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Population dynamics of *Rhipicephalus microplus* in dairy cattle: influence of the animal categories and correlation with milk production

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Abstract

Is well known the taurine and zebuine susceptibility to Rhipicephalus microplus. Few are the reports regarding tick population dynamics between the same herd/breed, and because of this, two experiments were performed. In the 1st, the cattle tick population dynamics in dairy nursing calves (reared collective and individually), weaning calves (4–16 months), heifers (17–29 months), cows in lactation and dry cows (≥30 months) from the same herd, tick burden and milk production correlation were performed, for two years. R. microplus females (4.5-8.0 mm) counts and the milk production were performed every 28 and 14 days, respectively. In the 2nd experiment, bovines belonging to different categories/age (newborn without previous contact with tick; 12-13 months with tick contact since birth; and 23-24 months with tick contact since birth) were experimentally infested with 30,000 R. microplus larvae, to quantify the number of fully engorged females detached from these animals. In the 1st experiment, when the mean counts of tick were ≥ 30 all animals of the group were treated. Nursing calves showed 3–4 peaks of ticks, animals reared individually showed smaller ($p \le 0.05$) tick burden than those reared collectively. Weaning calves (4-8 months) showed 5 tick peaks/year and higher mean tick burden was found than other categories. On the other hand, animals with 17–29 months of age showed smallest ($p \le 0.05$) tick burden, with 3 tick peaks/year. When the animal become lactating the tick burden increase, and 5 peaks/year occurred, and decrease again in dry cows ($p \le 0.05$) showing 4–5 tick peaks/year. Weaning calves and lactating cows received more acaricide treatments ($p \le 0.05$), 18 and 15, respectively. Nursing calves reared individually, and heifers (21–29 months) were the categories that received two acaricide treatments. The more milk the cow produce, more ticks it has ($p \le 0.05$). In the 2nd experiment, more ($p \le 0.05$) fully engorged females were recovered from younger animals than older ones. So, different tick control strategies need to be adopted in different dairy cattle categories, and the tick burden should be considered, once the effect may be more inherent to the animal rather than the strategy adopted.

Keywords Calves · Cattle tick · Daily milk · Lactating cows · Tick count · Tick peaks

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Introduction

The cattle tick, *Rhipicephalus microplus*, is widely distributed in tropical and subtropical regions where the dairy production is an important activity causing sanitary and economic problems due to its hematophagy, transmission of pathogenic agents to the host and costs with treatments and labor (Jonsson et al. 1998; Grisi et al. 2014; Perez de Leon et al., 2020). Depending on the region, the cattle tick can complete until five generation per year (Hernández-A et al. 2000; Cruz et al. 2020; Nicaretta et al. 2021a).

It is well known that taurine (*Bos taurus taurus*) breeds are more susceptible to tick burden than zebu breeds (*Bos taurus indicus*) (Jonsson et al. 2014). In addition, the physiological state of different animal categories, sex, fur color, can be more vulnerable to cattle tick parasitism (Silva et al. 2010; Silva et al., 2013). Tick strategic control measures are set up regarding epidemiological and biological knowledge. Life cycle bionomic aspects and population dynamics are some of important ones (Rodriguez-Vivas et al. 2018; Nicaretta et al. 2021b).

Journal articles about seasonal and population dynamics of R. microplus usually assessed a single animal category (Nava et al. 2013; Rocha et al. 2019; Nicaretta et al. 2021a). There is no specific study about tick population dynamics assessing all different categories from the same herd. In addition, there is little information regarding tick burden impact on milk production. Therefore, there were performed two experiments in the present work. The first aimed to verify the cattle tick population dynamics in nursing calves, weaning calves, heifers, and cows from the same herd and its tick burden and milk production correlation. The second experiment, aimed to quantify the fully engorged females of R. microplus detached from experimentally infested bovines belonging to different categories/age (newborn without previous contact with tick; 12-13 months and 23-24 months old both with tick contact since birth).

Materials and methods

Experiment 1 - Population dynamics of *Rhipicephalus* microplusin dairy cattle.

Study location, season of the year, animals and paddocks management

This study was conducted from September 2015 to August 2017, on a dairy farm located in the municipality of São José do Rio Pardo state of São Paulo, Brazil. According to the Koppen-Geiger classification (Alvares et al., 2013), the climate of the region is subtropical with annual rainfall

of approximately 1430 mm concentrated from October to March (spring-summer). A purebred Simmental cattle (n = 302) for milk purposes created in a grazing area were used. As the objective of the study was to evaluate the dynamics of *R. microplus* in different categories/ages, it was decided to use the Simmental breed that is a pure taurine breed with high susceptibility to the tick.

The animals were evaluated by category: nursing calves (until 3 months of age); weaning calves (4-16 months); heifers (17–29 months of age) and cows (\geq 30 months of age). The nursing calves were kept in individual (tropical) or collective rearing system, each animal received 4 L of milk daily until 2 months and 2 L of milk until the weaning plus 3 kg of feed and 5 kg of corn silage. The weaning calves were separated by age ($\approx 4-8$, 9–12 and 13–16 months of age), each receiving daily 1.5 kg of feed and 10 kg of corn silage. The heifers were subdivided in two groups, able to reproduction ($\approx 17-20$ months of age) and pregnant ($\approx 21-29$ months of age) receiving 2.5 kg of feed/ animal/day and 25 kg of corn silage/animal/day. The cows were separated in lactating and dry, both receiving Coast cross pasture and 3.5 kg of feed/animal/day plus 35 kg of corn silage/animal/day and 2.5 kg of feed/animal/day plus 30 kg of corn silage/animal/day, respectively. Each animal category described received water ad libitum and kept in different paddocks, as shown in Fig. 1.

Over the two years of study each dairy category had a mean number of animal and stocking rate (animal unit per hectare - AU/ha). The mean number of animals and AU/ha for each dairy cattle category is in board 1. The vegetation of each paddock, over the 23-24 months of the trial, was similar between the different category/paddock. Between the months of June and September, the forage mass of the pastures of all the paddocks became drier, due to the dry period of the year since low occurrence of rains during this time of the year. Anyway, there was the presence of forage mass during the whole period. This occurred because the animals were supplemented with corn silage and feed throughout the study. Although the animals in the older categories had a higher stocking rate, the amount of silage and feed for these animals was also higher, allowing paddocks to remain similar in relation to forage mass between groups throughout the study. There was no shaded area (natural or artificial) in any of the paddocks.

Dairy cattle category	Mean number	AU/
	of animals	ha
nursing calves - individual (≈3 months)	≈15	1.39
nursing calves – collective (≈ 3 months)	≈20	1.88
weaning calves (≈4–8 months)	≈25	1.77
weaning calves ($\approx 9-12$ months)	≈25	2.13
weaning calves (≈13–16 months)	≈25	4.20
heifers (≈ 17–20 months)	≈25	5.87

Fig. 1 Farm area showing the different paddocks where the animals were raised. Legend: 1 - nursing calves in individually rearing up to 3 months of age; 2 - nursing calves in collective rearing up to 3 months of age; 3 - weaning calves with 4 up to 8 months of age; 4 - weaning calves with 9 up to12 months of age; 5 - weaning calves with 13 up to 16 months of age: 6 - heifers able to reproduce with 17 up to 20 months of age; 7 - pregnant heifers with 21 up to 29 months of age; 8 - lactating $cows \ge 30$ months of age; 9 - dry $cows \ge 30$ months of age and 10 - corral facilities. Image from Google Earth



Dairy cattle category	Mean number of animals	AU/ ha
heifers (≈21–29 months)	≈25	7.32
lactating cow (\geq 30 months)	≈104	13.55
dry cow (\geq 30 months)	≈38	13.20

During the 24 months of the study, the animals were managed for the different paddocks according to their age/ category. For example, the animal X when born remained in the paddock "nursing calves" up to ≈ 3 months. After that, this same animal was transferred to the "weaning calves" paddock, where it remained until ≈ 8 months; and so on until the female gave birth for the first time, when they were placed in the "lactating cow" paddock. After the first calving, the animals were alternating between the paddock "lactating cows" (during lactation) and "dry cow" (during the dry period and pre-calving of the next lactation). That is, this management occurred with all animals, during the entire experimental period, and was conditioned to the date of birth of each animal evaluated during the study. However, every 30 days there were animals that were being managed for different paddocks, according to age or lactation status.

Weaning calves and heifers up to 20 months of age received preventive albendazole-based anthelmintic (Valbazen®, Zoetis) every four months according to the farm routine. Vaccines against Foot and Mouth Disease, Rabies and Clostridiosis (*Clostridium tetani*, *Clostridium botuli*num and *Clostridium perfringens*) were administered. In addition, all females were vaccinated against Brucellosis at 4 months of age and received in the months of May and November of both years vaccine against Foot-and-Mouth disease.

