RESEARCH



What is the optimal timing to initiate strategic control of *Rhipicephalus microplus* in taurine cattle in a tropical region?

Luccas Lourenzzo Lima Lins Leal¹ · Lorena Lopes Ferreira² · Igor Maciel Lopes de Morais¹ · Vanessa Ferreira Salvador¹ · Artur Siqueira Nunes Trindade¹ · Octaviano Pereira Neto³ · Vando Edésio Soares⁴ · Fernando de Almeida Borges⁵ · Caio Marcio de Oliveira Monteiro^{1,6} · Welber Daniel Zanetti Lopes^{1,6}

Received: 22 October 2023 / Accepted: 26 December 2023 / Published online: 8 February 2024 © The Author(s), under exclusive licence to Springer Nature Switzerland AG 2024

Abstract

Cattle tick control poses a significant challenge for livestock in tropical and subtropical regions. The objective of this study was to determine the most suitable timing to initiate a strategic tick control program and to identify the ideal number of acaricide treatments for adult taurine cattle (Bos taurus taurus) in a tropical region throughout the year. Three groups with 10 bovines each were performed: T01 (strategic treatment in late autumn/ winter/late spring, every 28 days), T02 (strategic treatment to act in the "first tick generation" – early spring/summer/early autumn, every 28 days) and T03 (control). Tick counts (females 4–8 mm) were conducted every 14 days. If the tick burden in any group reached 30 or more during these counts, we applied an additional treatment. Over the course of a year, T02 required significantly fewer (p < 0.05) acaricide treatments than T01, with nine treatments for T02 and eleven for T01. Furthermore, during the tick counts, animals in T02 showed a lower tick burden compared to those in T01. Initiating the strategic tick control program in early spring, corresponding to the first tick generation, proved more effective than starting in autumn. This approach not only required fewer acaricide treatments but also resulted in a reduced tick burden. These benefits are particularly valuable in terms of animal welfare and managing acaricide resistance issues.

Keywords Autumn · Bos taurus taurus · Cattle tick · Early-spring · First tick generation

Introduction

It is well known that the cattle tick, *Rhipicephalus microplus*, is widely recognized as a global challenge in livestock farming. It leads to reduced productivity in both beef and dairy cattle, resulting in significant economic losses for farmers (Jonsson 2006; Grisi et al. 2014; Melo-Junior et al., 2022). Literature has shown that this tick species can complete three to five generations in a year, depending on climatic conditions, making its control complex

Extended author information available on the last page of the article

(Gomes et al. 2016; Canevari et al. 2017; Nava et al. 2019; Cruz et al. 2020; Nicaretta et al. 2021a). To effectively manage tick infestations while preserving the longevity of acaricides and delaying the development of resistance, a strategic control protocol based on the cattle tick's life cycle, epidemiology, parasitic load, and animal breed is recommended (Morel et al. 2017; Zapa et al. 2020; Nava et al. 2021; Nicaretta et al. 2021b, 2023; Felippelli et al. 2022; Gomes et al. 2022).

In subtropical areas of Argentina, Australia and some regions of southern Brazil, authors suggest acaricide treatment during seasons when the tick population is less numerous and most vulnerable, typically in winter (Norris 1957; Evans 1992; Nava et al. 2014, 2015, 2019, 2021).

In tropical areas of Brazil (including some subtropical areas in Southern Brazil) and Australia, some authors suggest strategic tick control should focus on the first tick generation, often referred to as the "spring rise" (Evans 1992; Furlong et al. 2007; Pereira et al. 2008). In areas of these regions with distinct rainy and dry seasons, such as parts of Brazil, it's commonly recommended to begin strategic tick control at the start of the rainy season, typically in late spring or early summer when the tick burden on the host is lower (Magalhães 1989; Furlong et al. 2003, 2007; Pereira et al. 2008). On the other hand, authors like Bonatte-Junior et al. (2019) recommend concentrating *R. microplus* control efforts during periods of high tick burden on the host, often in the autumn.

Although there are differences among some researchers as to when to perform strategic control, there are no studies performed in a tropical region using taurine animals, that that compare different periods of the year and the required number of acaricide treatments to effectively manage cattle tick infestations throughout the year. Given this context, the objective of this study was to quantify these aspects to determine the most suitable time to initiate strategic control of *R. microplus* in *Bos taurus taurus* cattle in a tropical region where up to five generations of this tick can occur in a year.

Materials and methods

Location, animals, group formation, and management

The experiment was conducted between June 2021 and June 2022 on a farm located in São José do Rio Pardo, São Paulo, in the southeast region of Brazil. The area has a tropical climate with around 1430 mm of annual rainfall, mainly falling between October and March (spring-summer).

The study involved 40 purebred Simmental cows of the *Bos taurus taurus* breed, aged between 3 and 7 years, with an average weight of 580 kg, and they were primarily used for beef production. These cows were naturally infested with *R. microplus* ticks and were grazing on *Coast cross* grasses. They were divided into three groups, namely T01, T02, and T03, each consisting of 10 animals. Before the trial began, all the animals had been free of antiparasitic drugs for at least 35 days. The last formulation used on the animals prior to the study was a spray combining pyrethroids and organophosphate compounds.

The bovines were allocated to experimental groups on day 0, being randomly designated to treatments according to a masked complete block design. Block formation was based on arithmetic means of the number of female ticks (measuring 4.5–8.0 mm in length) counted

on three consecutive days (days -3, -2, and -1), as recommended by Wharton and Utech (1970). Animals were distributed into ten blocks containing three bovines each, which were randomly placed in one of the treatment groups inside each block. The number of cows per group (10 animals per group) adhered to the guidelines outlined by the Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento - MAPA), as specified in Ordinance number 48 (Brazil 1997). The cows in all groups were provided with a diet consisting of corn silage, sulfur-free commercial ration, and had access to water as needed. They were also vaccinated against Foot and Mouth disease virus, rabies, and clostridiosis, which includes vaccinations for *Clostridium tetani, C. botulinum*, and *C. perfringens*.

