralaner		T02: Control (palliative treatment)			T01
0 [*, α]	4.38	A	4.38	A	0.9861	–
3	0.07	B	13.00	A	< 0.001	99.5
7	0.00	B	8.00	A	< 0.001	100
14	0.00	B	7.73	A	< 0.001	100
28	0.00	B	38.40	A	< 0.001	100
35	0.00	B	12.40	A	< 0.001	100
42 [α]	0.00	B	2.00	A	< 0.001	100
56 [β]	0.00	B	62.00	A	< 0.001	100
70	0.00	B	19.00	A	< 0.001	100
84 [α]	0.00	B	5.67	A	< 0.001	100
98 [β]	0.00	B	214.20	A	< 0.001	100
112	0.00	B	6.53	A	< 0.001	100
126	0.00	A	14.33	A	< 0.001	100

1: Means followed by the same capital letter in the line do not differ from each other ($p > 0.05$)

[*] Mean counts between − 3, − 2 and − 1 days

[α] Treated group received fluralaner 2.5 mg/kg

[β] Control group received palliative treatment with a spray formulation of 125 ppm alphacypermethrin + 400 ppm ethion + 212 ppm chlorpyrifos



α indicates when treatment was performed in T01 with fluralaner (2.5mg/kg)
β indicates when palliative treatment (spray 125 ppm alphacypermethrin + 400 ppm ethion + 212 ppm chlorpyrifos) in T02
* Indicates when Control animals had *C. hominivorax* larvae parasitism

Fig. 1 Mean count of *Rhipicephalus microplus* females in naturally infested cattle submitted to different control schemes during 126 study days

were found in the chest region of infested cattle, where > 30 adult ticks were found. On the same day that the parasites were identified, the larvae were manually removed, and the wounds were treated with 30% dichlorfenthion. No worms were found in animals subjected to control with fluralaner (T01) throughout the study.

Animal weighing

The results of the average weight, average weight gain and average daily weight gain are described in Table 2. On Days 0, 70 and 126 of the study, the weights of animals in T01 and T02 were homogeneous. There was no difference (ANOVA $F_{(1,28)}=3.48$, $P=0.0723$) in the mean weight gain from Day 0 to Day 70 of the study between Groups T01 and T02. Between D70 and D126, both groups lost weight. The group treated with fluralaner lost less weight (ANOVA $F_{(1,28)}=18.99$, $P<0.001$) than the control group, at -17.5 kg and -39.1 kg, respectively. Over 126 days of study, the animals subjected to control with fluralaner (T01) and the control group (T02) showed average weight gains of 45.1 and 12.3 kg (ANOVA $F_{(1,28)}=14.23$, $P<0.001$), respectively. The daily weight gain did not differ from D0 to D70 (ANOVA $F_{(1,28)}=3.48$, $P=0.0723$) between the fluralaner-treated and control groups. There was daily weight loss between D70 and D126, with a significant difference (ANOVA $F_{(1,28)}=18.99$, $P<0.001$) between groups. From D0 to D126, the daily weight gain of the group treated with fluralaner was higher (ANOVA $F_{(1,28)}=14.23$, $P<0.001$) than that of the control group, at 0.36 kg and 0.10 kg, respectively.

Discussion

This is the first study conducted with fluralaner in a region of Brazil where there are reports of *R. microplus* resistant to six chemical classes. Fluralaner was highly efficacious against this tick in the evaluated period, with nearly 100% efficacy in all evaluations performed. In addition, animals treated with fluralaner did not exhibit *C. hominivorax* myiasis during the study period and gained more weight than those in the control group.

Although three annual tick generations have been reported in the region where the study was carried out, the challenge created by this ectoparasite in animals may be greater in this location because of the favorable climatic conditions for the development of cattle ticks, especially from the summer (January) through the autumn (April and May) [8, 9]. The trend indicates an increasing risk of cattle infestation by this tick, with the highest load recorded in the autumn, which may have clinical consequences, including mortality due to tick-borne pathogens [41]. For example, in regions where *R. microplus* completes four to five annual generations, four to seven treatments are performed per year [7, 12–14]. In the southern region of Brazil, where the present study was conducted, there are reports of 10 to 12 treatments per year to control *R. microplus* [10]. The reason for this high number of treatments in this region of Brazil may be related to the predominance of taurine cattle, which are highly sensitive to parasitism by *R. microplus* [11], but also to the adoption of an incorrect strategy by farmers.

The data obtained from the cattle in the control group reinforce previous conclusions about the challenge of controlling *R. microplus* ticks on cattle. The animals in the control group were affected by screwworm, especially at the time of year when the tick population increases. On the other hand, no cases of screwworm were observed among cattle treated with fluralaner, even during autumn (May), the period with the highest risk from *R. microplus* in cattle in this region [9, 10]. Similar data were obtained by Reck et al. [3], who found a risk ratio approximately four times greater for an animal to acquire myiasis when highly parasitized by *R. microplus*.