Ticks counts, treatment, tick peaks observation, rainfall, and environmental temperature

Counts of partially engorged females of *R. microplus*, between 4.5 and 8.0 mm in length, were quantified on the left side of each animal every 28 days, according to the methodology recommended by Wharton and Utech (1970). The tick counts in each category were performed synchronously to prevent differential tick exposure due to the temporal variation of tick abundance. When the mean counts of *R. microplus* females in the respective category were \geq 30, all animals of the group were treated with flumethrin (1mg/kg, Bayticol®, Elanco Animal Health) as the manufacturer recommendations. This product was chosen due to the absence of residual effect that could interfere in the tick population during the study (Nicaretta et al., 2021b).

The determination on the tick peaks were based on the high tick counts observed during the study period. In December 2016, categories that showed the higher (weaning calves with 4 to months) and lesser tick burden (heifers 20 to 29 months) had females ticks (n=10) collected to verify their biological parameter such as: female weight (g), egg mass weight (g), larvae hatchability visually estimated using a stereomicroscope with an ocular grid to compare the proportion of larvae in relation to the proportion of whole eggs for each group and reproductive efficiency X% hatching \times 20,000, where 20,000 being a constant corresponding to an estimate of the number of R. microplus larvae contained in 1 g of eggs - Labruna et al. 1997. In addition, rainfall and temperature records were obtained in the farm. For rainfall was used a pluviometer equipment (Incoterm 4755) and for environmental temperature was used HOBO data logger (MX2305).

Animal milk production

The cows were milked twice a day, in the morning (04:00 to 06:00h) and afternoon (14:00 to 15:30h), during the whole experimental period (two years). Every 14 days the milk produced per cow/day were measured, and the milk production of each animal and the lactation curve at 305 days of lactation were evaluated. The lactation curve considering each month of lactation was calculated in accordance the methodology described by Molento et al. (2004). The values of milk production were expressed in mean total per animal/ month, for example: cow X in the 14th days of lactation produced 16.5 liters and with 28 days of lactation it produced 21.6 liters, corresponding to a daily mean value of 19.05 liters of milk produced during this period, or corresponding

a total of 571.5 liters (19.05 liters x 30 days) produced in the first month of lactation. This value was calculated for each month of lactation for each animal, and subsequently the curve of lactation of cows throughout lactation was calculated.

The milk production of each animal at 305 days of lactation was evaluated, according to pre-established criteria by the IDEAGRI software (2021). This calculation was performed to compare the milk production of cows in different lactations. For both calculations, only milk data from cows that completed lactation two months before the next calving were considered. Animals that demonstrated any concomitant disease during lactation, with mastitis for example, were not included in this analysis.

In addition, to prevent a large infestation by *Haema-tobia irritans* that could influence in the milk production, each cow was treated with insecticide-containing ear tags containing diazinon 6 g (Top Tag® - Zoetis, Brazil), which were placed in the left ear of each animal. The ear tags were replaced with new tags every six months until the end of the study.

Experiment 2 - Experimental infestation of R. microplus in ¹⁵/₁₆ Girolando males *of different categories/age*

Due to the results obtained in the experiment 1, it was decided to perform a second experiment to evaluate the quantity of fully engorged females ticks detached from experimentally infested animals with different ages and previous contact or not with *R. microplus*.

Location, animals, infestations and counting of engorged *R. microplus* females detached from the animals

This trial was conducted from October - December 2020 in the Large Animal Barn of the School of Veterinary Science and Animal Husbandry of the Federal University of Goiás (EVZ-UFG). Twelve uncastrated male cattle $(^{15}/_{16})$ Girolando) of different categories/age from a dairy farm (Céu Azul Farm) located in the municipality of Silvânia, state of Goiás were used. These animals did not receive any antiparasitic treatment for the prior 90 days; in the case of animals with seven days old (newborn), they did not have contact with antiparasitic drugs since birth. All animals were identified using numbered ear tags. At the beginning of the experimental infestations with the cattle tick larvae, four calves were 11 days old without previous contact with R. microplus since birth, called newborns; another four with 12-13 months of age and with contact with the cattle tick since birth; and four with 23-24 months of age and rearing in contact with R. microplus since birth.

The category of newborn calves (with seven days of life at the beginning of the experiment) were born on the Céu Azul farm on a concrete floor, where they received colostrum, and within 48 h after that, they were transported to the EVZ-UFG barn (D-35). All animals were housed individually in pens of 9m² with slatted floor during the entire experiment and these newborn calves received seven liters of milk substitute (Nattimilk®-Auster) daily, in addition to water and feed *ad libitum*. From the 20th day of life, silage and 2 kg of commercial feed were provided *ad libitum* for these animals as well. The animals aged 12–13 and 23–24 months old were brought newborn from the farm and kept in pastures at EVZ-UFG where they have contact with ticks. To this second experiment, on D-35 and were allocated individually in pens with slatted floor in EVZ-UFG barn. The animals received corn silage, feed, and mineral supplement *ad libitum* during the entire period.

The period between D-35 and D-25 was considered as the animals' acclimatization period. After this initial stage, all animals were infested with ~10,000 *R. microplus* larvae (originating from 0.5 gram of eggs) with a mean age of seven to 14 days, on days -24, -22 and -21. In total, each animal received ~ 30,000 *R. microplus* larvae. The pen door was opened, and the animal's identification checked. Then the animals were restrained (tied) to be infested with ticks in the pen itself. Syringes with the tip cut containing the larvae were applied gently along the dorsal and/or lateral line of the animal. The bovines were restrained from approximately 60 min to avoid the animal remove the larvae and allowing them to move into the coat and choose an attachment site.

The *R. microplus* strain used was GYN (Duque et al., 2021) and is currently maintained in the Veterinary Parasitology Center – EVZ/UFG, fed on cattle during the parasitic phase and allocated in a BOD incubator (27 °C, 80%RU) during the free-living stage. On days 0 until + 10, fully engorged *R. microplus* females that detached from each animal were counted. All pens were washed daily in the morning period (between 08:00 and 09:00 a.m.). Then, the engorged females were counted manually. At day + 10, the animal phase was finalized, and all animals received spray acaricide treatment with cypermetrin + chlorpyriphos (Colosso® spray – Ouro Fino Animal Health), in accordance with the test results of the Adult Immersion Test (Drummond et al. 1973) performed.

Statistical analysis

Data obtained from the counts of *R. microplus* females between 4.5 and 8 mm of length (experiment 1), or fully engorged *R. microplus* females that detached from the animals (experiment 2), were log transformed using the equation $\ln (x+1)$ and analyzed in an entirely randomized design. The data complied with the assumptions of normality and homogeneity of variances and residuals after the transformation. Means counts of *R. microplus* obtained of each category during the two years of study, were compared using Tukey's test (Proc GLM, SAS, 2016). To the total of treatments performed in each category in the two years, was used the Fisher's Exact Test.

Means of tick counts, prevalence of animals infested with *R. microplus* and animal stocking rate were described per month for each animal category. Month x prevalence and stocking rate x prevalence data were analyzed within each category, in a completely randomized design, using the Kruskal-Wallis test. Correlation analyzes were performed, within the same category, between mean tick count x prevalence x stocking rate.

In addition, the variables mean tick counts, animal category and stocking rate considering over the two years of the study were submitted to multiple regression analysis. After that, the variables mean tick counts of each animal category of the two years and the mean stocking rate over the two years, for each animal category, were subjected to linear regression analysis with the calculation of Pearson's correlation coefficients. Variables with $P \le 0.05$ and with the highest coefficients of determination ($R^2 \ge 0.70$) were considered the best descriptors, where $R^2 \ge 0.70$ was considered a strong correlation and $R^2 \le 0.69$ a not so strong correlation.

The data regarding milk production of the animals and R. microplus counts met the prerogatives of homogeneity of variance, normality and residue analysis. Subsequently, three correlation and regression analyze were performed. The first was between the curve of lactation (total milk in liters produced per month per cow during lactation) and tick counts; the second between the total milk in liters produced by each cow in 305 days of lactation in different lactations, with tick counts; and the third, was between the total of milk in liters produced by each cow in 305 days of lactation, and tick counts, independently of the lactation. The first and the second analyses were performed to show if there is a correlation between milk production and tick counts during a lactation, or between the different lactations, and the third analyze was performed to determine if the cows that more produce milk are the ones that show more R. microplus. For these linear regressions, variables with $P \le 0.05$ and the highest coefficients of determination ($R^2 \ge 0.70$) were considered the best descriptors. The reliability level was 95%. Only milk and tick count data from cows that completed lactation two months before the next calving were included in the correlation and regression analyzes.

