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# Biological parameters of two strains (acaricide resistant and susceptible) of the tick *Rhipicephalus microplus*

# Parâmetros biológicos de duas cepas (sensível e resistente a acaricidas) do carrapato *Rhipicephalus microplus*

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## Abstract

The resistance of *Rhipicephalus microplus* to acaricides occurs in almost all regions where it is present and treatments with chemicals are the most frequent. Therefore the monitoring of ticks is crucial to diagnose resistance at an early stage to help slow down the spread of resistance and to obtain knowledge of the distribution of acaricide resistance. The objective of the present work was to know the biological parameters of *R. microplus* sensitive strain to the main acaricides used. We compared the number and weight of engorged females, as well as the mass of eggs obtained the percentage of hatching and reproductive efficiency of two strains (acaricide-resistant and susceptible) of the tick *R. microplus*, after passage in cattle. The results showed a reduced capacity to adjust the acaricide-susceptible strain, which presented the lowest number of ticks recovered ($p \leq 0.0001$), with less weight and egg mass. With respect to reproductive parameters, the ticks susceptible strain showed hatching rate eggs ($p=0.0002410$) and reproductive efficiency reduced when compared with those of the acaricide-resistant strain.
**Key words:** *Rhipicephalus microplus*, biology, reproductive rate

## Resumo

A resistência do *Rhipicephalus microplus* aos carrapaticidas ocorre em quase todas as regiões onde ele está presente e os tratamentos com produtos químicos são os mais frequentes. Portanto, monitoramento de carrapatos é fundamental para diagnosticar a resistência em um estágio inicial, diminuir a propagação e obter conhecimento da distribuição da resistência acaricida. Com o objetivo de se conhecer os parâmetros biológicos de uma cepa sensível de *R. microplus* aos principais carrapaticidas utilizados, o presente trabalho foi desenvolvido. Foram comparados o número e o peso de teleóginas, assim como a massa de ovos obtida, a percentagem de eclosão e a eficiência reprodutiva de duas cepas (sensível e resistente a acaricidas) do carrapato *R. microplus*, após passagem em bovinos. Os resultados demonstraram uma menor capacidade de adaptação da cepa sensível, que apresentou menor número de teleóginas recuperadas ($p \leq 0{,}0001$), com menor peso e massa de ovos. Com relação aos parâmetros reprodutivos, as teleóginas da cepa sensível apresentaram percentagem de eclosão ($p=0{,}0002410$) e eficiência reprodutiva reduzidas quando comparadas com àquelas da cepa resistente.
**Palavras-chave:** *Rhipicephalus microplus*, biologia, índice reprodutivo

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The tick *Rhipicephalus* (*Boophilus*) *microplus* is one of the major parasites of cattle because it not only causes reductions in weight gain, quality of coat, and production of calves and milk, but also has the capacity to transmit pathogens (ALONSO et al., 1992; PETER et al., 2005; DE LA FUENTE et al., 2008).

The main method of control involves the use of synthetic acaricides. The basis of an efficient control of *R. microplus*, and other species monoxenic, is to prevent the development of engorged females and limit their oviposition. However, continuous and indiscriminate use of acaricides leads to the selection of chemical-resistant ticks along with contamination of the environment and animal products (GRAF; GOGOLEWSKI; LEACH, 2004).

According Gonzáles (1975), the acaricides, mostly, allow a portion of the ticks survive and activate its enzymatic defense system, transmitting to future generations all characters of resistance acquired. As new product applications are made, the portion of the susceptible population dies and occur the predominance of resistant individuals. Consequently each successive treatment is a process of selection.

These facts have stimulated the development of methods for evaluating the efficacy of acaricides and the occurrence of resistance. Monitoring of ticks is crucial to diagnose resistance early stage, to help slow down the spread of resistance and to obtain knowledge of the occurrence of resistant ticks (LOVIS et al., 2011).

Although different mutations or metabolic resistance mechanisms cannot be excluded, Aguilar-Tipacamu et al. (2011) demonstrated that pyrethroid resistant tick can originate in the crossing between susceptible and resistant genotypes from the cattle tick *R. microplus*.