Another parameter evaluated that highlights the importance of the benefits of control with fluralaner was weight gain throughout the study. Notably, there was a marked weight loss between D70 and D126. A possible reason for this finding was that in the second half of the study period, the region was affected by a drought, with days with high temperatures close

Table 2 Statistical analysis regarding weight, weight gain and daily weight gain of cattle submitted to different control schemes against *Rhipicephalus microplus* during the experimental period of 126 days

Days of study	Variable analyzed	Experimental groups/average weight		*p*-value
		T01: treatments with fluralaner	T02: control (palliative Treatment)	
0	Weight (kg)	307.3	325.9	0.3860
70		369.9	377.3	0.8937
126		352.4	338.2	0.6839
0 to 70	Mean weight gain (kg)	62.5	51.4	0.0723
70 to 126		−17.5	−39.1	< 0.001
0 to 126		45.1	12.3	< 0.001
0 to 70	Daily weight gain (kg)	0.89	0.73	0.0723
70 to 126		−0.25	−0.55	< 0.001
0 to 126		0.36	0.10	< 0.001

to 40 °C. This fact could explain this weight loss, since the absence of rain and increase in temperature led to lower forage availability for the herd and fewer hours of grazing, because the animals sometimes sought out shady areas. However, analyzing the whole period, it was possible to verify that the animals treated with fluralaner gained 32.8 kg more than the cattle in the control group. It is well known that tick infestation has a negative effect on the weight gain of parasitized cattle. Studies have shown that each engorged *R. microplus* female is responsible for the loss of 1.37 ± 0.25 g bodyweight in taurine cattle [42]. The presence of gastrointestinal nematodes of cattle that also trigger a decrease in weight gain of animals should be highlighted, according to Zapa et al. [43]. In the present study, the presence of these endoparasites in cattle was not evaluated, and for this reason, the difference in weight gain of the animals should be interpreted with caution and not attributed only to cattle ticks.

Tick control protocols in which ticks are exposed to different chemical classes in each generation, called rotation grazing, is recommended in regions where *R. microplus* has few annual generations, such as where this study was performed. In Rio Grande do Sul, Uruguay and Argentina, a country that borders this region of Brazil, this treatment protocol has already been used and successfully described as a control method [10, 24, 44, 45]. Although the rotational grazing strategy is well studied and established in regions with subtropical climates, this study aimed to evaluate the effect of treatments exclusively with fluralaner to understand how this new molecule behaves in a region with high challenge by *R. microplus* throughout the year. This study does not intend to recommend sequential treatment with fluralaner throughout the year, which would have potential implications in terms of emergence of acaricidal resistance. Nevertheless, our data indicate that fluralaner may be another alternative to include in rotation with other acaricide groups to control *R. microplus* on cattle.

In summary, this study demonstrates important results that should be disseminated among veterinarians and technicians in subtropical climate regions. The regimen of three treatments performed with fluralaner pour-on starting during summer was efficacious to control tick infestation on taurine cattle until the autumn, the time of year in which the levels of *R. microplus* tend to peak in this region. In addition, treatment with the new molecule resulted in the absence of larval infestation due to tick control and greater average weight gain by cattle at the end of the experiment.

Conclusions

The use of fluralaner pour-on at 42-day intervals was efficacious to control of *R. microplus* on taurine cattle in southern Brazil. In addition, fluralaner prevented the occurrence of screwworms on treated cattle, which also gained more weight compared with untreated cattle.

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Author contributions
TG: project administration, investigation, data curation, writing—original draft. CSL: investigation; CM: investigation; LLF: writing—original draft, review & editing; FAB: writing—original draft, review and editing; TS: supervision, conceptualization; ST: supervision, conceptualization; DCR: supervision, conceptualization; EA: formal analysis; WDZL: formal analysis, writing—original draft, review & editing.

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Availability of data and materials
The data supporting the findings of this study are included in the manuscript and associated files. Raw data are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
The project was approved by the Ethics Committee on the Use of Animals (ECUA) of the Federal University of Pampa (UNIPAMPA) (Protocol 021/2020) in accordance with the ethical principles of animal experimentation, execution and use in experiments.

Consent for publication
The authors obtained consent from the responsible authorities at the institution where the work was carried out before the work was submitted.

Competing interests
DCR and TS are employees of MSD Animal Health, which is the manufacturer of Exzolt® 5% (investigational veterinary product). ST is employee of Merck Animal Health.

Author details
[1]Federal University of Pampa, Uruguaiana, Rio Grande Do Sul, Brazil. [2]Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. [3]Federal University of Mato Grosso Do Sul, Campo Grande, Mato Grosso Do Sul, Brazil. [4]MSD Animal Health, Montes Claros, Brazil. [5]MSD Animal Health, Isando, South Africa. [6]Merck Animal Health, Madison, USA. [7]Federal University of Goiás, Goiânia, Goiás, Brazil.

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