An in vitro adult immersion test (AIT) was conducted before the study to select the appropriate acaricide (Drummond et al., 1973). Based on the results of the test, chlorpyrifos at 375 ppm, fenthion at 185.5 ppm, and cypermethrin at 187.5 ppm (Colosso® FC 30, Ourofino Animal Health, Brazil) was chosen due to its 100% in vitro efficacy. Each treated animal received 5 L of the acaricide solution as per the manufacturer's instructions using a hand pump, ensuring that the entire body of the cattle was bathed in the solution.

Before day 0 of the study, 30 animals were kept in an experimental area encompassing 13 hectares (ha) containing *Coast cross* grasses. On day 0, each group was allocated to a different paddock within the same area, with each paddock covering approximately 4.3 hectares. Initially, the stocking rates were 2.92, 2.94, and 2.97 animal units per hectare (au/ha) for T01, T02, and T03, respectively.

Strategic tick control and tick counts during the experiment

The cattle belonging to group T01 (strategic treatment in late autumn/winter/late spring), received seven strategic treatments (Nicaretta et al. 2020; Melo-Junior et al., 2022) application of acaricide each 28 days (D0, D+28, D+56, D+84, D+112, D+140, and D+168) which started in June (late-autumn) until December (late-spring). The animals were regularly inspected, and if necessary, they received additional treatments for their well-being, based on numerical criteria, whenever the mean tick count (females between 4.5 and 8 mm) of the group was \geq 30 (Gomes et al. 2016) until D+350 (Fig. 1A). The interval between treatments was chosen because the acaricide formulation did not exhibit a long-lasting efficacy on the animals.

For T02, a strategic treatment plan was designed to target the "first tick generation." The group received one palliative treatment on day 0 (June) because the mean tick count on this day was \geq 30. The strategic treatment was initiated in October (early spring), at the start of the rainy season (October to April), and continued until April (early autumn). Seven strategic treatments were administered every 28 days on days D+112, D+140, D+168, D+196, D+224, D+252, and D+280. Similar to T01, these animals were regularly inspected and treated as needed based on numerical criteria up to the end of the study (D+350) (Fig. 1B).

T03 served as the control group and received palliative treatments whenever the mean tick count (females between 4.5 and 8 mm) of the group was \geq 30 (Gomes et al. 2016) (Fig. 1C). Palliative treatments were employed to prevent severe tick infestations and ensure the well-being of the cattle. Different strategies were applied to T01 and T02 to determine if the treatments were effective in reducing the tick burden on the animals during the second year of the study. To assess the tick burden, regular tick counts were conducted every 14



Fig. 1 Strategic and palliative treatment schemes designed for the study in taurine cattle naturally infested with *Rhipicephalus microplus* in a region with up to five tick annual generations. A: T01- Strategic treatment commenced in the late autumn and finalized in late spring. B: T02- Strategic treatment commenced in the early spring (to act on the first tick generation of the tick) and finalized in early autumn. C: T03-Control group: acaricide treatment when the group mean tick count (females between 4.5 to 8 mm) \geq 30

days, following the methodology of Wharton and Utech (1970), without multiplying by two, up to day D+350.

Statistical analysis

Data obtained from *R. microplus* females counts 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 analysis in time. The data complied with the assumptions of normality and homogeneity of variances and residuals. Treatment means for tick counts were compared using Tukey's test (Proc GLM, SAS, 2016).

For comparing the total of treatments performed in each treatment group in one year, was used the Fisher's Exact Test.

Results

Throughout the experimental period of 350 days, the animals in T01, which received acaricide treatments starting in late autumn and ending in late spring of year 1, a period when animals typically have a higher tick burden, were provided with a total of eleven treatments. This included the initial seven strategic treatments (D0, D+28, D+56, D+84, D+112, D+140, and D+168) and four additional acaricide palliative treatments in the autumn of year 2. These palliative treatments were administered based on tick burden criteria on days D+266, D+294, D+322, and D+350 (summarized in Tables 1 and 2, and Fig. 2A).

Day of the study		Experim =[∑log(nental x+1)]	groups /N /n	lean ¹			Value of P	CV ²
	Month of the year	T01		T02		Т03			
0*, α, β, μ	June	3.4840	А	3.4778	А	3.4755	А	0.9796	3.95
14	June	0.5347	А	0.7581	А	1.2060	А	0.1827	96.42
28 ^a	July	0.4883	В	1.6639	А	0.9624	А	0.0303	90.18
42	July	0.2485	В	1.5249	А	1.4645	А	0.0115	91.72
56 ^a	August	1.2332	А	1.8205	А	1.2733	А	0.1111	46.57
70	August	0.2773	В	0.7535	AB	1.2526	А	0.0229	97.1
84 α	September	0.0693	В	0.3401	AB	0.7860	А	0.0105	123.37
98	September	0.1386	А	0.3951	А	0.6579	А	0.2686	175.94
112 ^{α, β}	October	0.1386	В	1.7974	А	1.7054	А	0.0054	96.3
126 ^µ	October	0.2485	В	0.6292	В	3.6816	А	< 0.0001	36.01
140 ^{α, β}	November	0.5743	А	0.6985	А	0.4277	А	0.6710	118.85
154	November	0.1386	А	0.2485	А	0.6474	А	0.0759	145.65
168 ^{α, β, μ}	December	0.3871	В	0.2485	В	3.1695	А	< 0.0001	61.89
182	December	1.6414	AB	0.3584	В	0.8438	А	0.0037	82.09
196 ^{β, μ}	January	0.9039	в	0.5663	в	2.9898	А	< 0.0001	62.64
210	January	0.8489	А	0.5257	А	0.6356	А	0.5875	105.33
224 ^{β, μ}	February	1.8430	В	0.7966	С	3.7720	А	< 0.0001	34.36
238	February	2.3130	А	0.9125	В	0.3584	В	0.0002	76.72
252 ^{β, μ}	March	2.9046	А	0.7139	В	3.7413	А	< 0.0001	32.34
266 ^α	March	3.6571	А	0.8776	В	0.4277	В	< 0.0001	34.4
280 ^{β, μ}	April	1.6129	В	0.5663	С	3.8742	А	< 0.0001	35.04
294 ^α	April	3.5933	А	0.4682	С	1.1233	В	< 0.0001	33.27
308 ^µ	May	0.9980	в	1.1821	в	4.1346	А	< 0.0001	32.91
322 ^α	May	3.5875	А	1.1629	в	1.4912	в	< 0.0001	32.71
336 ^µ	June	1.2583	в	1.4270	в	4.3668	А	< 0.0001	36.43
350 ^α	June	3.4671	А	1.3655	В	2.0275	в	< 0.0001	38.46

 Table 1
 Rhipicephalus microplus females counts and acaricide treatments performed during a year in bovines submitted to different strategic tick control scheme

* Tick counts mean between days -3, -2 and -1.

