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Technical note

Rhipicephalus microplus infestation level and its association with climatological factors and weight gain in *Bos taurus* x *Bos indicus* cattle

Roberto Omar Castañeda Arriola [a]

Jesús Antonio Álvarez Martínez [b]

Carmen Rojas Martínez [b]

José Juan Lira Amaya [b]

Ángel Ríos Utrera [a]*

Francisco Martínez Ibáñez [c]

[a] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental La Posta, kilómetro 22.5 carretera federal Veracruz-Córdoba, Paso del Toro, Medellín, Veracruz, México.

[b] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), CENID-SAI, Morelos, México.

[c] Servicios de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA), Ciudad de México, México.

*Corresponding author: rios.angel@inifap.gob.mx

Abstract:

Tick infestation is an ongoing challenge in cattle production, but chemical control methods can pose a risk to both animals and handlers. An evaluation was done of natural *Rhipicephalus microplus* infestation, its correlation to climatological factors and its effect on weight gain in dual-purpose cattle. Individuals consisted of 31 *Bos taurus* x *Bos indicus* cattle of both sexes with an average age of 307 d. Every 28 d for 15 mo, counts of semi-engorged ticks (4.5 to 8.0 mm in diameter) were done and the animals weighed. Tick counts were done from the head to the base of the tail, including the fore and hind limbs, and the ventral region. Response variables were tick count and average weight per animal. Average tick count per animal was higher (P<0.05) in the hottest month (July) than in the other months. Calf's sex and breed group had no effect (P>0.05) on tick count. Individual weight gain decreased 34 g (P<0.05) for each semi-engorged tick per 28-day period. Tick count had a low correlation (P<0.01) with environmental temperature and relative humidity, but average weight gain was negatively and moderately correlated with tick count (-0.67; P<0.01). Animals with a high infestation level (61+ ticks) exhibited lower average weight gain (P=0.001) than those with a medium (31 to 60 ticks) or low level (0 to 30 ticks). *Boophilus microplus* infestation in dual-purpose cattle requires stricter control during high-temperature months (April to July).

Key words: Ticks, Infestation level, Weight gain, Environmental temperature, Linear regression.

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In Mexico, the tick *Rhipicephalus microplus* poses a serious risk to the livestock sector since it negatively affects both meat and milk production. It also threatens export of live cattle to the United States of America, activity that generates 700 million dollars annually[1]. This tick vector is commonly controlled by applying chemical compounds, although these are expensive and can be toxic to livestock and their handlers. Excessive and inadequate use of chemical tick control methods has generated resistance among ticks and caused a rethink of these methods[2]. Comprehensive pest management is considered the best option for controlling ticks but requires detailed knowledge of the interactions between the environment, the host and the parasite. However, this control method can maintain tick populations at low levels, keeping livestock healthy. These low levels are still sufficient to infect animals with hemotropic pathogens at an early age, thus generating immunity against them and achieving enzootic stability[3].

Adequate control of ticks in livestock requires thorough knowledge of variations in annual tick populations and of the influence of climate and management practices on these populations. The present study objective was to evaluate the degree of natural *Rhipicephalus microplus* infestation in dual-purpose cattle, identify any correlations between infestation and climatological factors, and its effect on weight gain.

Field work was done at La Posta Research Station, which belongs to the National Institute for Forestry, Agricultural and Livestock Research (Instituto Nacional de Investigaciones

Forestales, Agrícolas y Pecuarias - INIFAP), in the state of Veracruz, Mexico (19°00’49” N; 96°10’ W; 12 m asl). Regional climate is subhumid (Aw1), with a 25°C annual average temperature (35.3°C maximum, 15°C minimum), 1,641 mm annual average rainfall and 74.4% average relative humidity (RH).