Fig. 2 Mean tick count of each animal category over the two years and stocking rate in the period of the study: nursing calves reared individually up to 3 months of age; nursing calves reared collectively up to 3 months of age; weaning calves with 4 up to 8, 9 up to 12 and 13 up to 16 months of age; heifers with 17 up to 20, and with 21 up to 29 months of age, lactating cows and dry $cows \ge 30$ months of age



Fig. 3 Mean tick counts performed during September 2015 to September 2017 in Simmental nursing calves. A: nursing calves individual rearing up to 3 months of age; B: nursing calves collective rearing up to 3 months of age. * = A caricide treatment; red arrow = tick peak

Results

100

90

50 cont of R.

40

30

20

10 n

lost 80

microplus on 70 60

Mean

Experiment 1

The mean tick counts, tick peaks, and acaricide treatments performed in each group over the two years (September 2015 -August 2017) of the study are shown in Figs. 2, 3, 4 and 5, being Fig. 2 the results of mean tick count between the categories including the two years. Figures 3, 4 and 5; Tables 1, 2, 3, 4 and 5, showing the tick peaks and treatments over the years of the study from, nursing calves, weaning calves/heifers and lactating/dry cows, respectively.

The mean tick count of the categories including the two years was statistically different ($p \le 0.05$), where the weaning calves of 4-8 months of age showed the higher mean tick burden than other categories (Fig. 2). Between the nursing calves reared individually and collectively, the animals from the first category showed a tick burden less than the second category ($p \le 0.05$). Animals from 4 to 29 months (weaning calves/heifers) showed a tick burden inversely proportional to the age ($p \le 0.05$). In other words, younger animals from these mentioned categories were more infested by R. microplus. However, when the animal become lactating the tick burden increase, and decrease again in dry cows ($p \le 0.05$) (Fig. 2).

In some categories (both nursing calves and heifers 13-16 months of age) three and four tick peaks were observed in the year 1 and 2, respectively. The tick peaks stood out in the weaning calves of 4–8 months of age (Fig. 4 A), heifers of 9-12 months of age (Fig. 4B) and lactating cows (Fig. 5 A) with five peaks per year each. While heifers with 17-20 (Fig. 4D) and 21–29 months of age (Fig. 4E) showed the less quantity of tick peaks (n=3 peaks per year). The weaning calves (4-8 months of age, Fig. 4 A and Table 6) and lactating cows (\geq 30 months of age, Fig. 5 A and Table 6) received more treatments ($p \le 0.05$), being 18 and 15, respectively. The nursing calves reared individually (until 3 months of age, Fig. 3 A and Table 6) and the pregnant heifers (21-29



Fig. 4 Mean tick counts performed during September 2015 to September 2017 in Simmental weaning calves and heifers. A: weaning calves with 4 up to 8 months of age; B: weaning calves with 9 up to 12 months of age; C: weaning calves with 13 up to 16 months of age; D:

heifers able to reproduce with 17 up to 20 months of age; E: pregnant heifers with 21up to 29 months of age. * = Acaricide treatment; red arrow = tick peak



Fig. 5 Mean tick counts performed during September 2015 to September 2017 in Simmental $\cos \ge 30$ months of age. A: lactating cows; B: dry cows. * = Acaricide treatment; red arrow=tick peak

months of age, Fig. 4E; Table 6) were the categories that received only two acaricide treatments ($p \le 0.05$). The biological parameters of the ticks collected in December of 2016 from weaning calves 4–8 months of age were female weight of 2.190 g, egg mass weight of 0.933 g, 98.3% of hatchability and reproductive efficiency of 837,569.86. For the heifers 20–29 months of age the same parameters were 2.193 g, 0.672 g, 72.60% and 444,935.70, respectively.

During the experiment period the rainfall in the year 1, ranged from 1 to 451 mm and 5-321 mm in year 2 (Fig. 6 A). In the year 1 the rain volume (2,269 mm) was higher than the rain volume (1,659.25) of the year 2. However, the rain was more constant in year 2. It was observed a lack of rain in April of the year 1. The mean of the maximum temperature during the first year and second year of the study ranged

from 26 to 38 °C and 27–35 °C, respectively (Fig. 6B). The mean of the medium temperature was 19–28 °C and 19–27 °C (Fig. 6 C). While the mean of the minimum temperature was 11-21 °C and 11-19 °C (Fig. 6D).

There was no correlation (p>0.05) between the results of mean tick counts evaluated per month with the prevalence of cattle infested by *R. microplus*, stocking rate and prevalence. By the multiple regression analysis performed considering the two years of study, no correlation was observed between mean tick counts, animal category and the mean stocking rate [ticks=32.93+(category*-0.64) + (stocking rate*0.74); r=0.21; R²=0.05; p=0.8682]. Moreover, through regression analysis, no correlation (r=0.1746; R² = 0.0305; p=0.6529) was observed between mean tick

rate of nursing c	alves (indiv	vidual and co	ollective) during	the study					4		•	N)
Nursing calves -	individual						Nursing calves	s - collectiv	9				
Month	Tick mean	Range	Number of animals with	Total of animals	Prevalence of infested	Stocking rate	Month	Mean count	Range	Number of animals with	Total of animals	Prevalence of infested	Stock- ing
	count		ticks		anımals					ticks		anımals	rate
September	1.1	0 to 1	3	15	20.0	1.2	September	2.9	0 to 18	8	14	57.1	1.2
October	1.0	0 to 1	3	19	15.8	1.5	October	2.6	0 to 12	7	14	50.0	1.2
November	1.0	0 to 1	3	18	16.7	1.5	November	4.1	0 to 13	14	16	87.5	1.4
December*	46.5	0 to 123	15	17	88.2	1.5	December*	68.7	17 to 156	18	18	100.0	1.6
January	3.1	0 to 8	13	17	76.5	1.4	January	9.3	0 to 42	17	19	89.5	1.8
February	14.1	5 to 32	15	15	100.0	1.3	February*	39.1	1 to 82	16	16	100.0	1.4
March	6.0	1 to 12	13	14	92.9	1.4	March	10.2	0 to 45	20	24	83.3	2.3
April	1.9	0 to 4	8	14	57.1	1.4	April	2.5	0 to 10	20	28	71.4	2.7
May	25.4	0 to 141	15	17	88.2	1.4	May*	<i>T.T.</i>	0 to 210	20	22	9.09	2.1
June	1.5	0 to 4	5	21	23.8	1.8	June	5.8	0 to 78	6	29	20.7	2.8
July	1.1	0 to 0	2	17	11.8	1.5	July	0.4	0 to 5	4	21	19.0	2.0
August	1.1	0 to 0	2	18	11.1	1.5	August	0.3	0 to 5	2	24	8.3	2.3
September	5.9	0 to 14	14	16	87.5	1.3	September	11.9	0 to 76	15	24	62.5	2.3
October	1.1	0 to 0	0	19	0.0	1.5	October	0.2	0 to 1	4	21	19.0	2.0
November	6.6	0 to 21	14	14	100.0	1.3	November	15.1	0 to 45	15	17	88.2	1.5
December*	35.3	5 to 145	17	17	100.0	1.3	December*	176.1	46 to 471	19	19	100.0	1.8
January	7.7	1 to 14	13	13	100.0	1.3	January*	56.7	25 to 85	23	23	100.0	2.2
February	2.1	0 to 5	8	10	80.0	1.3	February	25.0	0 to 98	16	17	94.1	1.5
March	2.3	0 to 4	5	9	83.3	1.2	March*	38.7	0 to 201	13	14	92.9	1.2
April	2.0	0 to 5	5	9	83.3	1.2	April*	41.7	2 to 102	18	18	100.0	1.6
May	18.5	0 to 65	10	11	90.9	1.3	May^*	42.9	5 to 154	20	20	100.0	1.9
June	0.6	0 to 3	5	14	35.7	1.3	June	4.2	0 to 210	11	17	64.7	1.5
July	5.7	0 to 33	4	12	33.3	1.4	July	29.3	0 to 105	23	25	92.0	2.4
August	0.0	0 to 0	0	18	0.0	1.6	August	3.1	0 to 32	10	24	41.7	2.3
Mean	6.3		1	14.9	1	1.4	Mean	27.9	I	1	20.2	1	1.9
* Indicates when	n the anima	als of the cat	tegory were trea	ted with aca.	ricide								