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

² Coefficient of variation.

^a When T01 received acaricide treatment.

^β When T02 received acaricide treatment.

^µ When T03 received acaricide treatment.

T01: Strategic control late-autumn/winter/late-spring.

T02: Strategic control early-spring/summer/early-autumn.

T03: control (palliative treatment when necessary).

In contrast, T02, which received acaricide treatments aimed at targeting the "first tick generation" during early spring, summer, and early autumn of year 1, received a total of eight treatments in one year. This included one initial palliative treatment at the beginning of the study and seven strategic treatments (summarized in Tables 1 and 2, and Fig. 2B).

The T03 group, which served as the control and received palliative treatments, underwent nine palliative acaricide treatments during the experimental trial. These treatments

Table 2 Counts of h	hipicet	halus n	nicro	plus fe	male	s (betw	/een	4.5 and	8.0 m	m in l	ength) (in the	side lef	t side o	f cattle,	subjec	ted to di	ferent	control	scheme	SS					
Animal	Group	Month.	. day c	of study	and n	umber	of Rhi	picephal	us mici	oplus i	emales I	present	in cattle													
		Jun	Jun	Jul	Jul	Aug	ЗuА	g Sep	Sep	Oct	Oct	Nov	Nov D	ec D	ec Jan	Jan	Feb	Feb	Mar	Mar	Apr	Apr	May	May	Jun	Jun
		•0	14	28	42	56	70	84	98	112	126	140	154 16	58 1.	82 196	210	224	238	252	266	280	294	308	322	336	350
16,027	T01	33.3	2	0	0	3	-	0	0	0	1	0	0 2	6	2	2	2	26	2	42	12	35	0	34	5	25
18,004		36.0	-	10	0	S	0	0	0	-	0	-	1 0	7	0	0	13	12	36	62	5	45	0	25	0	16
18,141		35.0	0	0	0	7	0	0	0	0	0	0	0 0	6	5	0	б	5	13	35	1	67	0	47	2	14
18,144		31.0	0	0	0	-	-	0	0	0	0	0	0 0	9	2	7	4	16	5	27	0	29	2	78	0	27
18,145		32.3	4	0	0	9	0	0	0	0	0	-	0 0	ŝ	0	-	2	4	16	22	5	15	_	65	4	54
19,031		28.7	0	0	0	4	0	-	б	-	2	0	0 1	2	0	7	0	52	23	18	8	37	4	42	4	26
19,067		27.0	0	5	0	0	-	0	0	0	-	0	0 0	4	2	0	19	7	42	64	1	46	3	45	2	35
20,020		29.7	9	0	0	7	0	0	0	0	0	2	0 1	-	б	6	9	4	36	57	4	4	5	23	5	41
20,054		31.0	0	0	-	7	0	0	0	0	0	-	1 0	-	0	8	12	1	6	52	14	57	4	15	5	82
20,072		33.0	0	-	5	4	-	0	0	0	0	12	0 3	6	12	0	21	38	67	31	7	15	5	21	5	32
Total		317	13	16	9	29	4	-	3	5	4	17	2 7	5	1 26	24	82	160	249	410	57	390	24	395	32	352
Mean		31.70a	1.3(0 1.60	a 0.6) 2.90a	1 0.4() 0.10a	0.30	0.20a	0.40	1.70a	0.20 0.	70a 5.	.10 2.60	2.4(8.20	16.00	24.90	41.00b	5.70	39.00b	2.40	39.50b	3.20	35.20b
18,005	T02	33.0	4	7	7	7	-	0	0	0	0	2	0 0	2	2	0	1	0	2	3	2	0	2	7	2	2
18,007		35.7	9	26	13	S	0	4	0	21	0	-	0 0	0	S	0	2	0	г	0	1	7	1	-	-	12
18,029		36.0	0	4	7	4	7	0	0	5	0	0	0 0	2	-	ŝ	0	0	0	-	0	0	0	2	0	0
18,073		35.7	0	7	0	7	0	0	0	21	1	-	2 0	1	ŝ	0	ŝ	0	5	2	0	0	0	0	0	0
18,086		29.3	0	-	32	18	-	0	0	2	2	2	-	0	0	-	4	7	9	0	1	0	2	-	12	13
19,011		30.7	ŝ	10	-	16	5	г	12	23	5	4	0 0	0	0	0	0	4	0	2	б	1	3	2	2	2
19,051		30.0	9	7	0	9	0	0	0	~	0	0	0 0	0	0	0	5	ŝ	0	4	5	7	5	4	_	1
20,006		29.0	-	34	17	10	12	7	0	0	4	0	0 1	0	0	-	-	7	0	8	0	0	4	12	21	19
20,036		28.3	0	S	9	S	-	0	ю	16	0	0	0 2	-	0	7	-	7	4	0	0	1	9	-	16	0
20,071		27.7	0	0	З	-	0	0	0	-	2	5	1 0	0	-	Э	0	16	0	-	0	7	8	7	8	12
Total		315	20	86	76	69	22	٢	15	76	14	15	4	9	12	10	17	29	18	21	12	8	31	32	63	61
Mean		31.53b	2.0	0 8.60	7.6	06.9 (2.2(0.70	1.50	9.70a	1.40	1.50a	0.40 0.	40a 0.	60 1.20	a 1.0(0 1.70a	2.90	1.80a	2.10	1.20a	0.80	3.10	3.20	6.30	6.10