The identification, maintenance and knowledge of the biological parameters of a *R. microplus* strain sensitive to acaricides are of major importance for it can monitor the effectiveness of available molecules, and bases future that will be developed to control this important bovine parasite

The object of this work was to study the biological parameters of two strains (acaricide resistant and susceptible) of the tick *R. microplus*, after passage in cattle.

A total of four Holstein cattle, aged 10-14 months, were maintained in individual stables for collection of adult ticks, receiving food and water *ad libitum*. These animals were without any acaricide treatment for a minimum of 21 days and were washed with mild soap and water two days before the start of infestations.

The maintenance of ticks populations of acaricide-susceptible and acaricide-resistant for amidine, organophosphate and pyrethroid was performed in the Laboratório de Parasitologa e Doenças Parasitárias, of Centro de Ciências Agroveterinárias of Universisade do Estado de Santa Catarina (UDESC) in Lages, SC, and Laboratório de Quimioterapia Experimental em Parasitologia Veterinária, of Instituto de Veterinária of Universidade Federal Rural do Rio de Janeiro (UFRJ) in Seropédica, RJ, respectively.

The larvae infesting, acaricide-resistant and susceptible strains, were obtained from engorged females collected after engorgement and detachment from cattle and incubated on Biochemical Oxygen Demand (BOD) under conditions at 27±1°C and relative humidity (RH) of 80±10%, for oviposition. At the end of laying, egg mass was weighed and aliquots taken at 140mg (125mg + 15% safety margin for unhatched eggs). The eggs were packed in plastic syringes with a capacity of 5 mL, cut at the end next to the cannon, sealed with tissue thin plot and maintained on BOD (27±1ºC and RH=80±10%), until the hatch of the larvae that were used to infect cattle.

Animals were divided into two groups of two animals each (G1 e G2) and they were experimentally infected with approximately 2500 larvae, with 15±5 days of age, of *R. microplus*,

acaricide and susceptible strain, respectively, every two days, for 10 days for a total of five infestations for animal.

After infestation, were measured the recovery rate and weight of engorged females ticks. In order to measure the mass of ticks were counted only those who were alive, excluding the crushed and / or killed.

After weighing the ticks from each animal, we selected ten specimens with sufficient force that were then fixed dorsally, in Petri dishes and incubated for 25 days on BOD (27±1ºC e RH=80±10%), to perform the posture. At the end of laying, egg mass was weighed and recorded. The eggs obtained were packed in plastic syringes with a capacity of 5 mL, cut at the end next to the cannon, sealed with tissue thin plot and maintained on BOD (27±1ºC and RH=80±10%), until the hatch of the larvae. The hatching rate, assessed visually, was then used to calculate the reproductive efficiency (RE), using the formula:

RE = (weight of eggs/weight of engorged female) X % hatching eggs X 20,000 Data were tabulated and analyzed by the averages test by Student t test for evaluation of differences ($p \leq 0.01$) between the parameters of the sensitive and resistant strains of *R. microplus*.

In Figures 1A and 1B shows the results for the number (n) and weight average (mg) of ticks obtained per animal per day, over the period of infestation of cattle. The number of engorged female acaricide-susceptible strain recovered from cattle was smaller than that of the acaricide-resistant strain, being highly significant ($p \leq 0.0001$). This result is suggesting that the acaricide-susceptible strain ticks are more susceptible to immune response in cattle. In Table 1 are shown the numbers of engorged female recovered of acaricide-susceptible and resistant strains, during the experiment.

**Table 1**. Average of engorged females of *Rhipicephalus microplus*, resistant and susceptible strains, detached from experimentally infected cattle, per day and total average, 2010.

| Day | Average females engorged (n) | |
|---|---|---|
| | Resistant Strain | Susceptible Strain |
| 1 | 24.5 | 2 |
| 2 | 146.5 | 24.5 |
| 3 | 75 | 26 |
| 4 | 156 | 31.5 |
| 5 | 153.5 | 37 |
| 6 | 152 | 45 |
| 7 | 123.5 | 24 |
| 8 | 102 | 25 |
| 9 | 155.5 | 41.5 |
| 10 | 147 | 45.5 |
| 11 | 176 | 102.5 |
| 12 | 145.5 | 115 |
| **Total** | **129.8** | **43.3** |

