

Comparison of efficiency and reproductive aptitude indexes between a reference and field strains of the cattle tick *Rhipicephalus (Boophilus) microplus*, in Sinaloa, Mexico

Comparação dos índices de eficiência e aptidão reprodutiva entre uma cepa de referência e outra de campo do carrapato do gado, *Rhipicephalus (Boophilus) microplus*, em Sinaloa, México

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Abstract

The objective of this work was to study the reproductive behavior of two strains of *R. (B.) microplus*, one wild-caught (Native) and one reference strain (Media Joya) for two years, measuring the reproductive efficiency index (REI) and reproductive aptitude index (RAI). Engorged ticks from each strain were collected monthly from February 2002 to February 2004. These were weighed and kept in the laboratory until egg-laying. Oogenic masses were individually weighed and incubated until larvae emergence. REI and RAI were calculated from each sample, grouping ticks by weight in 100 mg range classes. An analysis of variance and Duncan's multiple rank tests were carried out on the data ($P < 0.05$). Pearson's correlation coefficients were estimated ($P < 0.01$) in order to determine the relationship between engorgement weight and the weight and number of laid and hatched eggs. The Native strain showed a higher engorgement weight than Media Joya strain. In both strains REI and RAI were similar in all weight classes; with a quadratic tendency in Media Joya and linear-quadratic in the Native strain ($P < 0.05$). Significant differences were observed when REI and RAI of both strains were compared along the duration of the study ($P < 0.01$) with the Native strain performing better in both indexes, including better performance during the dry season. All parameters studied showed highly significant correlation; eggs laid and eggs hatched were notably highly correlated, 0.94 and 0.91, for Media Joya strain and Native strain respectively.

Keywords: *R. (B.) microplus*, Mexico, no-parasitic stage, reproductive efficiency index, reproductive aptitude index.

Resumo

O objetivo deste trabalho foi estudar a conduta reprodutiva de duas cepas de *R. (B.) microplus*, uma coletada no campo (Nativa) e outra de referência (Média Jóia), por dois anos, calculando o índice de eficiência reprodutiva (REI) e o índice de aptidão reprodutiva (RAI). Foram coletados mensalmente carrapatos fêmeas engurgitadas de cada cepa no período de Fevereiro de 2002 a Fevereiro de 2004. Estas foram pesadas e conservadas no laboratório até ovipositar. As massas de ovos foram pesadas individualmente e incubadas até a eclosão das larvas. O REI e o RAI foram calculados para cada coleta, agrupando os carrapatos em classe de peso de 100 mg. Os dados foram submetidos a uma análise de variância e prova de classe múltipla de Duncan ($P < 0,05$). Foram calculados os coeficientes de correlação de Pearson ($P < 0,01$) para estimar a associação entre o peso dos carrapatos engurgitados e o peso e número de ovos postos e eclosionados. A cepa Nativa mostrou um peso ao engurgitamento mais alto do que a cepa Média Jóia. Os valores de REI e de RAI em ambas cepas foram similares em todos as classes de peso, mostrando tendência quadrática na Média Jóia e linear-cuadrática na cepa Nativa ($P < 0,05$). Ambos os índices por classe de peso no engurgitamento foram sempre mais altos na cepa Nativa ($P < 0,05$). Os valores de REI e de RAI de ambas cepas ao longo do estudo mostraram diferenças ($P < 0,01$), sendo a cepa Nativa a de melhor desempenho em ambos índices, incluindo um melhor desenvolvimento durante a estação seca. Observou-se uma correlação altamente significativa em todos os parâmetros, sobressaindo o número de ovos postos com o número de ovos eclodidos, 0,94 e 0,91, para a cepa Média Jóia e a Nativa, respectivamente.

Palavras-chave: *R. (B.) microplus*, México, fase exógena, índice de eficiência reprodutiva, índice de aptidão reprodutiva.

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Introduction

The cattle tick, *Rhipicephalus (Boophilus) microplus*, is widely distributed in tropical and subtropical regions of America, causing direct damage to the animals by bloodsucking as well as by transmission of diseases such as bovine babesiosis; economic losses are important due to these direct causes as well as due to chemical control costs (BYFORD; CRAIG; CROSBY., 1992; HOMER et al., 2000).