inued)
(cont
2
<u>e</u>
P
Та

Animal G	hroup 1	Month. d٤	ay of stu	udy an	d numb	er of R	hipicep	halus n	nicroplus	s females	present	t in catt	le												
	د. ا	i un'	lun Jı	i li	Iul A	iA gu	ug Seț	> Set) Oct	Oct	Nov	Nov	Dec	Dec J _i	ur.	Ian Feb	Feb	Mar	Mar	Apr	Apr	May	May	Jun	Jun
	. –	•	14 2	8	42 5(5 70) 84	98	112	126	140	154	168	182 1	. 96	210 224	238	252	266	280	294	308	322	336	350
18,003 T	03	31.0	1		2 5	-	2	ŝ	-	45	2	_	12	2 4	4) 42	0	36	0	32	9	83	0	149	9
18,025	. 4	28.0 (0 0	-) 2	4	ŝ	0	0	65	-	0	34	4	6	2 25	0	82	0	52	5	80	5	23	5
18,039	. 4	29.0	2 2	. 4	2 1	2	2	2	21	35	0	0	26	6 6	5	3 54	2	16	1	42	4	16	2	52	4
18,110		30.3 4	4	. –	1 6	2	0	2	0	45	0	-	52	0 8.	2) 62	-	42	0	35	2	46	-	49	23
19,029		33.0 .	7 5	-) 2	5	2	0	32	85	0	0	42	21 2	5	1 47	0	27	2	16	0	27	10	109	1
19,032		32.7 4	4 6	. –	12 6	4	ю	4	0	16	-	0	32	0 1.	2	3 82	5	65	1	45	0	79	2	137	2
19,064		33.0 ±	5 2		3 7	5	2	0	12	32	0	-	45	0 3.	5	2 35	0	22	0	62	-	105	8	67	15
20,025	. 4	27.0 (0 1	~	8 0	16	5 1	0	-	28	0	7	36	0 2		3 42	0	49	0	54	7	23	4	57	11
20,033	. 4	28.7 2	2 0	. –	16 3	0	0	ŝ	24	32	-	2	36	1 2	-	0 15	0	57	2	85	3	57	9	116	6
20,059	7	13.0	7 3	. –	15 1	0	0	0	26	41	2	8	0	0 1.	5	1 62	0	63	1	105	4	92	7	137	10
Total		316	32 2	3	59 35	35	15	14	117	424	7	15	315	34 3	02	12 466	80	459	7	528	27	683	45	896	86
Mean		31.57b	3.20 2.	30	5.90 3.	30 3.	90 1.5	0 1.4	0 11.76	42.40	0.70	1.50	31.50b	3.40 3	0.50b	1.20 46.	60b 0.8t	(45.9	b 0.70	52.801	0 2.70	68.30t	4.50	409.68	8.60
*0=mean counts between	days –.	32 and -	 																						
a Indicate strategic treatme	snt																								
b Indicate palliative treatm	lent																								
T01: Strategic treatment i	in late-	autumh	winter/	late-sp	oring, e	very 28	3 days																		
T02: Strategic treatment t	to act i	n the "fir	st tick §	genera	tion"-(early-sl	pring/sı	ummer	/early-a	utumn, e	svery 28	days													

T03: control (palliative treatment when necessary)

were administered on days D+0, D+126, D+168, D+196, D+224, D+252, D+280, D+308, and D+336. Most of these treatments occurred during the rainy season, with six during the rainy season (October to March), two during spring, three in the summer, and three in the autumn (summarized in Tables 1 and 2, and Fig. 2C).

A total of 26 tick counts were conducted during the study (summarized in Table 1). In three of these counts (D+28, D+42, and D+112), T01 had a lower tick burden compared to T02 ($P \le 0.05$). On the other hand, T02 had lower tick counts ($P \le 0.05$) than T01 in eight counts (D+224, D+238, D+252, D+266, D+280, D+294, D+322, and D+350). Additionally, the mean tick counts for T01 and T02 were both lower ($P \le 0.05$) than T03 in 12 and 10 instances, respectively.

When comparing the number of acaricide treatments received by each group, T01 received a higher number of treatments than T02 (P=0.0062) (as shown in Table 3). However, the total number of treatments performed per year did not significantly differ between T02 and T03 (p>0.05) (as shown in Table 3).

Discussion

The current work provides valuable insights into the timing of initiating strategic control against *R. microplus* in taurine adult animals within a tropical region. Our findings align with the approach recommended by some authors in tropical areas, which suggests commencing *R. microplus* strategic control in early spring, coinciding with the onset of the rainy season and the first tick generation (Evans 1992; Furlong et al. 2003, 2007; Pereira et al. 2008). However, our results do not support the idea proposed by Bonatte-Junior et al. (2019), suggesting that it might be more effective, in a tropical area, to concentrate acaricide treatments in autumn when animals have a higher tick burden.

Studies conducted in subtropical areas of Argentina, where the major tick peak typically occurs in autumn, have demonstrated that implementing strategic treatments aimed at the first tick generation in this region, starting during the period of lower tick abundance and greater vulnerability (late winter) and concluding these treatments in late spring, proved sufficient to prevent the development of more tick generations during late summer and autumn (Nava et al. 2014, 2015, 2019, 2020, 2021).

The consensus regarding the commencement of tick control in early spring for tropical areas and late winter for subtropical areas is that these periods correspond to lower tick burdens. This decrease in tick burdens during these times is attributed to abiotic factors such as temperature, relative humidity of the air, saturation deficit of the atmosphere, and rainfall, which adversely affect the biological parameters of the non-parasitic phase of the cattle tick, including factors such as no hatching of eggs and shorter larval longevity. Consequently, this naturally reduces the number of ticks and the number of larvae infesting the pasture during these specific times of the year in various regions (Gomes et al. 2016; Canevari et al. 2017; Mastropaolo et al. 2017; Cruz et al. 2020; Nicaretta et al. 2021a).