Experimental animals consisted of 31 *Bos taurus* x *Bos indicus* cattle (13 heifers and 18 bulls), which were 11/16 Holstein x 5/16 Zebu (3.2%), 11/16 Brown Swiss x 5/16 Zebu (3.2%), 3/4 Holstein x 1/4 Zebu (16.1%), 3/4 Brown Swiss x 1/4 Zebu (25.8%), 5/8 Holstein x 3/8 Zebu (25.8%), 5/8 Brown Swiss x 3/8 Zebu (6.5%), undefined Holstein x Zebu crosses (16.1%), and undefined Brown Swiss x Zebu crosses (3.2%). At the beginning of the experiment average animal age was 307 d. Bulls and heifers were kept separate, under a rotational grazing system in contiguous pastures of established grasses: Tanzania (*Megatyrsus maximus*), Signal (*Urocholoa decumbens*), Pangola (*Digitaria decumbes*) and Mombaza (*Megatyrsus maximus*). Mineral salts and water were offered freely year round. During the dry season (December to May) sorghum (*Sorghum vulgare*) silage was provided *ad libitum*. The animals were vaccinated annually (August) against paralytic rabies and semi-annually (March and September) against clostridiasis. They were also treated for gastroenteric nematodes every 6 mo. A feed concentrate (18% crude protein, 70% total digestible nutrients) was provided at 1 kg per animal per day.

The population dynamics of *R. microplus* on the sampled animals was documented by counting all the semi-engorged ticks (4.5 to 8.0 mm in diameter) found on them. Samples were collected every 28 d for 15 mo, from August 2014 to October 2015. Tick collection and counting were done by the same field technician. Collections were done in the morning (08:00 h) by placing an animal in a covered handling pen and inspecting it from the head to the base of the tail, including the anterior and posterior limbs and the ventral region(4). At each sampling, animal body weight was measured using an electronic scale. No chemical tick control methods were applied during the study period. As ticks were removed from the animals they were placed in a 70% alcohol solution. All were identified and taxonomically classified at La Posta’s Animal Health Laboratory, following established criteria(5). Data on environmental temperature and relative humidity were obtained from La Posta’s weather station, which belongs to the National Network of Automated Agricultural Weather Stations of INIFAP. Rainfall data were not available for the study period.

The studied response variables were number of semi-engorged ticks (tick count) per animal, individual weight gain and average weight gain per animal. Tick count was the number of ticks collected from the left side of the animal after a period of 28 d. This variable has a high correlation (>0.90) with the number of ticks on the animal’s entire body(6). Overall average tick count per animal was 39.4, with a range of 0 to 264. Individual average tick counts were used to classify tick infestation level: 1) low (0 to 30 ticks); 2) moderate (31 to 60 ticks); and 3) high (61+ ticks). Individual weight gain consisted of the body weight (kg) gained by an animal after a 28-d period. Average weight gain per animal was calculated by dividing the sum of individual weight gain results per 28 d by the total number of measurements.

All statistical analyses were run using the SAS statistical package[7]. Tick count per animal was analyzed with the GENMOD procedure (PROC GENMOD), using a repeated measurements model that included the fixed effects of sampling month (period), calf's sex and breed group. For this variable, a Poisson distribution was declared as a subroutine in GENMOD, and a first-order autoregressive covariance structure [AR(1)] model was applied. Individual weight gain was analyzed with the MIXED procedure (PROC MIXED), using a repeated measurements model that included calf's sex and breed group as categorical variables, and tick count, environmental temperature and RH as covariates; the previously mentioned covariance structure was applied. In the analysis of individual weight gain, environmental temperature and RH were included in the statistical model, rather than month (period) of sampling. This was done because the focus was not on differences in weight gain between months, but rather on more accurate adjustment for climatological factors and production of a linear regression coefficient of individual weight gain on tick count per animal. Average weight gain was analyzed with the GLM procedure (PROC GLM), using a model that included infestation level (high, medium and low). In a preliminary analysis, the effects of calf's sex and breed group were found not to be significant (P>0.05), and were therefore not included in the definitive model. In the analyses of tick count per animal and average weight gain the differences between means were tested with the PDIFF option. A Pearson's correlation coefficient, run with the CORR procedure (PROC CORR), was used to estimate degree of association between tick count per animal and environmental temperature and RH, as well as between average tick count and average weight gain.

Average tick count per animal varied widely from a low of 6 to a high of 94, and infestation level varied correspondingly (Table 1). Average individual weight gain also varied broadly from a low of 8 kg to a high of 18 kg, with an overall average of 11.9 kg.