It is known that in Ixodidae ticks, the number of eggs produced is correlated with the weight of the engorged female i.e., heavier females produce more eggs or these are heavier (DIEHL; AESCHLIMANN; OBENCHAIN, 1982; CHILTON; BULL, 1991). Drummond and Whetstone (1970) created the Reproductive Efficiency Index (REI), which is defined as the number of eggs laid divided by the weight of the engorged female at the time of leaving the host, in order to estimate the reproductive aptitude of different females. Later, Chilton (1992) proposed the Reproductive Aptitude Index (RAI), which is defined as the number of eggs hatched as larvae divided by the weight of the engorged female at the time it leaves the host. RAI has the advantage over REI that it takes into account the viability of laid eggs.

The object of this work was to study the reproductive behavior of two strains of *R. (B.) microplus*, one which was collected in the field and the other a reference strain, for a period of two years, measuring the REI and RAI.

Materials and Methods

Between February 2002 and February 2004, monthly samples of engorged *R. (B.) microplus* ticks were collected from two sources: a) naturally infested cattle (Native Strain) located in a livestock farm in the Culiacan municipality, Sinaloa, Mexico; and b) from susceptible Holstein cattle that are kept artificially infested in the Cenid-PaVet (INIFAP), located in Jiutepec, Morelos, Mexico, with the Media Joya reference strain, that is susceptible to acaricides, organophosphates, pyrethroid and amidines, and free of *Anaplasma* and *Babesia* infection. The Media Joya strain was collected at the municipality of Talpalpa in the state of Jalisco, Mexico, and it has been maintained in laboratory conditions for 20 generations, being used only in research protocols. A total of 30 engorged females were obtained from each strain in each collection, with the following protocol: the Media Joya

strain was collected 21 days post-infestation and for the Native strain, on the day of the visit, ticks were collected ticks that were observed to be more engorged, taken from 10 animal crosses of Zebú (*Bos indicus*) × Holstein (*Bos taurus*), selected at random; in both cases the ticks were removed directly from different body regions of the cattle. In each strain, of the total of collected ticks only those were selected that had ≥10 mm of length. Later, these were weighed and placed individually in glass vials and incubated at 27 °C and 80% relative humidity until eggs were laid. The oogenic masses were individually weighed and incubated under the previous conditions until larvae hatched. Finally a portion of approximately 100 eggs was taken from the egg mass in each vial and observed under a stereoscopic microscope in order to classify them as hatched, when only the shell was present, or not hatched, when the whole egg remained.

REI and RAI measures were calculated from each monthly sample in matched pairs according to the recommendations in the literature (DRUMMOND; WHETSTONE, 1970; CHILTON, 1992), grouping the engorged ticks in 100 mg weight ranges. Data was analyzed with an analysis of variance and Duncan's multiple rank tests ($P < 0.05$). Pearson's correlation coefficients were calculated ($P < 0.01$) in order to estimate the association between engorged tick weight, and the weight and number of eggs laid or hatched (STEEL; TORRIE, 1992).

Results

The Native strain showed a higher engorgement weight than the Media Joya strain (Table 1), with the latter within a range of 100 to 200 mg in weight ($n = 449$, 62.6%), while the former had a range of 210 to 300 mg ($n = 410$, 58.6%).

The REI in the Media Joya strain (Table 1) was similar in the three engorgement weight ranges ($P > 0.05$), with a quadratic trend ($P < 0.02$). This index was similar in the Native strain in the four weight ranges ($P > 0.05$) and showed a linear ($P < 0.001$) as well as quadratic ($P < 0.007$) trend. When REI was compared between strains by weight range, the Native strain invariably had higher REI ($P < 0.05$) than the Media Joya strain. The RAI of the Media Joya strain (Table 1) was similar among the three engorgement weight ranges ($P > 0.05$) with a quadratic trend ($P < 0.04$); a similar result was obtained for the Native strain with no differences among weight ranges ($P > 0.05$), although a linear and quadratic trend was observed ($P < 0.02$). When RAI

Table 1. REI and RAI values for each range of engorgement weight (IW) in two strains of *R. (B.) microplus* (Media Joya and Native).