When we compare the reality between the current study of Brazil (tropical) and Argentina (subtropical), the number of treatments in Argentina (is no more than three or four per year (Nava et al. 2014, 2015, 2019, 2021; Morel et al. 2017). This phenomenun is possible in subtropical latitudes, because the constriction that winter imposes on the development of a *R. microplus* population in these areas, and by the bovine breed (*B. taurus indicus* x



Fig. 2 Mean tick counts and acaricide treatments performed in bovines naturally infested with *Rhipicephlaus microplus*. A: T01- Strategic treatment commenced in the late autumn and finalized in late spring. B: T02- Strategic treatment commenced in the early spring (to act on the first generation of the tick) and finalized in early autumn. C: T03- Control group: acaricide treatment when the group mean tick count (females between 4.5 to 8 mm) \geq 30

Dairy cattle category	Total of treatments*
T01: Strategic treatment to act in the "first tick generation" – early-spring/summer/early- autumn, each 28 days	8 ^b
T02: Strategic treatment in late-autumn/winter/late-spring, each 28 days T03: Control	11 ^a 9 ^{ab}
P value	0.0062
Coefficient of variation	42.7

 Table 3
 Total acaricide treatments performed against *Rhipicephalus microplus* per group during the study

Total followed by the same letter in the column do not differ from each other (p>0.05)

B.taurus taurus crosses). In tropical areas, where the conditions are more favorable for cattle tick development, with the presence of susceptible bovine breed for *R. microplus* infestation, it notably imply a higher number of treatments must be carried out, and above all, with a strong selection pressure for resistance (Zapa et al. 2020; Nicaretta et al. 2021a, b; Felippelli et al. 2022; Gomes et al. 2022; Trindade et al. 2023). It clearly makes the chemical treatment unsustainable over time in tropical regions of Brazil, when compared to subtropical regions of Argentina. Nevertheless, there is a consensus that strategic control should target the first generation of *R. microplus*.

In our study conducted in a tropical region, even though we initiated tick strategic control in late autumn, which is a period of significant tick challenge as suggested by Bonatte-Junior et al. (2019), and continued the applications when the tick population was less numerous and most vulnerable, extending until late spring (December in the region where the study was conducted), unfortunately, this approach was insufficient to effectively reduce the tick burden in cattle by the following autumn. Similar findings to ours were reported by Nicaretta et al. (2021b) with *B. t. taurus* cattle and Trindade et al. (2023) with animals having a genetic composition of 96.8% B.t. taurus in a tropical region. They used a strategic treatment scheme during spring/early summer (November to January and October to December, respectively) with long-lasting formulations such as fluazuron, fluazuron+abamectin, fluazuron+fipronil, and high concentrations of ivermectin or moxidectin.

However, it's worth noting that the fact that initiating tick strategic control during the period of greatest challenge (autumn) and continuing it when the tick population is less numerous and most vulnerable wasn't the most effective strategy in our study doesn't necessarily apply to all situations in regions with a tropical climate. In the study mentioned earlier by Trindade et al. (2023), they also compared different control schemes in crossbred cattle ($\frac{1}{2}$ Angus and Nellore). In that case, acaricide treatments performed on one group in late autumn/middle winter/middle spring of year 1 were sufficient to suppress the tick burden in the autumn of year 2. For the other group of crossbred animals, a single tick treatment applied in late autumn of year 1 was enough to control the tick infestation in the autumn of year 2. In any case, it's essential to consider that the results obtained with *B. t. taurus* animals in the study conducted by Trindade et al. (2023) may have been influenced by the genetic composition of the cattle rather than solely the acaricide treatment scheme.

One practical challenge that may arise on farms when determining the appropriate timing for initiating strategic tick control against cattle ticks is related to the timing of the reproductive season of beef cattle in many regions of South America. In most of these regions, calf weaning occurs between April and July each year, coinciding with the period of peak tick challenge for cattle (Nicareta et al., 2021; Gomes et al. 2022; Nicaretta et al. 2023; Trindade

et al. 2023). Additionally, it's worth noting that animals are generally more vulnerable during this time of the year, which can exacerbate the tick burden (Junior et al., 2019; Martins et al. 2002; Trindade et al. 2023). Therefore, it's advisable to implement palliative treatments against *R. microplus* for these animals during this period, as was done for T02 on day 0 of our study. An operational obstacle on the farm that may occur when ideating the appropriate time to initiate strategic control against the cattle tick may be related to the period that the reproductive season of beef cattle occurs in several regions of South America. In most of these regions, calf weaning occurs between April and July of each year, when the greatest challenge of cattle by ticks occurs (Nicaretta et al., 2021; Gomes et al. 2022; Nicaretta et al. 2023; Trindade et al. 2023). Allied with this, we highlight that animals are more debilitated at this time of year, which can further aggravate the tick burden (Junior et al., 2019; Martins et al. 2002; Trindade et al. 2023). For this reason, palliative treatments against *R. microplus* should be performed on these animals at this time, as well as performed for T02 on day 0 of the present study.

It is notorious that the susceptibility of R. microplus population to chemicals, as well as the residual effect of the formulation used, may interfere with these previously mentioned peaks. In studies conducted in Argentina, (Morel et al. 2017; Nava et al. 2019, 2021) taurine animals and taurine x zebuine animals were used, the mean stocking rate ranged from 1 AU/hectare to 2.4 AU/hectare and prevails long-lasting formulations. In the present study, the taurine breed and similar stocking rate were used, however, the formulations were not. In Argentina (Nava et al. 2014, 2015, 2019, 2020, 2021; Morel et al. 2017), fluazuron, ivermectin, fipronil, and flumetrin were used, while in the present study, a spray formulation containing chlorpyrifos+fenthion+cypermethrin. However, in a study previously conducted at the same farm and by this same team of researchers, as the current study was conducted, a strategic treatment scheme starting in early-spring until middle-summer, with fluazuron+abamectin (pour-on), fluazuron+fipronil (pour-on), ivermectin (injectable) and a spray association of chlorpyrifos+fenthion+cypermethrin was not sufficient to suppress the population peak of *R. microplus* in cattle in the autumn after the sequence of treatments (Nicaretta et al. 2021b). Probably, the variation in results between these tropical and subtropical regions is likely attributable to environmental and climatic factors rather than differences in the formulation used.