Table 1: Average semi-engorged tick (*Boophilus microplus*) count per animal (ATC), infestation level per animal (IL) and average weight gain per animal (AWG; kg)

Animal	ATC	IL	AWG	Animal	ATC	IL	AWG
1	27	Low	12	17	87	High	3
2	47	Medium	15	18	48	Medium	12
3	45	Medium	12	19	58	Medium	12
4	94	High	9	20	63	High	8
5	30	Low	16	21	53	Medium	11
6	45	Medium	13	22	65	High	12
7	15	Low	13	23	50	Medium	12
8	62	High	10	24	72	High	12
9	25	Low	13	25	22	Low	15
10	14	Low	15	26	36	Medium	14
11	6	Low	14	27	65	High	10
12	6	Low	13	28	39	Medium	11
13	33	Medium	12	29	64	High	10
14	32	Medium	18	30	62	High	10
15	62	High	13	31	48	Medium	11
16	15	Low	13				

Sampling month had a significant effect on tick count (P=0.0199), and the linear effect of tick count was significant for individual weight gain (P=0.0212). Infestation level (IL) had a significant effect on average weight gain (P=0.001). Average tick count was higher ($P<0.05$) in the hottest month (July) than in all other sampling months (Table 2).

Table 2: Least square means (± standard errors) for tick count, and mean temperature (Temp) and relative humidity (RH), by month

Month[£]	Tick count	Temp	RH
January	20.7 ± 2.5[ef]	18.6	86.9
February	18.5 ± 2.7[ef]	19.0	86.1
March	32.4 ± 4.5[cd]	20.8	88.0
April	45.0 ± 6.8[b]	25.2	86.2
May	43.0 ± 4.4[b]	26.2	85.6
June	45.1 ± 4.3[b]	25.9	85.4
July	62.9 ± 6.9[a]	26.8	87.7
August	39.3 ± 7.5[bc]	26.0	87.0
August 2	27.9 ± 3.2[cd]	26.5	87.8
September	24.5 ± 2.9[de]	25.1	91.0
September 2	16.3 ± 3.3[f]	25.8	89.9
October	14.4 ± 3.2[f]	24.4	87.5
October 2	5.9 ± 1.6[g]	25.2	87.3
November	32.5 ± 3.4[c]	21.5	86.0
December	17.4 ± 2.5[f]	20.6	89.4

[£]August 2, September 2 and October 2 indicate these months in 2015.

[a,b,c,d,e,f,g] Different letter superscripts in the same column indicate significant difference (P<0.05).

For example, in the most extreme cases, in the month of July the animals exhibited 46.6 and 57.0 more ticks than in September (2015) and October (2015), respectively. Tick count did not differ (P>0.05) between April (45.0), May (43.0) and June (45.1), months which had similar values for environmental temperature and RH. Again, tick count did not differ (P>0.05) between January (20.7) and February (18.5), both months with relatively low temperatures. Of note is that the tick counts in these cooler months was lower (P<0.05) than in relatively warmer months such as April, May and June. This trend is supported by the corresponding Pearson's correlation coefficient results, which indicated that tick count per animal was higher at higher temperatures (0.21; P<0.0001). There was a weaker correlation between lower RH and higher tick counts (-0.19; P<0.0001).

The annual fluctuations in tick population observed in the present data coincide with previous studies in which variations responded to regional climate conditions. For example, in a study using ¾ *Bos taurus* x ¼ *Bos indicus* cattle *R. microplus* infestation (average ticks per animal) was found to be highest in May (93) and June (82) but lowest (<10) in November and March when RH was lower than in May and June[(8)]. A more recent study using Criollo Lechero Tropical cattle in central Veracruz found that tick (*Amblyomma cajennense* + *Boophilus microplus*) infestation (ticks per animal) was highest in August (11.1 ± 0.6) and October (12.0 ± 0.6), both high rainfall months (11.9 and 17.9 mm, respectively), but lowest (2.9 ±

0.6) in May which has low rainfall (1.4 mm); environmental temperatures did not differ between these months[9]. In a study done in the dry tropics of Mexico (Culiacán, Sinaloa), infestation levels (average number of ticks per animal) in cattle were affected by temperature, with high levels (50) during the hottest months from July to October and lower levels (30) as temperatures dropped in November and December[10]. This contrasts with a report on terminal cross calves in which *B. microplus* infestation levels (ticks per animal) were higher (155 ± 10) in February and March, which are cooler and drier (22°C average temperature; 49 mm rainfall), than levels (26 ± 15) in September and October, which are hotter and have more precipitation (26.4°C average temperature; 271 mm precipitation)[11].