Weight range (mg)	n		IW		REI		RAI	
	Media Joya	Native	Media Joya (X ± S.D)	Native (X ± S.D)	Media Joya (X ± E.E)	Native (X ± E.E)	Media Joya (X ± E.E)	Native (X ± E.E)
100 – 200	449	87	172.7 ± 19.7	182.9 ± 15.3	7.5 ± 0.14 ^a	12.5 ± 0.27 ^a	4.6 ± 0.10 ^a	9.1 ± 0.23 ^a
201 – 300	263	410	236.3 ± 23.2	262.7 ± 25.1	6.6 ± 0.14 ^a	10.4 ± 0.08 ^b	4.1 ± 0.11 ^a	8.1 ± 0.07 ^b
301 – 400	8	213	309.9 ± 8.9	331.9 ± 19.7	7.9 ± 1.08 ^a	9.7 ± 0.10 ^{bc}	5 ± 0.075 ^a	8 ± 0.09 ^b
401 – 500	0	10		403.9 ± 3.1		9.4 ± 0.37 ^c		7.9 ± 0.31 ^b
Linear			0.001	0.001	0.457	0.001	0.319	0.042
Quadratic			0.250	0.497	0.023	0.007	0.045	0.023

*Values with same literal in a column indicate no significant difference ($P > 0.05$).

was compared between engorgement weight ranges, the Native strain always showed higher values than the Media Joya strain ($P < 0.05$).

The behavior of REI and RAI along the time of this study can be seen in Table 2, with significant differences between both strains ($P < 0.01$), while the Native strain showed better performance than the Media Joya strain in both indexes. The lowest REI and RAI in the Native strain were observed in September, 9.72 and 6.9% respectively, whereas the lower REI in the Media Joya strain was observed in September (6.49%) and lower RAI in July (3.38%). The highest REI were observed during the dry season, from October to May, with 10.95 to 11.87% in the Native strain, and 7.54 to 7.75% in the Media Joya strain. Furthermore, the highest RAI in the Native strain were 9.30 to 9.95% in March and April, respectively, and 5.01 to 5.25% from January to March in the Media Joya strain.

Pearson's correlation coefficients are shown in Table 3. A highly significant correlation can be observed among all tested parameters, with the relationship between number of eggs laid and the number of eggs hatched being noteworthy (0.94 and 0.91, Media Joya and Native, respectively).

Discussion

This study found that the Native strain had engorgement weights higher than the Media Joya strain; 88% of the Native strain

ticks had weights above 200 mg. This could be due to the fact that Media Joya has been maintained for several years in artificial infestation conditions. This is carried out in a single day and with a constant number of larvae, which causes higher competition for attachment sites on the host and consequently, a tendency for the larvae to be lighter in weight. On the other hand, the Native strain comes from natural infestations where larvae climb freely onto the host and only the most capable reach engorgement, showing their full reproductive capacity. Bennett (1974) reported weight ranges similar to those in this study, although higher maximums occurred in this study. Furthermore, it is noteworthy that the collection site of the Native strain has an ideal climate conditions for the development of *R. (B.) microplus*.

The egg-laying potential of an engorged female is directly related to her capacity to feed; therefore heavier females show highest values in the number of eggs laid as well as the weight of the egg mass, and after that a higher hatch rate. This would explain higher REI and RAI in the native strain during this study. It has been suggested by Passos dos Santos and Furlong (2002) that heavier females produce more eggs and thus have a higher REI. The REI and RAI observed in this study were higher than those reported by other authors (BORGES et al., 2001; CHILTON, 1992; LIU et al., 2005). The quadratic and linear trend observed in this study should be considered normal due to existent variations in female weight increase and the decrease of their capacity to lay eggs. The existence of a curved relationship between female

Table 2. REI and RAI values from two strains of *R. (B.) microplus* (Native and Media Joya) determined for each month of the study period.

Month	REI					RAI				
	Strain					Strain				
	Native		Media Joya		p	Native		Media Joya		p
Mean	EE	Mean	EE	Mean		EE	Mean	EE		
March	11.87	0.20	7.38	0.45	<0.001	9.95	0.17	5.25	0.28	<0.001
April	11.49	0.32	7.19	0.44	<0.001	9.3	0.25	4.6	0.28	<0.001
May	10.45	0.16	6.84	0.27	<0.001	8.33	0.11	3.88	0.21	<0.001
June	9.68	0.22	7.01	0.27	<0.001	7.76	0.17	3.83	0.15	<0.001
July	9.74	0.23	6.94	0.26	<0.001	7.69	0.18	3.38	0.15	<0.001
August	9.52	0.15	6.93	0.29	<0.001	8.37	0.14	3.68	0.17	<0.001
September	9.72	0.10	6.49	0.27	<0.001	6.9	0.16	3.8	0.14	<0.001
October	10.95	0.30	7.75	0.40	<0.001	8.21	0.18	4.8	0.26	<0.001
November	10	0.19	7.21	0.33	<0.001	8	0.19	4.57	0.22	<0.001
December	10.09	0.17	7.54	0.36	<0.001	8.16	0.17	4.73	0.24	<0.001
January	10.22	0.16	7.53	0.49	<0.001	8.19	0.15	5.01	0.34	<0.001
February	9.32	0.29	7.33	0.45	<0.001	7.36	0.24	4.88	0.33	<0.001