**Source**: Elaboration of the authors.

The immune response of the tick *R. microplus* has a behavior similar to that of other vertebrates and invertebrates such as crustaceans, insects and mollusks, which is demonstrated by cells in the hemolymph of the tick (PEREIRA et al., 2008). Carvalho (2006) evaluating the humoral immune and inflammatory response in cattle infested with *R. microplus* found no differences in levels of IgG1 anti-saliva on evaluated breeds. But found that during periods of high infestation the levels of antibodies are minimal, with a significant increase in minor infestations. Therefore, the levels of infestation by ticks are capable of mediating the production of antibodies by animals, indeed justified due to the presence of immunosuppressive molecules in saliva.

Although not statistically significant, the average weight of engorged female of acaricide-susceptible strain obtained after passing on cattle was, in most cases, was lower those of acaricide-resistent strain (Figure 1B). This fact is important since the weight of engorged female is strongly related to the number of eggs produced by female (DAVEY et al., 1980). Thus, females with lower body mass produce fewer

eggs (Figure 1C) and thus, this factor contributes to a lower occurrence of susceptible ticks in environment when compared with the resistant strain.

Borges et al. (2001) correlated the weight of females of *R. microplus* with its conversion into eggs and evaluated the effect of the seasons on this parameter in female engorged ticks, from naturally infested cattle in State of Goiás, Brazil, under controlled environmental conditions, and verified the existence of a curvilinear relationship between weight and reproductive efficiency. Females weighing between 31 and 150mg showed a reproductive efficiency rate of 31.4 to 39%, while females weighing between 151 and 360mg had higher values (44.9 to 51.4%) and females weighing less than 150mg showed significantly lower ($p < 0.05$) than the other.

The hatching rate eggs (Figure 1D) obtained from engorged females of the strain sensitivity was lower ($p=0.0002410$) as well as reproductive efficiency (Figure 1E), although no statistical difference for the latter parameter.

There are few studies that evaluated reproductive parameters of susceptible "versus" resistant strains of *R. microplus*.