It's important to emphasize that the goal of this study is not to determine the specific number of sequential tick control treatments that should be administered when implementing a particular control strategy. To identify the optimal number of sequential treatments, several factors must be considered. These include the choice of the commercial product, its residual effectiveness, the category of the animals (as well as their genetic composition), and the climatic conditions of the region. The regional climate plays a significant role in shaping the population dynamics of *R. microplus* (Nava et al. 2021; Melo-Junior et al., 2022; Trindade et al. 2023). In areas where this tick species can complete up to five generations or peaks per year, more sequential acaricide treatments are typically required (Gomes et al. 2016, 2022; Cruz et al. 2020; Nicaretta et al. 2021a, 2023). On the other hand, the number of sequential acaricide applications may be lower in regions of southern South America, such as South Brazil and Argentina. This is due to the shorter "tick season" that characterizes these areas throughout the year. This shorter season is a result of the lower temperatures that prevail in these regions, particularly around May and June, leading to fewer tick generations (Martins et al. 2002; Canevari et al. 2017; Mastropaolo et al. 2017; Nava et al. 2019, 2021). Commencing the strategic control protocol at the right time, which results in fewer treatments throughout the year as demonstrated in this study, offers several advantages. It positively impacts the resistance of ticks to commercially available chemical compounds, reduces expenses related to acaricides, and enhances animal management. The global prevalence of *R. microplus* resistance is concerning, with reports spanning all acaricide classes (except isoxazolines) and the emergence of multi-resistant tick strains. This is accompanied by an increase in the number of tick generations, a consequence of global warming, leading to the spread of tick-borne diseases (Cruz et al. 2015; Gomes et al. 2015; Maciel et al. 2016; Fular et al. 2018; Marques et al., 2020; Cavalcante et al. 2021; Nicaretta et al. 2021a; Githaka et al. 2022; Obaid et al. 2022; da Costa et al. 2023). Reducing the number of treatments not only lessens the selection pressure for drug resistance in tick populations, which is highly desirable, but it also decreases the financial burden on farmers in terms of maintaining herd health and productivity. It also minimizes the stress animals experience due to handling during treatment (Graf et al. 2004; Jonsson 2006; Willadsen 2006; Pereira et al. 2008; Rodriguez-Vivas et al. 2018; Obaid et al. 2022).

Another crucial point to emphasize is that this study specifically involved adult animals (*Bos t. taurus*) in a region characterized by a tropical climate. Therefore, the results obtained should be applied to similar situations, such as the one examined in this study. Future studies should consider variations in animal age within the same regions. It's essential to recognize that a deep understanding of the biology and ecology of cattle ticks in each region, as well as the genetic composition and category of animals, plays a pivotal role in determining the right timing to initiate control strategies. This is especially important in the context mentioned above.

Conclusion

Initiating strategic tick control in taurine cattle within a tropical region during early spring, coinciding with the first tick generation and the onset of the rainy season, is more effective than starting in the autumn when tick infestations are higher. By commencing the protocol in early spring, the study observed a reduced number of required acaricide treatments (only 8) and a lower tick burden over the course of a year in cattle. This translates to cost savings on products and animal handling, as well as less pressure exerted on the tick population, thereby contributing to more sustainable tick control practices.

Acknowledgements We thank the owner of the farm, João Luis Cobra Monteiro, and the employees Claudemir de Paula Coelho, Lindo Andre ("Sr Lino"), and Valdir da Costa ("Tomate" – *in memoriam*) for their support in the study conduction.

Author contributions L.L.L.L., I.M.L.M, V.A.S and A.S.N.T investigation, data curation. L.L.F writing - original draft and review. O.P.N., F.A.B. and C.M.O.M. writing - review and editing. V.E.S. formal analysis. W.D.Z.L. conceptualization, methodology, supervision, project administration and writing - review & editing.

Funding none.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval The project was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Goiás (UFG) (Protocol 072/18), being in accordance with ethical principles in animal experimentation.

Consent to participate The authors obtained consent from the responsible authorities at the institute/organization where the work has been carried out before the work is submitted.

Consent for publication All authors gave explicit consent to submit the work.

References

- Bonatti-Junior P, Rodrigues VDS, Garcia MV, Higa LOS, Zimmermann NP, Barros JC, Andreotti R (2019) Economic performance evaluation of Brangus and Nellore cattle breed naturally infested with *Rhipicephalus microplus* in an extensive production system in Central-West Brazil. Exp Appl Acarol 78:565– 577. https://doi.org/10.1007/s10493-019-00404-1
- Brazil (1997) Ministério da Agricultura e Abastecimento, Secretaria de Defesa Agropecuária, Portaria n.48, 12/05/1997. Avaiable on: https://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method =visualizarAtoPortalMapa&chave=143662794
- Canevari JT, Mangold AJ, Guglielmone AA, Nava S (2017) Population dynamics of the cattle tick *Rhipicephalus (Boophilus) microplus* in a subtropical subhumid region of Argentina for use in the design of control strategies. Med Vet y Entomol 31:6–14
- Cavalcante ASA, Ferreira LL, Couto LFM, Zapa DMB, Heller LM, Nicaretta JE, Cruvinel LB, Junior RDM, Soares VE, de Souza GRL, Monteiro CMO, Lopes WDZ (2021) An update on amitraz efficacy against *Rhipicephalus microplus* after 15 years of disuse. Parasitol Res 120:1103–1108. https://doi.org/10.1007/ s00436-021-07063-5
- Cruz BC, Lopes WDZ, Maciel WG, Felippelli G, Favero FC, Carvalho RS, Ruivo MA, Colli MHA, Sakamoto CAM, da Costa AJ, de Oliveira GP (2015) Susceptibility of *Rhipicephalus (Boophilus) microplus* to ivermectin (200,500 and 630 µg/kg) in field studies in Brazil. Vet Parasitol 207:309–317
- Cruz BC, Mendes AFL, Maciel WG, Santos IB, Gomes LVC, Felippelli G, Teixeira WFP, Ferreira LL, Soares VE, Lopes WDZ, Costa AJ, Oliveira GP (2020) Biological parameters for *Rhipicephalus microplus* in the field and laboratory and estimation of its annual number of generations in a tropical region. Parasitol Res 119:2421–2430
- Da Costa AJ, de Souza Martins JR, de Almeida Borges F, Vettorato LF, Barufi FB, de Arriero Amaral O (2023) First report of the efficacy of a fluralaner-based pour-on product (Exzolt® 5%) against ectoparasites infesting cattle in Brazil. Parasit Vectors 16:336
- Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Grsham OH (1973) Boophilus Annulatus and Boophilus microplus: laboratory tests of insecticides. J Econ Entomol 66:130–133
- Evans DE (1992) Tick infestation of livestock and tick control methods in Brazil: a situation report. Int J Trop Insect Sci 13:629–643. https://doi.org/10.1017/S1742758400016234
- Felippelli G, Teixeira WFP, Gomes LVC, Maciel WG, Cruz BC, Buzzulini C, Santos D, Ferreira TR, Soares LL, Rodrigues VE, Monteiro DC, da Costa CMO, Lopes AJ, W.D.Z (2022) Tick infestation level interferes with spray formulation (organophosphate+pyrethroid) efficacy against *Rhipicephalus microplus*. Ticks Tick Borne Dis 13:101903. https://doi.org/10.1016/j.ttbdis.2022.101903
- Fular A, Sharma AK, Kumar S, Nagar G, Chigure G, Ray DD, Ghosh S (2018) Establishment of a multiacaricide resistant reference tick strain (IVRI-V) of *Rhipicephalus microplus*. Ticks Tick Borne Dis 9:1184–1191. https://doi.org/10.1016/j.ttbdis.2018.04.014
- Furlong J, Martins JR, Prata MCA (2003) Carrapato dos bovinos: controle estratégico nas diferentes regiões brasileiras (in Portuguese). Embrapa Gado de Leite - Comunicado Técnico 36, 2003. Disponível em: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/594909/1/COT36Carrapatodosbovinos.pd
- Furlong J, Martins JR, Prata MCA (2007) O carrapato dos bovinos e a resistência: temos o que comemorar? (in Portuguese). A Hora Veterinária. 27, 26–32