Table 1 Month of the year, mean tick counts, range of the tick counts, number of animals with ticks, total of animals of the paddocks, prevalence of animals infested by *R. microplus* and stocking

rate of weaning (calves (4-	8 months and	9–12 months) di	uring the stud	ly				mond (moon		to page 10		0
Weaning calves	4-8 mont	JS					Weaning calve	s 9–12 mo	nths				
Month	Tick mean count	Range	Number of animals with ticks	Total of animals	Prevalence of infested animals	Stocking rate	Month	Tick mean count	Range	Number of animals with ticks	Total of animals	Prevalence of infested animals	Stock- ing rate
September*	57.0	0 to 121	27	28	96.4	2.1	September	8.2	0 to 39	12	24	50.0	1.9
October*	49.6	32 to 62	17	17	100.0	0.9	October	2.9	0 to 21	8	34	23.5	3.0
November*	81.9	37 to 161	21	21	100.0	1.3	November	19.1	0 to 32	19	24	79.2	1.9
December*	45.1	34 to 75	21	21	100.0	1.3	December	9.7	0 to 21	20	21	95.2	1.6
January*	88.0	32 to 210	20	20	100.0	1.2	January*	31.4	2 to 116	24	24	100.0	1.9
February*	42.9	25 to 64	23	23	100.0	1.5	February	14.9	5 to 32	23	23	100.0	1.8
March*	101.7	34 to 147	25	25	100.0	1.8	March*	46.5	16 to 63	23	23	100.0	1.8
April*	79.4	25 to 167	25	25	100.0	1.8	April	13.9	0 to 21	22	23	95.7	1.8
May*	282.4	85 to 174	29	29	100.0	2.2	May*	181.7	24 to 650	24	24	100.0	1.9
June*	52.9	32 to 92	27	27	100.0	2.0	June	8.6	0 to 21	25	28	89.3	2.3
July	22.7	0 to 123	27	29	93.1	2.2	July	5.5	0 to 33	21	26	80.8	2.1
August	13.2	0 to 35	28	30	93.3	2.3	August	19.9	0 to 34	21	24	87.5	1.9
September*	85.0	12 to 145	21	21	100.0	1.3	September*	97.8	14 to 275	23	23	100.0	1.8
October*	139.5	23 to 216	26	26	100.0	1.9	October*	139.3	45 to 275	27	27	100.0	2.2
November*	46.6	12 to 121	26	26	100.0	1.9	November*	44.3	15 to 72	25	25	100.0	2.0
December*	114.7	32 to 185	24	24	100.0	1.7	December*	138.9	41 to 198	24	24	100.0	1.9
January*	36.8	24 to 621	28	28	100.0	2.1	January*	41.3	25 to 56	24	24	100.0	1.9
February*	35.5	12 to 62	25	25	100.0	1.8	February*	32.2	16 to 75	31	31	100.0	2.7
March*	55.2	35 to 72	25	25	100.0	1.8	March*	59.1	21 to 85	31	31	100.0	2.7
April**	21.5	0 to 61	26	27	96.3	2.0	April	18.1	5 to 32	27	27	100.0	2.2
May*	77.4	62 to 102	28	28	100.0	2.1	May*	76.4	41 to 105	27	27	100.0	2.2
June	3.4	0 to 12	19	29	65.5	2.2	June	4.1	0 to 21	21	29	72.4	2.4
July	18.2	4 to 78	24	24	100.0	1.7	July	18.0	2 to 21	28	28	100.0	2.3
August	1.9	0 to 61	17	24	70.8	1.65	August	1.4	0 to 5	22	32	68.8	2.8
Mean	64.7	ı	·	25.1	ı	1.8	Mean	43.0	ı	,	26.1	ı	2.1
* Indicates when	n the anin	als of the cate	gory were treat	ed with acari	icide								

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rate of weaning (calves (13-	-16 months) ar	nd heifers (17–2)	0 months) du	ring the study			I	I			I	
Weaning calves	13-16 mor	ıths					Heifers 17–20	months					
Month	Tick mean	Range	Number of animals with	Total of animals	Prevalence of infested	Stocking rate	Month	Tick mean	Range	Number of animals with	Total of animals	Prevalence of infested	Stock- ing
	count		ticks		animals			count		ticks		animals	rate
September	13.5	0 to 71	19	27	70.4	4.6	September	1.9	0 to 20	7	27	25.9	6.3
October	2.0	0 to 10	8	19	42.1	2.8	October	4.1	0 to 42	14	24	58.3	5.6
November	8.4	0 to 45	7	25	28.0	4.2	November	2.6	0 to 16	17	26	65.4	6.0
December	3.0	0 to 32	8	26	30.8	4.4	December	4.2	0 to 29	7	26	26.9	6.0
January	13.4	0 to 41	24	25	96.0	4.2	January	1.2	0 to 14	11	28	39.3	6.5
February*	36.5	2 to 72	25	25	100.0	4.2	February	9.1	0 to 42	26	31	83.9	7.2
March	28.3	15 to 62	24	24	100.0	3.9	March	12.5	0 to 53	25	28	89.3	6.5
April	26.3	15 to 41	24	24	100.0	3.9	April	1.5	0 to 10	13	28	46.4	6.5
May*	123.4	34 to 470	25	25	100.0	4.2	May^*	49.7	16 to 206	26	26	100.0	6.0
June	23.5	5 to 42	25	25	100.0	4.2	June	0.2	0 to 2	4	25	16.0	5.8
July	25.8	0 to 175	12	26	46.2	4.4	July	0.1	0 to 1	4	24	16.7	5.6
August	5.7	0 to 15	19	20	95.0	3.0	August	0.1	0 to 1	2	25	8.0	5.8
September	22.5	5 to 51	22	22	100.0	3.5	September	0.5	0 to 4	8	25	32.0	5.8
October*	64.3	16 to 105	22	22	100.0	3.5	October	0.5	0 to 2	7	22	31.8	5.1
November*	34.7	29 to 42	24	24	100.0	3.9	November	3.9	0 to 18	15	23	65.2	5.3
December*	61.2	34 to 121	24	24	100.0	3.9	December	15.0	3 to 21	23	23	100.0	5.3
January	1.3	0 to 5	15	24	62.5	3.9	January*	48.7	21 to 78	24	24	100.0	5.6
February	3.8	0 to 12	23	24	95.8	3.9	February	2.4	0 to 12	15	24	62.5	5.6
March*	45.0	24 to 89	30	30	100.0	5.3	March*	35.8	28 to 46	23	23	100.0	5.3
April	24.4	15 to 35	30	30	100.0	5.3	April	6.5	0 to 21	22	23	95.7	5.3
May*	73.0	45 to 110	31	31	100.0	5.5	May^*	32.2	16 to 42	22	22	100.0	5.1
June	3.4	0 to 12	19	28	67.9	4.8	June	5.0	0 to 16	20	25	80.0	5.8
July	10.1	0 to 23	25	28	89.3	4.8	July	0.2	0 to 2	5	27	18.5	6.3
August	2.2	0 to 20	15	27	55.6	4.6	August	0.2	0 to 2	3	27	11.1	6.3
Mean	27.3	ı	·	25.2	ı	4.2	Mean	6.6	ı	ı	25.3		5.9
* Indicates when	n the anima	als of the cate;	gory were treate	d with acaric	ide								

Table 3 Month of the year, mean tick counts, range of the tick counts, number of animals with ticks, total of animals of the paddocks, prevalence of animals infested by R. microplus and stocking