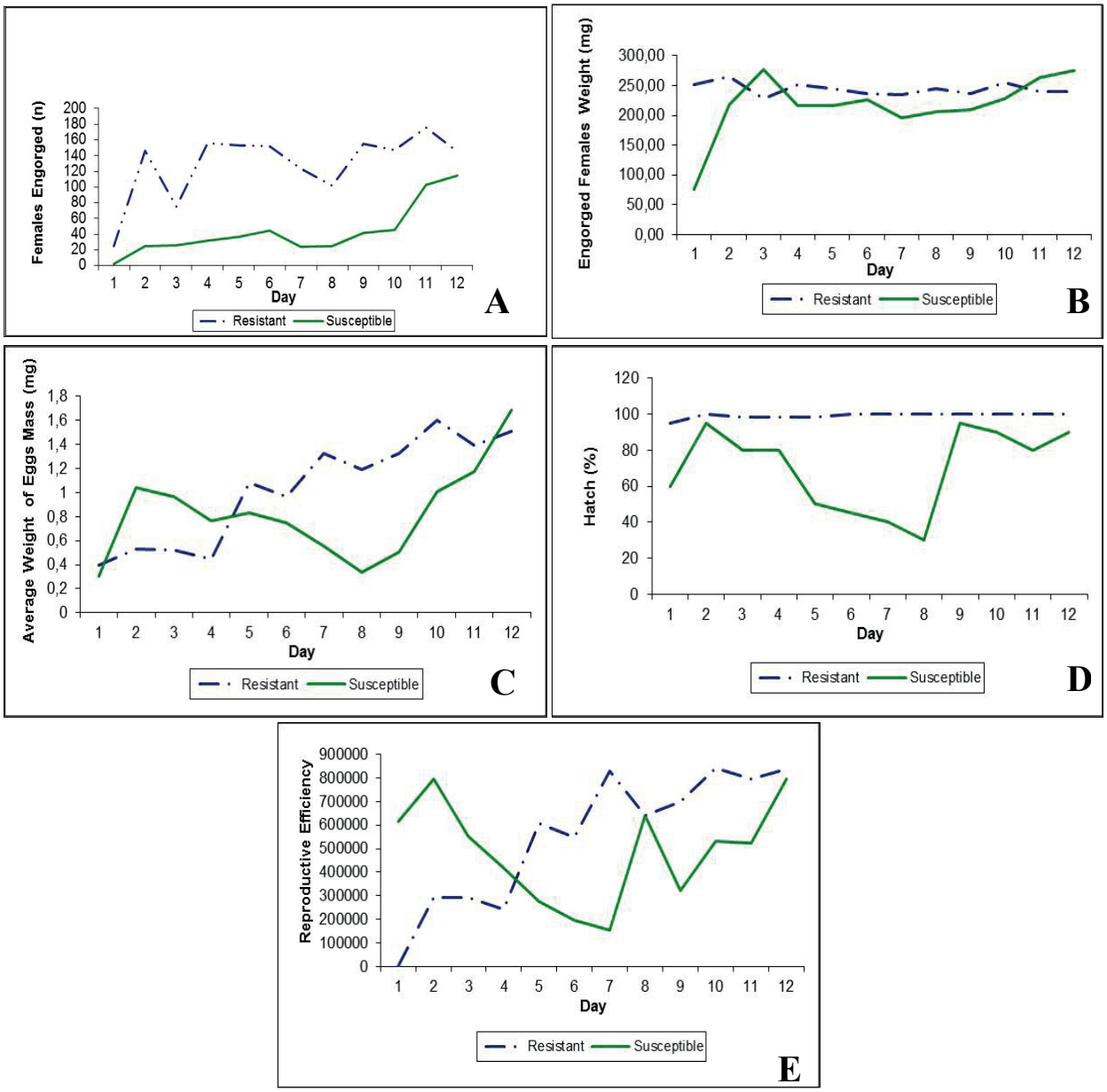
Analyzing whether acaricide resistance causes changes in the biology of *R. microlpus*, Gloria, Faccini e Daemon (1993) observed a bigger weight of engorged females of the susceptible strain (195.6 to 378.3mg) compared to the resistant strain (173.5 to 329.9mg), without significant differences in percentages on the eggs hatch between strains. The variation of Reproductive Efficiency Index was 43.93 to 64.96%, for the resistant strain, and 34.64 to 64.35% for the sensitive, and significant correlation between the weight of the egg mass and weight of engorged female was observed. They concluded that we cannot say that biology is altered with tick resistance to acaricides and there is need for more research in this area.

Davey, George e Miller (2006), making this comparison, concluded that the acaricide resistance contributes to a decrease in reproductive performance of resistant strains of *R. microplus*. Unlike, Gaxiola-Camacho et al. (2009), in Mexico and in the present study was observed that, according to the parameters analyzed, lower reproductive efficiency was observed in ticks of the susceptible strain. These contradictory results may be explained by the pressure acaricide that ticks have undergone over time in the field (DAVEY; GEORGE; MILLER, 2006).

So far there are no economic and practices alternatives for replacing the use of acaricides in the cattle tick. The selection of susceptible strains, pasture management, livestock vaccination and early detection of the occurrence of resistant strains are tools that can help in combat. The high costs to develop new molecules are limiting factors for the emergence of new products in the short term.

The results of this study indicate an adaptation of susceptible strain less compared to resistant, so the use of acaricides should exercise caution while preserving its effectiveness for longer periods in order to delay resistance.

**Figure 1.** A) *Rhipicephalus microplus* females engorged (n), susceptible and resistant strains, detached from experimentally infected cattle per day; B) Average weight (mg) of *R. microplus* engorged females, susceptible and resistant strains, detached from experimentally infected cattle per day, C) Average weight (mg) of mass of eggs of *R. microplus*, susceptible and resistant strains, obtained from experimentally infected cattle per day; D) Percentage of hatching eggs of *R. microplus*, ssusceptible and resistant strains, per day; E) Reproductive efficiency of *R. microplus* engorged females, of susceptible and resistant strains, detached from experimentally infected cattle per day.




**Source**: Elaboration of the authors.

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