- Githaka NW, Kanduma EG, Wieland B, Darghouth MA, Bishop RP (2022) Acaricide resistance in livestock ticks infesting cattle in Africa: current status and potential mitigation strategies. Curr Res Parasitol Vector Borne Dis 2:100090. https://doi.org/10.1016/j.crpvbd.2022.100090
- Gomes LV, Lopes WD, Cruz BC, Teixeira WF, Felippelli G, Maciel WG, Bichuette MA, Ruivo MA, Alcantara MHC, Carvalho RS, Martinez AC, Soares VE, da Costa AJ (2015) Acaricidal effects of fluazuron (2.5 mg/kg) and a combination of fluazuron (1.6 mg/kg)+ivermectin (0.63 mg/kg), administered at different routes, against *Rhipicephalus (Boophilus) microplus* parasitizing cattle. Exp Parasitol 153:22–28. https://doi.org/10.1016/j.exppara.2015.02.004
- Gomes LVC, Lopes WDZ, Teixeira WFP, Maciel WG, Cruz BC, Felippelli G, Buzzulini C, Soares VE, Melo DP, Bichuette MA, Junior GA, da Costa AJ (2016) Population dynamics and evaluation of the partial selective treatment of crossbreed steers naturally infested with *Rhipicephalus (Boophilus) microplus* in a herd from the state of Minas Gerais in Brazil. Vet Parasitol 220:72–76
- Gomes LVC, Teixeira WFP, Maciel WG, Felippelli G, Buzzulini C, Soares VE, de Melo DP, Cruz BC, Rodrigues DC, Ferreira LL, Monteiro CMO, Lopes WDZ, da Costa AJ (2022) Strategic control of cattle co-parasitized by tick, fly and gastrointestinal nematodes: is it better to use ecto+endoparasiticide or just endectocide formulations? Vet Parasitol 301:109622. https://www.sciencedirect.com/science/article/abs/pii/S030440172100282X#:~:text=The%20ROI%20of%20the%20co,endectocide%20 formulation%20(Table%203)
- Graf JF, Gogolewski R, Leach-Bing N, Sabatini GA, Molento MB, Bordin EL, Arantes GJ (2004) Tick control: an industry point of view. Parasitol 129:S427–S442. https://doi.org/10.1017/s0031182004006079
- Grisi L, Leite RC, Martins JRS, Barros ATM, Andreotti R, Cançado PHD, Leon AAP, Pereira JB, Vilella HS (2014) Reassessment of the potential economic impact of cattle parasites in Brazil. Rev Bras Parasitol Vet 23:150–156
- Jonsson NN (2006) The productivity effects of cattle tick (*Boophilus microplus*) infestation on cattle, with particular reference to *Bos indicus* cattle and their crosses. Vet Parasitol 137:1–10. https://doi. org/10.1016/j.vetpar.2006.01.010
- Maciel WG, Lopes WDZ, Gomes LVC, Cruz BC, Felippelli G, Santos IBD, Borges FA, Gonçalves WA, Scarpa AB, Nicaretta JE, Bastos TSA, da Costa AJ (2016) Susceptibility of *Rhipicephalus (Boophilus) microplus* to fluazuron (2.5 mg/kg) and a combination of novaluron (2.0 mg/kg)+eprinomectin (0.36 mg/kg) in field studies in Brazil. Prev Vet Med 135:74–86. https://doi.org/10.1016/j.prevetmed.2016.10.019
- Magalhães FEP (1989) Aspectos biológicos e ecológicos e de controle do Boophilus microplus (Canestrini, 1887) no Município de Pedro Leopoldo-MG, Brasil (in Portuguese). 1989. 117 f. Tese (Doutorado)— Instituto de Ci^encias Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte
- Marques R, Krüger RF, Peterson AT, Melo LF, Vicenzi N, Jiménez-García D (2020) Climate change implications for the distribution of the babesiosis and anaplasmosis tick vector, *Rhipicephalus (Boophilus) microplus*. Vet Res 51:81
- Martins JR, Evans DE, Ceresér VH, Corrêa BL (2002) Partial Strategic Tick Control within a herd of European Breed Cattle in the state of Rio Grande do sul, Southern Brazil. Exp Appl Acarol 27:241–251
- Mastropaolo M, Mangold AJ, Guglielmone AA, Nava S (2017) Non-parasitic life cycle of the cattle tick Rhipicephalus (Boophilus) microplus in Panicum maximum pastures in northern Argentina. Res Vet Sci 115:138–145
- Melo-Júnior RD, Ferreira LL, Zapa DMB, Heller LM, Iuasse HV, Naves RB, Trindade ASN, Gontijo LMA, Scarpa AB, Rodrigues DS, Soares VE, Monteiro CMO, Lopes WDZ (2022) Population dynamics of *Rhipicephalus microplus* in dairy cattle: influence of the animal categories and correlation with milk production. Vet Res Commun. https://doi.org/10.1007/s11259-022-10002-z
- Morel N, Signorini ML, Mangold AJ, Guglielmone AA, Nava S (2017) Strategic control of *Rhipicephalus* (Boophilus) microplus infestation on beef cattle grazed in *Panicum maximum* grasses in a subtropical semi-arid region of Argentina. Prev Vet Med 144:179–183
- Nava S, Mangold AJ, Canevari JT, Morel N, Guglielmone AA (2014) Strategic treatments with systemic biocides to control *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in northwestern Argentina. InVet 16:23–30
- Nava S, Mangold AJ, Canevari JT, Guglielmone AA (2015) Strategic applications of long-acting acaricides against *Rhipicephalus* (*Boophilus*) *microplus* in northwestern Argentina, with an analysis of tick distribution among cattle. Vet Parasitol 208:225–230. https://doi.org/10.1016/j.vetpar.2015.01.015
- Nava S, Tofalletti JR, Morel N, Gugliemone AA, Mangold AJ (2019) Efficacy of winter–spring strategic control against *Rhipicephalus (Boophilus) microplus* infestations on cattle in an area with ecological conditions highly favorable for the tick in northeast Argentina. Med Vet Entomol 33:312–316
- Nava S, Toffaletti JR, Rossner MV, Morel N, Mangold AJ (2021) Alternative applications of the strategic control against the cattle tick *Rhipicephalus (Boophilus) microplus* in a subtropical area. Parasitol Res 120:3653–3661. https://doi.org/10.1007/s00436-021-07324-3