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	Heifers 21–29 1	months			IIOIIIIIs, uuiii	g uic study		Lactation cow>	×30 month	ŝ				
September 8.3 01072 6 29 2071 80 September* 3651 01073 107 109 982 141 Gotober 010 0102 0 34 30 317 01073 107 109 982 111 12 November 112 0102 14 313 9103 912 971 112 292 91112 121 292 91112 121 2912 <t< th=""><th>Month</th><th>Tick mean count</th><th>Range</th><th>Number of animals with ticks</th><th>Total of animals</th><th>Prevalence of infested animals</th><th>Stocking rate</th><th>Month</th><th>Tick mean count</th><th>Range</th><th>Number of animals with ticks</th><th>Total of animals</th><th>Prevalence of infested animals</th><th>Stock- ing rate</th></t<>	Month	Tick mean count	Range	Number of animals with ticks	Total of animals	Prevalence of infested animals	Stocking rate	Month	Tick mean count	Range	Number of animals with ticks	Total of animals	Prevalence of infested animals	Stock- ing rate
October 3.77 0.023 0.0 3.47 0.023 9.0 102 9.71 12 Nevember 0.2 0.023 4 3.47 0.023 9.0 102 9.12 9.012 9.71 12 Nevember 0.2 0.012 18 3.2 0.012 18 9.0 10.37 9.94 106 9.7 100 9.2 100 100	September	8.3	0 to 72	6	29	20.7	8.0	September*	36.51	0 to 173	107	109	98.2	14.6
November 0.2 0.02 4 34 11.8 9.2 November $8.2.37$ 9.0374 87 87 100.0 9.2 December 1.3 0.012 10 23 30.3	October	0.0	0 to 0	0	34	0.0	9.2	October*	34.77	0 to 243	66	102	97.1	12.9
	November	0.2	0 to 2	4	34	11.8	9.2	November*	82.37	9 to 374	87	87	100.0	9.5
January 3.6 0.012 18 29 6.21 8.0 January* $5.4.80$ 0.0234 97 103 94.2 113 Rebruary 3.1 0.063 19 28 67.9 7.8 April 2.551 0.0239 22 106 86.8 133 April 3.3 0.061 10 28 $3.7.1$ 7.8 April 21.4 0.0239 22 106 94.3 114 116 98.3 113 May* 92.9 23.61 0.073 114 116 98.3 16 May* 92.9 23.69 0.073 114 116 98.3 16 May* 0.1 0.01 0.01 22 24 42.3 6.9 1008 1068 106 93.3 16 May* 0.1 0.01 0.01 22 26.9 1008 1008	December	1.3	0 to 12	10	33	30.3	9.0	December	16.74	0 to 89	94	106	88.7	13.9
February 214 0 to 34 19 28 67.9 7.8 February 13.13 0 to 89 92 106 86.8 13 March 3.0 0 to 31 9 28 3.2.1 7.8 March** 36.51 0 to 209 92 104 88.5 13 33 April 3.3 0 to 1 0 to 1 2 28 3.7 7.8 April 21.45 0 to 78 114 116 98.3 16 Juy 0.1 0 to 1 10 to 1 2 29 6.9 July 16.94 0 to 187 106 118 89.3 16 Juy 0.1 0 to 1 1 1 24 4.2 6.9 July 16.94 107 116 93.1 16 93.1 16 93.1 16 93.1 16 93.1 16 93.1 16 93.1 16 93.1 16 93.1 16 93.1 16 <t< td=""><td>January</td><td>3.6</td><td>0 to 12</td><td>18</td><td>29</td><td>62.1</td><td>8.0</td><td>January*</td><td>54.80</td><td>0 to 234</td><td>97</td><td>103</td><td>94.2</td><td>13.2</td></t<>	January	3.6	0 to 12	18	29	62.1	8.0	January*	54.80	0 to 234	97	103	94.2	13.2
	February	21.4	0 to 34	19	28	67.9	7.8	February	13.13	0 to 89	92	106	86.8	13.9
April 3.3 0 to 16 10 28 35.7 7.8 April 21.45 0 to 102 100 106 94.3 113 May* 92.9 33.0 0 to 1 2 2 2 66.7 7.6 May* 94.68 0 to 737 114 116 93.3 16 Muy 0.1 0 to 1 2 2 2 8.0 Junc* 58.62 0 to 377 114 116 93.3 16 Muy 0.1 0 to 1 1 2.4 4.2 6.9 Junc* 58.62 0 to 376 110 111 99.1 15 August 0.1 0 to 1 1 2.4 4.2 6.9 Junc* 58.04 0 to 187 108 116 93.1 16 August 0.2 0 to 2 2.4 2.5 0 sottober* 32.13 0 to 187 109 92.1 14 November 5.5 0 to 13 1.7 <t< td=""><td>March</td><td>3.0</td><td>0 to 31</td><td>6</td><td>28</td><td>32.1</td><td>7.8</td><td>March*</td><td>36.51</td><td>0 to 209</td><td>92</td><td>104</td><td>88.5</td><td>13.4</td></t<>	March	3.0	0 to 31	6	28	32.1	7.8	March*	36.51	0 to 209	92	104	88.5	13.4
May* 92.9 23 10 235 18 27 667 7.6 May* 94.68 0.0783 114 116 98.3 16 Jure 0.1 0 to 1 2 2 2 9 6.9 8.0 June* 58.62 0 to 376 110 111 99.1 15 Jury 0.1 0 to 1 2 24 8.3 6.9 July 16.94 0 to 154 108 116 91.1 15 91.1 16 93.2 16 16	April	3.3	0 to 16	10	28	35.7	7.8	April	21.45	0 to 102	100	106	94.3	13.9
June 0.1 </td <td>May*</td> <td>92.9</td> <td>23 to 235</td> <td>18</td> <td>27</td> <td>66.7</td> <td>7.6</td> <td>May*</td> <td>94.68</td> <td>0 to 783</td> <td>114</td> <td>116</td> <td>98.3</td> <td>16.2</td>	May*	92.9	23 to 235	18	27	66.7	7.6	May*	94.68	0 to 783	114	116	98.3	16.2
July 0.1 0.1 0.1 0.1 0.1 0.1 0.1 0.1 1 2 2 4 3 6 9 $July$ 1694 0.187 106 118 89.8 16 August 0.1 0.11 1 24 4.2 6.9 $August$ 27.08 0.154 108 116 93.1 16 September 0.5 0.03 6 24 25.0 6.9 $August$ 27.08 10.178 101 109 95.4 14 November 7.5 0.02 0.2 0.2 0.2 0.2 102 114 109 92.7 14 November 7.5 0.027 102 10 24 41.7 6.9 $November*$ $8.2.1$ 0.0178 101 109 92.7 14 November 7.5 0.027 102 10 101 109 92.7 14 November* 30.2 0.0212 2.9 0.027 114 116 98.2 16 January 15.6 0.089 15 22 6.9 $January*72.970.023711411698.216March4.60.010313225.25.27.30.0234899296.716March1.60.013132231.30.0234899294.610May1.2$	June	0.1	0 to 1	2	29	6.9	8.0	June*	58.62	0 to 376	110	111	99.1	15.0
August 0.1 $0(1$ 1 24 4.2 6.9 August 27.08 $0(0.154$ 108 116 93.1 16 September 0.5 $0(0.3$ 6 24 25.0 6.9 September* 32.21 $0(0.198$ 104 109 95.4 14 October 0.2 $0(0.2$ 5 28 17.9 7.8 $October*$ 32.13 $0(0.178$ 101 109 92.7 14 November 7.5 $0(0.78$ 10 24 41.7 6.9 $November*$ 58.21 $0(0.237)$ 114 116 98.3 16 Secomber* 30.2 $0(0.121)$ 25 26 96.2 7.3 $December*$ 58.04 $0(0.237)$ 114 116 98.3 16 January 15.6 $0(0.89)$ 15 24 6.5 5.9 $January*$ 72.97 $0(0.237)$ 114 116 98.3 16 January 15.6 $0(0.013)$ 13 25 52.0 7.1 $February*$ 38.76 $0(0.149)$ 87 92 96.7 16 March 4.6 $0(0.010)$ 211 213 $0(0.147)$ 96 92 96.7 10 March 1.2 $0(0.12)$ 21 22 31.8 6.6 $Amrh*$ 77.33 $0(0.147)$ 96 92 96.7 10 March 1.2 $0(0.12)$ 21 22 10.0 112 22	July	0.1	0 to 1	2	24	8.3	6.9	July	16.94	0 to 187	106	118	89.8	16.7