Males tended to have more ticks than females (29.9 *vs* 22.1 ticks; Table 3), although the difference was not significant (*P*<0.055). This generally coincides with a study done in Australia in which tick count was 90% higher in male than in female cattle, suggesting that sex hormones exercise a strong effect on parasite resistance[12]. However, the present results contrast slightly with the lack of difference in infestation levels between eight-month-old male and female calves under humid tropical conditions[11]. Another study also reported a lack of difference in tick count between bulls and heifers (3.2 ± 0.6 *vs* 3.5 ± 0.4) of the Criollo Lechero Tropical breed in Mexico[9].

Table 3: Least square means (± standard errors) and 95% confidence intervals for tick (*Boophilus microplus*) count by sex

		Confidence interval	
Sex	**Mean**	**Lower limit**	**Upper limit**
Females	22.1 ± 2.3	18.1	27.1
Males	29.9 ± 3.2	24.2	37.0

(*P*>0.05).

Infestation levels did not differ between the evaluated breed groups (*P*>0.05), although 11/16 Brown Swiss x 5/16 Zebu calves tended to have fewer ticks than those of the other breed groups (Table 4). This lack of difference may be an artifact of sample size in the present study. If the overall sample size had been larger the means probably would have had lower standard errors, allowing identification of inter-breed group differences in tick count. Previous reports do identify inter-breed group variation in tick count. A study comparing ¾ *B. taurus* x ¼ *B. indicus* to ½ *B. taurus* x ½ *B. indicus* cattle found that the former had higher tick counts than the latter in nine months of the year[8]; that is, the higher the proportion of genes from European breeds the higher the count. This is noteworthy since the 62.5 to 75.0% European breed percentage interval in the present study is lower than the 50 to 75% interval in the cited study[8], which would imply greater genetic diversity in the latter. However, inter-breed group differences in tick count have been reported between crosses with uniform proportions of *B. taurus versus B. indicus*; one study found that ½ Braunvieh-¼ Holstein-¼ Zebu calves had higher natural tick infestation levels than ½ Black Angus-¼ Holstein-¼ Zebu and ½ Red Angus-¼ Holstein-¼ Zebu calves[11].

Zebu breeds (*B. indicus*) have been reported to be more resistant to ticks than European breeds (*B. taurus*)[13,14]. For example, in one study with Nelore cattle counts for engorged *B. microplus* females increased progressively as the proportion of European genes increased: 3.3 in Nelore; 25.2 in ½ Nelore x ½ Fleckvieh; 22.5 in ½ Nelore x ½ Chianina; 21.0 in ½ Nelore x ½ Charolais; and 59.7 ticks in 3/8 Nelore x 5/8 Angus[15]. A study done in South Africa also reported higher tick counts in cattle as the proportion of European genes increased: 5.3 in Nguni (tropically adapted African breed, *B. taurus* x *B. indicus*); 24.1 in Bonsmara (5/8 Afrikaner and 3/8 Hereford or Shorthorn); and 37.4 in *B. taurus* (Hereford). A study in South Africa[16] found that the average number of ticks was 37.4, 24.1 and 5.3 in Hereford, Bonsmara (5/8 Afrikaner and 3/8 Hereford or Shorthorn) and Nguni (tropically adapted African breed, product of the combination of breeds B. taurus and B. indicus), respectively.

In one study under field conditions (i.e. natural infestation) in Australia *B. taurus* x *B. indicus* cattle were found to carry fewer ticks than *B. taurus* (Shorthorn x Hereford) cattle[12]. It has been argued that this disparity in IL may be due to Zebu breeds' exhibition of behavior aimed at avoiding ticks, their greater skin sensitivity and more frequent and thorough grooming habits in comparison to exotic *B. taurus* breeds[17]. Differences in tick counts by breed have also been reported between *B. indicus* breeds; for instance, in a study of one-year-old animals, Brahman cattle were found to have twice as many *B. microplus* ticks as Nelore cattle[18].