- Nicaretta JE, Dos Santos JB, Couto LFM, Heller LM, Cruvinel LB, de Melo Júnior RD, de Assis Cavalcante AS, Zapa DMB, Ferreira LL, Monteiro CMO, Soares VE, Lopes WDZ (2020) Evaluation of rotational grazing as a control strategy for *Rhipicephalus microplus* in a tropical region. Res Vet Sci 131:92–97. https://doi.org/10.1016/j.rvsc.2020.04.006
- Nicaretta JE, Zapa DMB, Couto LFM, Heller LM, Cavalcante ASA, Cruvinel LB, Melo-Júnior RD, Ferreira LL, Nascimento RM, Soares VE, Borges LMF, Monteiro CMO, Lopes WDZ (2021a) *Rhipicephalus microplus* seasonal dynamic in a Cerrado biome, Brazil: an update data considering the global warming. Vet Parasitol 296:109506. https://doi.org/10.1016/j.vetpar.2021.109506
- Nicaretta JE, Couto LFM, Heller LM, Ferreira LL, Cavalcante ASA, Zapa DMB, Cruvinel LB, Júnior RDM, Gontijo LMA, Soares VE, Mello IAS, Monteiro CMO, Lopes WDZ (2021b) Evaluation of different strategic control protocols for *Rhipicephalus microplus* on cattle according to tick burden. Ticks Tick Borne Dis 12:101737. https://doi.org/10.1016/j.ttbdis.2021.101737
- Nicaretta JE, de Melo Junior RD, Naves RB, de Morais IML, Salvador VF, Leal LLLL, Teixeira ALC, Ferreira LL, Klafke GM, Monteiro CMO, Borges FA, Costa Junior LM, Rodrigues DS, Lopes WDZ (2023) Selective versus strategic control against Rhipicephalus microplus in cattle: a comparative analysis of efficacy, animal health, productivity, cost, and resistance management. Vet Parasitol 321:109999. https://doi.org/10.1016/j.vetpar.2023.109999
- Norris KR (1957) Strategic dipping for control of the cattle tick. Aust J Agric Res 8:768-787
- Obaid MK, Islam N, Alouffi A, Khan AZ, da Silva IV, Tanaka T, Ali A (2022) Acaricides resistance in ticks: selection, diagnosis, mechanisms, and mitigation. Front Cell Infect Microbiol 12:941831. https://doi. org/10.3389/fcimb.2022.941831
- Pereira MC, Labruna MB, Szabó MPJ, Klafke GM (eds) (2008) Rhipicephalus (Boophilus) microplus: biologia, controle e resistência (in Portuguese). Medicina Veterinária, São Paulo, p 169
- Rodriguez-Vivas RI, Jonsson NN, Bhushan C (2018) Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitol Res 117:3–29
- Trindade ASN, Couto LFM, Heller LMH, Zapa DMB, Aquino LMA, Ferreira LL, Morais IML, Salvador VFS, Leal LLLL, Ruivo MA, Scarpa AB, Pereira-Neto O, Soares VE, Lopes WDZ (2023) Cattle tick and gastrointestinal nematodes strategic control in dairy ³¹/₃₂ gyr x holstein and beef ½ Brangus: is the same way? Livest. Sci 268:105154
- Wharton RH, Utech KBW (1970) Relation between engorgement and dropping of *Boophilus microplus* to assessment of tick number in cattle. Aust Entomol Soc 9:171–182
- Willadsen P (2006) Tick control, thoughts on a research agenda. Vet Parasitol 138:161-168
- Zapa DMB, Couto LFM, Heller LM, Cavalcante AA, Nicaretta JE, Junior CLBM, Ferreira RD, Bastos LL, Soares TSA, Mello VE, Lopes IAS, W.D.Z (2020) Do rainfall and tick burden affect the efficacy of pour-on formulations against *Rhipicephalus (Boophilus) microplus*? Prev. Vet Med 177:104950. https:// doi.org/10.1016/j.prevetmed.2020.104950

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Authors and Affiliations

Luccas Lourenzzo Lima Lins Leal¹ · Lorena Lopes Ferreira² · Igor Maciel Lopes de Morais¹ · Vanessa Ferreira Salvador¹ · Artur Siqueira Nunes Trindade¹ · Octaviano Pereira Neto³ · Vando Edésio Soares⁴ · Fernando de Almeida Borges⁵ · Caio Marcio de Oliveira Monteiro^{1,6} · Welber Daniel Zanetti Lopes^{1,6}

Welber Daniel Zanetti Lopes wdzlopes@hotmail.com

- ¹ Centro de Parasitologia Veterinária, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Goiânia, Goiás, Brazil
- ² Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
- ³ Veterinarian, Fazenda Curupira, Pedro Osório, Rio Grande do Sul, Brazil
- ⁴ Universidade Brasil, Descalvado, São Paulo, Brazil
- ⁵ Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil
- ⁶ Departamento de Biociências e Tecnologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Goiás, Brazil