September 0.5 $0to3$ 6 24 25.0 6.9 September* 32.21 $0to 198$ 104 109 95.4 14 October 0.2 $0to78$ 10 24 41.7 6.9 November* 32.13 $0to 178$ 101 109 92.7 14 November 7.5 $0to 78$ 10 24 41.7 6.9 November* 68.21 $0to 238$ 112 114 116 98.3 16 January 15.6 $0to 121$ 25 26 96.2 7.3 $December*58.040to 23711411698.316January15.60to 13132552.07.1February38.760to 149879294.610Amch4.60to 10212391.36.6March*51.200to 33311011298.215April12.30to 122391.36.6March*51.200to 348899296.710April12.20to 12212231.86.6March*51.200to 343899296.710March4.60to 10212221.30ta 147969296.710March1.20ta 122221.000.06.6March*77.33$	August	0.1	0 to 1	1	24	4.2	6.9	August	27.08	0 to 154	108	116	93.1	16.2
October 0.2 $0 to 7$ $0 to 78$ 17.9 7.8 October* 32.13 $0 to 178$ 101 109 92.7 14 November 7.5 $0 to 78$ 10 24 41.7 6.9 November* 88.21 $0 to 288$ 112 114 98.2 15 December* 30.2 $0 to 121$ 25 26 96.2 7.3 December* 58.04 $0 to 237$ 114 116 98.3 16 January 15.6 $0 to 89$ 15 24 6.5 5.0 7.1 February 38.76 $0 to 373$ 110 112 98.2 16 March 4.6 $0 to 10$ 21 23 91.3 6.6 $March*$ 51.20 $0 to 234$ 89 92 96.7 10 April 12.3 $0 to 10$ 21 21.32 $0 to 147$ 96 96.7 10 111	September	0.5	0 to 3	6	24	25.0	6.9	September*	32.21	0 to 198	104	109	95.4	14.6
November 7.5 0 to 78 10 24 41.7 6.9 November* 68.21 0 to 237 112 114 98.2 15 December* 30.2 0 to 121 25 26 96.2 7.3 December* 58.04 0 to 237 114 116 98.3 16 January 15.6 0 to 13 13 25 52.0 7.1 February 38.04 0 to 237 114 116 98.3 16 January 15.6 0 to 13 13 25 52.0 7.1 February 38.76 0 to 373 110 112 98.2 15 Arch 4.6 0 to 10 21 23 91.3 6.6 April 21.20 0 to 147 96 97.0 12 May 1.2 0 to 12 7 222 31.8 6.4 May* 77.33 0 to 363 88 95 92.6 91.1 June 10.8 8 to 16	October	0.2	0 to 2	5	28	17.9	7.8	October*	32.13	0 to 178	101	109	92.7	14.6
	November	7.5	0 to 78	10	24	41.7	6.9	November*	68.21	0 to 288	112	114	98.2	15.7
January 15.6 $0 \ to 89$ 15 24 62.5 6.9 $January^*$ 72.97 $0 \ to 373$ 110 112 98.2 15 February 2.0 $0 \ to 13$ 13 25 52.0 7.1 $February$ 38.76 $0 \ to 149$ 87 92 94.6 10 March 4.6 $0 \ to 10$ 21 23 91.3 6.6 $March^*$ 51.20 $0 \ to 234$ 89 92 96.7 10 April 12.3 $0 \ to 10$ 21 23 91.3 6.6 $April$ 21.32 $0 \ to 147$ 96 99 97.0 12 April 12.3 $0 \ to 21$ 21 23 91.3 6.6 $April$ 21.32 $0 \ to 147$ 96 99 97.0 12 May 11.2 $0 \ to 12$ 7 222 31.8 6.4 May^* 77.33 $0 \ to 363$ 88 95 92.6 11 June 10.8 $8 \ to 16$ 222 211 0.00 6.4 May^* 77.33 $0 \ to 363$ 88 95 92.6 112 June 10.8 $8 \ to 16$ 222 $210 \ 0.0$ 5.3 $July$ 3.20 $0 \ to 783$ 73 77.9 84.8 12 June 0.0 $0 \ to 0$ $0 \ to 0$ $0 \ to 783$ 73 71.3 $0 \ to 783$ 73 101 72.3 12 June 9.1 $ 25.9$ <t< td=""><td>December*</td><td>30.2</td><td>0 to 121</td><td>25</td><td>26</td><td>96.2</td><td>7.3</td><td>December*</td><td>58.04</td><td>0 to 237</td><td>114</td><td>116</td><td>98.3</td><td>16.2</td></t<>	December*	30.2	0 to 121	25	26	96.2	7.3	December*	58.04	0 to 237	114	116	98.3	16.2
February 2.0 $0 to 13$ 13 25 52.0 7.1 February 38.76 $0 to 149$ 87 92 94.6 10 March 4.6 $0 to 10$ 21 23 91.3 6.6 March* 51.20 $0 to 234$ 89 92 96.7 10 April 12.3 $0 to 21$ 21 23 91.3 6.6 April 21.32 $0 to 147$ 96 99 97.0 12 May 1.2 $0 to 21$ 21 22 31.8 6.4 May* 77.33 $0 to 363$ 88 95 92.6 11 June 10.8 $8 to 16$ 22 22 31.8 6.4 May* 77.33 $0 to 363$ 88 95 92.6 112 June 10.8 $8 to 16$ 22 22 1100.0 6.4 June 8.30 $0 to 83$ 89 105 92.6 112 June 10.8 $8 to 16$ 22 22 1100.0 6.4 June 8.30 $0 to 83$ 89 105 82.6 112 June 0.0 $0 to 0$ 0 0 0 0 0 0 0 75.3 12 August 0.0 0 0 0 0 0 77.3 0 0 77.3 77.9 83 June 9.1 $ 25.9$ $ 7.3$ $Mean$ 3.9 $ 101$ 72.3 15 <td>January</td> <td>15.6</td> <td>0 to 89</td> <td>15</td> <td>24</td> <td>62.5</td> <td>6.9</td> <td>January*</td> <td>72.97</td> <td>0 to 373</td> <td>110</td> <td>112</td> <td>98.2</td> <td>15.3</td>	January	15.6	0 to 89	15	24	62.5	6.9	January*	72.97	0 to 373	110	112	98.2	15.3
March 4.6 0 to 10 21 23 91.3 6.6 March* 51.20 0 to 234 89 92 96.7 10 April 12.3 0 to 21 21 23 91.3 6.6 April 21.32 0 to 147 96 99 97.0 12 May 1.2 0 to 12 7 22 31.8 6.4 May* 77.33 0 to 363 88 95 92.6 11 June 10.8 8 to 16 22 21.1 0.0.0 6.4 June 8.30 0 to 83 89 105 84.8 13 July 0.0 0 to 0 0 17 0.0 5.3 July 3.20 0 to 76 63 83 75.9 8. August 0.0 0 to 0 0 117 0.0 5.3 August 1.20 0 to 783 73 101 72.3 12 August 0.1 - 25.9	February	2.0	0 to 13	13	25	52.0	7.1	February	38.76	0 to 149	87	92	94.6	10.6
April 12.3 0 to 21 21 23 91.3 6.6 April 21.32 0 to 147 96 99 97.0 12 May 1.2 0 to 12 7 22 31.8 6.4 May* 77.33 0 to 36.3 88 95 92.6 11 June 10.8 8 to 16 22 22 100.0 6.4 June 8.30 0 to 83 89 105 84.8 13 July 0.0 0 to 0 0 17 0.0 5.3 July 3.20 0 to 76 63 83 75.9 8. August 0.0 0 to 0 0 17 0.0 5.3 August 1.20 0 to 783 73 101 72.3 12 August 9.1 - 25.9 - 7.3 Mean 39.9 - 104.6 - 15	March	4.6	0 to 10	21	23	91.3	6.6	March*	51.20	0 to 234	89	92	96.7	10.6
May 1.2 0 to 12 7 22 31.8 6.4 May* 77.33 0 to 363 88 95 92.6 11 June 10.8 8 to 16 22 22 100.0 6.4 June 8.30 0 to 83 89 105 84.8 13 July 0.0 0 to 0 0 17 0.0 5.3 July 3.20 0 to 76 63 83 75.9 8. August 0.0 0 to 0 0 17 0.0 5.3 August 1.20 0 to 783 73 101 72.3 12 Mean 9.1 - - 25.9 - 7.3 Mean 39.9 - 104.6 - 15	April	12.3	0 to 21	21	23	91.3	6.6	April	21.32	0 to 147	96	66	97.0	12.2
June 10.8 8 to 16 22 22 100.0 6.4 June 8.30 0 to 83 89 105 84.8 13 July 0.0 0 to 0 0 17 0.0 5.3 July 3.20 0 to 76 63 83 75.9 8.3 August 0.0 0 to 0 0 17 0.0 5.3 August 1.20 0 to 783 73 101 72.3 12 Mean 9.1 - - 25.9 - 7.3 Mean 39.9 - - 104.6 - 15	May	1.2	0 to 12	7	22	31.8	6.4	May*	77.33	0 to 363	88	95	92.6	11.3
July 0.0 0 to 0 0 17 0.0 5.3 July 3.20 0 to 76 63 83 75.9 8.1 August 0.0 0 to 0 0 17 0.0 5.3 August 1.20 0 to 783 73 101 72.3 12 Mean 9.1 - - 25.9 - 7.3 Mean 39.9 - 104.6 - 12	June	10.8	8 to 16	22	22	100.0	6.4	June	8.30	0 to 83	89	105	84.8	13.6
August 0.0 0 to 0 0 17 0.0 5.3 August 1.20 0 to 783 73 101 72.3 12 Mean 9.1 - - 25.9 - 7.3 Mean 39.9 - 104.6 - 12	July	0.0	0 to 0	0	17	0.0	5.3	July	3.20	0 to 76	63	83	75.9	8.5
Mean 9.1 25.9 - 7.3 Mean 39.9 104.6 - 12	August	0.0	0 to 0	0	17	0.0	5.3	August	1.20	0 to 783	73	101	72.3	12.7
	Mean	9.1		ı	25.9		7.3	Mean	39.9	ı		104.6	ı	13.6