Table 4: Least square means (± standard errors) and 95% confidence intervals for tick (*Boophilus microplus*) count by breed group

		Confidence interval	
Breed group£	**Mean**	**Lower limit**	**Upper limit**
11/16 HO	28.7 ± 2.1[a]	24.9	33.0
11/16 BS	15.2 ± 0.4[a]	14.5	16.0
3/4 HO	48.4 ± 3.2[a]	42.4	55.2
3/4 BS	37.3 ± 5.6[a]	27.8	50.0
5/8 HO	20.7 ± 3.3[a]	15.1	28.3
5/8 BS	30.8 ± 18.4[a]	9.5	99.6
X HO	34.6 ± 5.5[a]	25.4	47.2
X BS	32.4 ± 2.4[a]	28.0	37.4

£11/16 HO= 11/16 Holstein x 5/16 Zebu; 11/16 BS= 11/16 Brown Swiss x 5/16 Zebu; 3/4 HO= 3/4 Holstein x 1/4 Zebu; 3/4 BS= 3/4 Brown Swiss x 1/4 Zebu; 5/8 HO= 5/8 Holstein x 3/8 Zebu; 5/8 BS= 5/8 Brown Swiss x 3/8 Zebu; X HO= undefined Holstein x Zebu crosses; X BS= undefined Brown Swiss x Zebu crosses.

[a](P>0.05).

The linear regression coefficient for weight gain on tick count (-0.03442 kg/tick; $P<0.05$) represents a 34 g weight loss per tick during a 28-d period. Under the studied conditions this means that in the month of July each evaluated animal lost an average of 2.1 kg of body weight. This weight loss is only slightly higher than the 28 g per adult *Amblyomma hebraeum* tick reported in Brahman, Brahman x Simmental, Sanga and Hereford bulls(19). However, the coefficient calculated here is lower than the -0.42 kg/tick/10 mo (i.e. 420 g weight loss over ten months) reported elsewhere(20). Of note is that the present results and those of the aforementioned studies are far in excess of the 4.4 g weight loss per engorged tick reported for *Rhipicephalus appendiculatus* in cattle(21).

The Pearson's correlation coefficient (-0.67; $P<0.0001$) supported the above findings in that it showed average weight gain to be negative as tick count increased. This coincides with the coefficient of -0.61 estimated between weight gain and cumulative tick count (*Amblyomma americanum*) in Angus x Zebu cattle in Texas(22). A study done in Zambia in two herds of Sanga breed cattle found that weight gain was negative (-0.72 and -0.70) and moderately correlated to tick count (*Amblyomma variegatum*)(23). Another study reported a negative and moderate correlation (-0.52) between weight gain and tick count (*A. variegatum*) in the Gudali breed (*B. indicus*) in Cameroon(24).

Compared to the animals with a high IL, those with a medium level gained 2.88 kg more weight per sampling period while those with a low level gained 3.87 kg more ($P<0.05$; Table 5). A study done in Brazil with Holstein-Zebu cattle also reported greater weight gain at low infestation levels than at medium and high levels, although it did not specify the criteria for classifying infestation level(25). In an evaluation of the effects of engorged tick load (*Boophilus microplus*; diameter >5 mm) in Norman cattle, highly-infested animals (138 to 300 ticks) weighed 24 kg less at d 125 of the test than lightly-infested animals (0 to 33 ticks)(26). There are multifold reasons why some animals have higher tick counts than others in the same environment, although several authors suggest that an animal's immunological response to ticks may affect their tick count(27-29).

Table 5: Least square means (± standard errors) and 95% confidence intervals for average weight gain (kg) by infestation level

		Confidence interval	
Infestation level	**Mean**	**Lower limit**	**Upper limit**
High	9.82 ± 0.66[a]	8.46	11.18
Medium	12.70 ± 0.61[b]	11.46	13.94
Low	13.69 ± 0.70[b]	12.25	15.12

[a,b]Different letter superscripts indicate significant difference (P<0.01).

The present results indicate a lack of adequate control of *B. microplus* infestation during the warmest months (April to July) in the study area. Calf's sex and breed group had no effect on tick counts. The correlation between average tick count and average weight gain was negative and moderate, meaning animals with high infestation levels had lower weight gain than those with medium and low levels. Tick infestation clearly affects animal productive potential and needs to be effectively controlled.

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