EE = standard error.

Table 3. Pearson's correlation coefficients of reproductive characteristics of *R. (B.) microplus*.

	Native strain			
	Female weight (mg)	Egg weight (mg)	Egg number	Hatched eggs
Female weight (mg)	1.00	0.40**	0.66**	0.67**
Egg weight (mg)	0.61**	1.00	0.33**	0.35**
Egg number	0.35**	0.59**	1.00	0.91**
Hatched eggs	0.33**	0.57**	0.94**	1.00

**P < 0.001.

Media Joya strain

weight and weight of the egg mass produced has been previously shown in *R. (B.) microplus* (BENNETT, 1974; CHILTON; ANDREWS, 1988; CHILTON, 1992; BORGES et al., 2001), as well as in other tick species (KOCH; DUNN, 1980; KOCH, 1982; BORGES; RIBEIRO; OLIVEIRA, 1997).

The importance of RAI stems from the fact that it demonstrates the capacity to generate a new larvae generation, which serves as evidence of the reproductive aptitude of the tick (CHILTON; BULL, 1991). The success or failure in egg viability has important biological ramifications, for example, in models that calculate reproductive success of females located at the edges of the distribution range for the species (PRICE, 1977) where not only egg-laying values are considered, but also hatchability and final larvae production. Reproductive capacity of the studied strains provides reference values that should be considered for different uses, as the information generated in the laboratory can be useful to predict behavior in the field (DE LA VEGA; DIAZ, 1996; DIAZ; DE LA VEGA, 2000; OGDEN et al., 2004).

It was observed that REI and RAI along this study were higher during the dry season, which agrees with the results of other authors (BORGES et al., 2001). Sutherst et al. (1983) and Sutherst and Bourne (2006) suggest an influence of the length of day in the natural resistance of cattle to infestations, which would allow a higher presence of adult ticks in autumn and winter, and of temperature on the emergence of larvae in grasses. Climatic conditions would favor the development of adult ticks during autumn and winter (DAVEY et al., 1980a, b; DAVEY; COOKSEY; DESPINS, 1991; DAVEY, 1988; BARRIGA; DA SILVA; AZEVEDO, 1995). Davey, Pound and Cooskey (1994) found more eggs laid in spring and autumn and the egg hatchability were essentially equal during the study. Furthermore, when the relative humidity (RH) is equal or above 75%, temperature becomes the main factor that determines larvae survival; in contrast, when RH is equal or less than 63%, it becomes the main factor substituting temperature (DAVEY; COOKSEY; DESPINS, 1991).

Davey et al. (1980a, b), Garris, Popham and Zimmerman (1990), and Gallardo and Morales (1999), when studying the non-parasitic stages of *R.(B.) microplus*, found high correlations between tick weight and the number of eggs laid and eggs hatched. Other authors have found similar relationships in other species of ticks (DRUMMOND; WHETSTONE, 1970, 1975; DRUMMOND et al., 1971; WANCHINGA; BARKER, 1986; KOCH, 1982; LIU et al., 2005). The results of this study agree with these previous reports in the literature.

A lack of standardization of initial weight in female ticks in other studies could generate unreliable conclusions; the results of this study suggest that a female *R. (B.) microplus* selected for validation or assessment of ixodicides or for epidemiological studies should weigh at least 160 mg. Females with less weight would not be a reliable representation in tests, as a lighter female will have a lighter egg mass or decreased number of eggs and a corresponding decreased viability.

To conclude, this study detected better REI and RAI in the Native strain than the Media Joya reference strain, and demonstrated that engorged females with weights greater than 160 mg have a better reproductive behavior. We suggest use of REI and RAI when attempts are made to assess this life stage in *R. (B.) microplus*.

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