 Table 5
 Month of the year, mean tick counts, range of the tick counts, number of animals with ticks, total of animals of the paddocks, prevalence of animals infested by *R. microplus* and stocking rate of dry cows (>30 months) during the study

 Derivative 20 months
 20 months

Month	Tick mean count	Range	Number of animals with ticks	Total of animals	Prevalence of infested animals	Stock- ing
						rate
September	19.8	0 to 223	46	53	86.8	16.6
October	12.3	0 to 110	46	53	86.8	16.6
November	20.5	0 to 111	53	54	98.1	16.8
December	11.7	0 to 51	37	38	97.4	13.1
January*	33.6	20 to 53	24	24	100.0	9.9
February	13.1	0 to 56	41	50	82.0	15.9
March	8.9	1 to 21	32	32	100.0	11.7
April	28.7	0 to 145	38	51	74.5	16.1
May	25.3	3 to 78	49	49	100.0	15.7
June	10.3	0 to 138	25	46	54.3	15.0
July	15.2	0 to 150	31	53	58.5	16.6
August	1.3	0 to 7	12	34	35.3	12.2
September	0.7	0 to 6	12	32	37.5	11.7
October	15.1	0 to 102	18	33	54.5	12.0
November	5.9	0 to 69	32	48	66.7	15.4
December*	36.3	0 to 81	42	45	93.3	14.7
January	29.0	0 to 134	28	38	73.7	13.1
February	28.6	0 to 89	29	31	93.5	11.5
March*	63.3	45 to 85	23	23	100.0	9.6
April	13.4	9 to 31	30	30	100.0	11.3
May*	81.1	32 to 175	30	30	100.0	11.3
June	15.8	10 to 31	21	21	100.0	9.2
July	3.1	0 to 23	19	26	73.1	10.3
August	1.3	0 to 13	24	26	92.3	10.3
Mean	20.6	-	-	38.3	-	13.2

 Table 6
 Number of acaricide treatment performed against the cattle tick in the different dairy cattle categories during the experiment period of two years

Dairy cattle category	Total
	of treatments*
nursing calves - individual	2 ^d
nursing calves - collective	8 ^{bc}
weaning calves (4 to 8 months)	18 ^a
weaning calves (9 to12 months)	11 ^b
weaning calves (13 to 16 months of age)	7 ^{bc}
heifers able to reproduce (17 to 20 months)	4 ^c
pregnant heifers (21 to 29 months)	2 ^d
lactating cows (\geq 30 months)	15 ^{ab}
dry cows (\geq 30 months)	4 ^c
P value	0.0002
Coefficient of variation	36.5

*Total of treatments followed by the same letter in the column does not differ (P > 0.05)

counts of each animal category of the two years and the mean stocking rate over the two years.

The lactation curve in liters of milk production and the total of milk production in liters in 305 days per lactation of the cows over a two-year period are describe in Figs. 7 and

8, respectively. The regression analysis regarding the curve of lactation during one lactation showed a positive linear correlation. It was observed that from the fourth month of the lactation the milk production and the tick counts were directly proportional (R^2 =0.99, r=0.99, p<0.0001) and declined (Fig. 7). Evaluating the total of milk production of cows for 305 days in different lactation, and total of tick counts, there is a positive linear correlation too (R^2 =0.92, r=0.96, p=0.0001). The third regression analysis performed regarding of total of milk production and total of tick counts of the lactation, showed a positive linear correlation too (Y=0,2648*X+75,42; r=0.9814; R^2 =0.9632), in other words, when more milk the cow produce, more ticks this cow presented.

Experiment 2

Table 7 describes the results regarding the counts of R. *microplus* fully engorged females detached from the animals. It was possible to observe that the mean count of females quantified from newborn calves, 12–13 months old and 23–24 months old was 447.61, 119.9 and 40.4,



Fig. 6 Rainfall (mm) and temperature ($^{\circ}$ C) in the study region during the experiment per year. A: rainfall (mm³); B: mean of the maximum temperature ($^{\circ}$ C); C: mean of the medium temperature ($^{\circ}$ C) and D: mean of the minimum temperature ($^{\circ}$ C)

Fig. 7 Multiple regression analysis for milk production during one lactation (lactation curve) and *Rhipicephalus microplus* burden in relation to the month of lactation of the cows over a two-year period



respectively. The younger the cattle, the greater $(p \le 0.05)$ was the number of ticks quantified.

Newborn calves were treated with the spray formulation based on cypermethrin + chlorpyrifos on D+6, due to the high tick burden on these animals by ticks (mean of 779.7 fully engorged females/animal). In bovines aged 12–13 and 23–24 months, tick recovery occurred until D+10, when these animals also received acaricide spray treatment containing cypermethrin + chlorpyrifos.

Discussion

This study brings new results regarding tick counts, tick peaks and acaricide treatment in nine different categories from the same herd, including nursing calves to dry cows raised in the same production system. In addition, a correlation between tick burden and milk production was evaluated. It was verified that the tick count and the population dynamic varied according to the animal category within the same property. Other point is that the more productive cows showed higher tick burden. It is important to highlight



Fig. 8 Multiple regression analysis for total milk in liters production in 305 days of lactation and *Rhipicephalus microplus* burden in relation to the number of the cow's lactation over a two-year period

that some of these results has been seen in practice, but not yet scientific reported. Moreover, this fact limits the trial finding's utility for herds managed in a different way. Even that, it was observed that newborn animals are more susceptible to tick burden than older ones after an experimentally infestation.

In the current study, different dairy cattle categories presented different tick burden. The weaning calves of 4-8 months of age showed a higher tick count than others and the tick counts reduced until calving. However, the number of ticks found in the lactating cows increased being statically equal to the weaning calves of 4-8 months, and different from the dry cows. Possibly the susceptibility to tick parasitism, in the same breed, can be explained by the age, physiological and immunological state that the animals are as caused by intrinsic (hormones) and extrinsic factors (environment, management) (Jonsson 2006; Silva et al. 2010; Silva et al., 2013; Rocha et al. 2019). More ticks found in newly weaned category may be related to stressful factors that these animals suffer in the field, for example the milk diet. Nursing calves ingest about 20% of body weight per day and reach up to 1 kg of daily weight gain (Flower et al., 2001). Preventive management measures, such as the gradual withdrawal of milk from the calf are adopted to avoid health problems after weaning (Lorenz et al. 2011). Regardless, the simple removal of milk already reduces the mean daily weight gain of post-weaning calves (Jasper and Weary, 2002), in addition to these animals are usually modified from environment/paddock. Possibly these aspects end up depressing the immune system of the animals in this category, what make them more susceptible to tick parasitism and tickborne diseases (Souza et al. 2021).

On the other hand, in the present study, as the weaned animals became older, the tick burden decreased. The heifers able to reproduce (17–20 months of age) and the pregnant heifers (21–29 months of age) demonstrated lower tick burden and fewer peaks of *R. microplus*. Three peaks of *R.*

microplus were identified in years 1 and 2 of these categories, and in addition, following the pre-established criteria in this study, for the heifers able to reproduce one tick treatment was needed in year 1, and three treatments in year 2. For the pregnant heifers category, only one tick treatment was performed each year of the study. It is well known the differences in the immunological response of Bos t. taurus and Bos t. indicus breeds related to the cattle tick parasitism (Carvalho et al. 2008; Piper et al. 2009, 2010; Constantinoiu et al. 2010). However, in the literature known to us, there are no studies that have observed these aspects in different categories of the same herd/breed, which makes it difficult to discuss the results found in the present study. Anyway, a possible explanation regarding the degree of infestation of the different categories may be related to some immunological/hormonal factor in animals between 17 and 29 months of age, which is responsible for maintaining a low tick burden in these animals, when compared to other more susceptible categories. Other aspect observed in the current study which can reinforce this hypothesis are the biological parameters obtained from the engorged females ticks recovered from the most parasitized cattle in experiment 1 (weaning calves, 4-8 months) and the less one (heifers, 21-29 months) and the results obtained in the experiment 2.

In the present study, by experiment 2, possibly for immunological reasons of the host, ticks have greater difficulty in completing their life cycle, and becoming a fully engorged female in older animals ($\pm 23-24$ months of age) than in younger cattle. It is known that the host can develop an acquired resistance to tick after repeated infestation in which result in decreased numbers of engorged ticks or tick death (Wikel 1996; Piper et al. 2010; Karasuyama et al. 2020). Consequently, this situation could provide less environmental contamination by larvae. In other words, less larvae on pastures, keeping the tick burden on these animals lower. These findings are extremely important for the field, warning that the tick control in the same property needs to be performed in a personalized way for the different animal categories. Despite exist different tick susceptibility within the same breed as verified in the present study and by other works (Wambura et al. 1998; Veríssimo et al., 2002; Silva et al. 2010) and the tick burden should be considered when a control method is proposed (Nicaretta et al. 2021b). Sometimes the farmers prejudge the efficiency of a control measure adopted, however they do not consider the animal tick burden or animal category when choose this method. The results of the current study suggest that the effect may be more inherent in the animal rather than the strategy adopted. However, new studies are necessary to evaluate the process in each dairy cattle category.

In addition, the increase in the stocking rate can increases the herd tick burden (Labruna and Veríssimo 2001; Nava

Day of study	Cattle category - Ag rally detached	ge/ Mean numh	ver ¹ of fully engorg	ed Rhipicepha	lus microplus femal	es natu-	Varianc	e Analysis
	Newborns – withou since birth	t tick contact	12-13 months old tick contact	- with prior	24–25 months old prior tick contact	- with		
	Mean tick counts	Range	Mean tick counts	Range	Mean tick counts	Range	CV	Value of p
-24 -22 and -21 [*]		1	-					
0**	21.7 A	(2-37)	26.7 A	(17 - 35)	10.3 A	(5-14)	31.37	0.4638
1	301.7 A	(100–529)	85.7 AI	3 (58–119)	25.7 B	(15-46)	14.45	0.0110
2	509.7 A	(341–826)	127.3 B	(105 - 143)	21.3 C	(14-26)	7.51	0.0001
3	445.7 A	(310–562)	137.3 B	(115-141)	38.7 C	(25–56)	6.17	0.0002
4	627.3 A	(395-835)	146.0 B	(98–175)	49.7 C	(35–72)	7.01	0.0004
5	A 779.7	(658–756)	158.7 B	(97 - 201)	65.7 C	(41 - 84)	6.16	0.0002
9	NP	NP	127.3 A	(102 - 145)	60.3 B	(41-75)	6.99	>0.001
7	NP	NP	131.7 A	(135 - 145)	59.0 B	(49–75)	4.88	>0.001
8	NP	NP	139.0 A	(109 - 187)	47.3 B	(35–65)	8.34	>0.001
6	NP	NP	126.7 A	(87 - 195)	31.7 B	(28 - 35)	9.35	>0.001
10	NP	NP	112.7 A	(75–172)	35.0 B	(12-41)	18.56	>0.001
0-10	447.61 A		119.9 B		40.4 C		8.70	0.0014
¹ Mean values followed by the same letter in the line does not differ (P	> 0.05)							
* = Each animal was experimentally infested with 10.000 R. microplus	larvae in each of thos	e days						
** = Beginning of fully engorged <i>R. microplus</i> females detachment fro	m bovines and countin	<u>8</u>						

rally detached from animals experimentally infested with the cattle tick larvae belonging to different categories/age	
Table 7 Mean number of fully engorged Rhipicephalus microplus females natural	with or without previous tick contact

NP=Not performed, animals were treated due to high tick burden

et al. 2013; Teel et al. 1998, Nicaretta et al., 2020). In the present study, there was no correlation between the stocking rate of the animals and the tick burden. It is important to reinforce that probably the stocking rate is a factor that can influence the tick burden if we consider the same category/age of animal, and the period that these categories are in paddocks with high or low stocking rate. In the present study, the greatest variation in stocking rate within the same category occurred in lactating cows (8.5 to 16.7 AU/hectare; mean deviation of 1.7 including the entire study period) and dry cows (9.2 to 16.8 AU/hectare; mean deviation of 2.3 including the entire study period), while in the other categories the mean stocking rate during the study varied by approximately 0.5 AU/hectare. However, it is important to highlight that the lowest stocking rates observed for the lactating and dry cows occurred during a short period of time. For example, in October, November and December 2015, the stocking rate of lactating cows was 12.9, 9.5 and 13.9 AU/hectare, respectively. A scenario like this, occurred again for this same category between June, July, and August 2017. That is, the period of 30 days in which the animals maintained a lower stocking rate, possibly, was not enough to change the tick load on the animals of this category during this period. The results found by Nicaretta et al., (2020), in the same property in which this study was carried out, help to reinforce the hypothesis described above. In the work published by these researchers, the tick burden increased significantly in the group of cattle that were subjected to a higher stocking rate (considering only the grazing area/day), after approximately 60 days of grazing. Moreoever, if only grazing area/day is considered in the work of Nicaretta et al. (2020), the stocking rate designated to animals with more ticks, was 20.9 times higher compared to the other group of animals.

The calves raised individually showed less ticks than the calves raised collectively. The dairy calves raised individually, in a tropical system, has a limited area while those raised collectively has free access to the paddock area in which allow them a higher contact with ticks spread on the pasture by different animals. In addition, studies reported that collective housing is a risk factor to increase the problem caused by parasites in the herd (Cruvinel et al., 2020), while animals at individual system can limit the spread of diseases (Waele et al. 2010; Weiller et al. 2020). Possibly, it could explain why calves raised individually had less tick burden. Regarding the number of tick peaks and treatments, weaning calves (4-8 months) and lactating cows were the most treated animals during the experimental period. It is important to highlight that the acaricide treatments were performed when animals from the same category showed a mean tick burden \geq 30 females ticks (4.5-8 mm). In some categories, the tick peaks varied between the experimental years. This could be related with the different levels of rainfall in each year of the study, besides the animal tick susceptibility, once the non-parasitic stage of the cattle tick is influenced by temperature and humidity/rainfall and can accelerate the egg incubation, for example (Cruz et al. 2020; Nicaretta et al. 2021a). A hypothesis that could explain this in the current study, is that the constancy of rainfall occurred between September to May was more relevant than the volume of rainfall in this same period. In the first year of the study, despite the more volume of rainfall (2,269 mm), no rainfall occurred in April. While in the second year, a total volume of rainfall was lesser than year (1,659.25 mm), but there was a constant volume of rainfall between September to May. Probably, this may have influenced in more peaks of the tick in some categories in July in the second year.

In the lactating cow category, the most productive animals were the most parasitized ones as observed in other studies (Jonsson et al., 2006; Silva et al., 2013). An effect of peripartum, defined between five weeks before and five weeks after the cow birth (Silva et al., 2013), did not influenced the tick burden in the present study. In beef cattle, Hereford and Braford, was concluded that in general, the tick counts did not show relationship with productivity (Biegelmyer et al., 2017). Maybe it could be related to the age of the animals once in the study performed by these authors, the animal's age was about 18 months. In the current study, the animals with 17 up to 29 months of age naturally presented a smaller tick burden, and possibly it can be influenced the results. However, future studies should be performed. Mapholi et al. (2016) studying markers associated to host resistance to ticks in Nguni cattle (South Africa) detected polymorphisms genomic in regions associated with variation in tick burden highlighting that the genetic approach to tick control need to be carefully evaluated to select markers to have productivity animals. These results highlight that the interrelationships between genes influencing tick resistance and animal productivity until now are not yet well understood, which makes clear the need for further studies related to this subject. An alternative that could help to better understand this theme would be the use of commercially available products that evaluate the genome of dairy and beef cattle for productive characteristics (Carvalho et al. 2019; Lima et al. 2019), which aims to select genetically more and less productive animals, and then carry out studies of tick experimental infestations. In addition, these same cattle can be genetically evaluated for the presence of chromosomal segments associated to a phenotypic characteristic of resistance to ectoparasites (Gasparin et al. 2007; Regitano et al. 2008; Machado et al. 2010; Turner et al. 2010; Porto Neto et al. 2011; Mapholi et al. 2014).

Conclusion

In the present study, the weaning calves (4-8 months of age) and the lactating cows were the dairy cattle categories which presented the higher tick burden, and five tick peaks per year were observed in these categories. On the other hand, the animals with 17 to 29 months of age showed the smallest tick burden, with three tick peaks per year. Into the lactating cow's category, the animals most productive in milk were those that presented more ticks. In situations such as verified in this work, different control strategies against R. microplus should be adopted in different categories. For example, weaned calves 4 to 8 months and lactating cows need more acaricide treatment than the nursing calves individual: heifers between 17 and 29 months and dry cows like discussed in this study. These results were reinforced by the results obtained in the experiment 2, where less fully engorged R. microplus detached from older animals $(\pm 23-24 \text{ months old})$, than compared to youngers animals (newborn and 12-13 months old).

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Authors' contribution RDMJ: investigation; data curation. LLF: writing – original draft writing, review & editing. DMBZ, LMH, HVI, RBN, ASNT, LMAG: investigation. ABS: methodology, formal analysis. DSR, CMOM: writing - review & editing. VES: formal analysis, WDZL: conceptualization; methodology; data curation, writing - review & editing.

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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.

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