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# SUMÁRIO

Interactions between ants and plants: the role of extrafloral nectaries of *Aegiphila sellowiana* Cham., 1831 (Verbenaceae) \_\_\_\_\_ **277**

**Leonardo Carvalho de Paula**  
**Kleber Del Claro**  
**Fábio Prezoto**

Analysis of biological parameters of *Boophilus microplus* (Canestrini, 1887) exposed to entomopathogenic nematode *Steinernema carpocapsae* Weiser, 1955, santa rosa and all strains \_\_\_\_\_ **279**

**Gláucia Marques de Freitas**  
**John Fulong**  
**Viviane de Oliveira Vasconcelos**

Action of the entomopatogenic nematodes *Steinernema glaseri* Santa Rosa (Steiner, 1929) and heterorhabditis bacteriophora cca Poinar, 1975 in the biological behavior of engorged females of *Boophilus microplus* (Canestrini, 1887) (Acari, Ixodidae) \_\_\_\_\_ **281**

**Viviane de Oliveira Vasconcelos**  
**John Furlong**

Effect of immersion of engorged females of *Boophilus microplus* (Canestrini, 1887) (Acari, Ixodidae) in distilled water on the biological parameters of the oviposition \_\_\_\_\_ **283**

**Gabriella Lyra Louzada**  
**Erik Daemon**

Moluscicidal and repelent activity of three *Euphorbia* (Euphorbiaceae) species on *Leptinaria unilamellata* d'Orbigny, 1835 (Gastropoda, Subulinidae) \_\_\_\_\_ **285**

**Inês Scassa Afonso Neto**  
**Geraldo Luiz Gonçalves Soares**  
**Elisabeth Cristina de Almeida Bessa**

Embryo development in the oviduct microenvironment of rats (*Rattus norvegicus* Berkenhout, 1769) treateds with *Hypericum perforatum* Linnaeus, 1753 \_\_\_\_\_ **287**

**Nathália Barbosa do Espírito Santo Borges**  
**Vera Maria Peters**  
**Martha de Oliveira Guerra**

Influence of substrate on the life cycle and behaviour  
of *Subulina octona* (Brugüiere, 1789)  
(Mollusca, Subulinidae), under laboratorial conditions \_\_\_\_\_ **289**

***Sthefane D'ávila***  
***Elisabeth Cristina de Almeida Bessa***

Moluscicidal and fago-inhibitory activity of cafein  
and thymol on three species of terrestrial gastropod  
molusks in laboratory conditions \_\_\_\_\_ **291**

***Helenize Eliza de Souza***  
***Geraldo Luiz Gonçalves Soares***  
***Elisabeth Cristina de Almeida Bessa***

Biological parameters of the parasitary phasis and of free  
life of engorged females of *Amblyomma cajennense*  
(Fabricius, 1787) (Acari, Ixodidae) in experimental infestation  
of capybaras (*Hydrochaeris hydrochaeris* L.) \_\_\_\_\_ **293**

***Márcio Caetano Brügges***  
***Erik Daemon***

# Interactions between ants and plants: the role of extrafloral nectaries of *Aegiphila sellowiana* Cham., 1831 (Verbenaceae)

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**ABSTRACT:** The natural history of the ant-plant association has been getting the researchers' attention in the world in the last centuries due to evidences that these mutualistic organisms can affect each other in several ways in their lives. About these interactions, many are formed by association of ants with extrafloral nectaries (EFNs) bearing plants. EFNs are glands of varied morphology, nectar secretor and that are not directly linked to the pollination. Extra-nectaried plants common in tropics, and in the cerrado vegetation get to represent 25% of the arboreal species. Due to the evolutionary pressure that the vegetables suffer for they be base of trophic webs, several strategies of defenses were developed, and one of them is the association with ants that can reduce the foliar herbivory, increase the amount and quality of the produced fruits, and remove herbivores. *Aegiphila sellowiana* Cham. 1831 (Verbenaceae) is a plant found in Central and South Americas and presents EFNs in the leaf blade. This study, accomplished in a transition area between cerrado vegetation and atlantic forest, in the campus of the Universidade Federal de Juiz de Fora (MG), had as main objectives to verify if EFNs attract ants, and if they supplied benefits to the plant. 51 trees were marked and divided in two groups: control (n=31) and treatment (n=20) that were isolated of

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ants for the application of resin in the stem. Between April and June of 2001 measures of the herbivory and counting of herbivores and ants were made, and among January to April of 2002 the behavior of ants visitors were registered. The results showed that the foliar herbivory maintained low percentages and didn't differ among the groups due to little abundance of chewer herbivores. But the ants were demonstrated efficient in the suckers removal that were in larger number. In plants with ants, the medium number of this phytophagous was significantly smaller. *Camponotus* was the most abundant ant genera and also the most aggressive as *visa* in works done in Brazil. The ants were efficient in the removal of the main herbivore of the plant, a Homoptera: Cicadellidae no mutualistic. The relationship between the ants and this Verbenaceae is characterized as a mutualism and these results are subject to abiotics and biotics factors as, for instance, the behavior, abundance and aggressiveness of the ants as well as the abundance and the alimentary habit of the herbivores. This way, methods that esteem with efficiency the suckers effect are fundamental. The subject of the relationship among these organisms is not closed, because the evaluation of physical and meteorological variations along the time can implicate in changes in the result of the interaction.

# Analysis of biological parameters of *Boophilus microplus* (Canestrini, 1887) exposed to entomopathogenic nematode *Steinernema carpocapsae* Weiser, 1955, santa rosa and all strains

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**ABSTRACT:** *Boophilus microplus* (Canestrini, 1887) is a species of cattle louse that causes liabilities of almost two billion dollars per year in Brazil. Due to the overall resistance of tick to commercial tick-killers, other chemical-controlled alternatives have been in the foreground, as is the case with the application of biologically controlling entomopathogenic nematodes. Ingurgitated and partially-ingurgitated females of *B. microplus* were exposed to 600, 3 000, 6 000 and 30 000 infected juveniles of *Steinernema carpocapsae*, Santa Rosa and ALL strains, per plate, under lab conditions. Exposition tests were undertaken in 5-cm diameter petri dishes, with 15 g of sterile sand. Each plate received six ingurgitated and partially-ingurgitated females. Material was kept in temperature-controlled buffer at  $27 \pm 1^\circ\text{C}$  and over 80% relative humidity. Tick were exposed to nematodes for 72 h. After this period tick were transferred to clean plates, without sand or nematodes, so that the biological parameters of *B. microplus* could be analyzed. Mass weight of eggs, pre-laying period, laying period, survival time, reproductive efficiency rate, percentage of eclosion of larvae and lethal concentrations 50% and 90%

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(CL<sub>50</sub> and CL<sub>90</sub>) were calculated for ingurgitated females. In the case of partially ingurgitated females, only mass weight of eggs, survival time and CL<sub>50</sub> and CL<sub>90</sub> were undertaken. All biological parameters of ingurgitated or partially ingurgitated females have been altered by exposition of infected juveniles of *S. carpocapsae*, Santa Rosa and ALL strains ( $p < 0.05$ ). Difference in parameter results were reported according to concentration increase in infected juveniles per plate ( $p < 0.05$ ). The CL<sub>50</sub> and CL<sub>90</sub> of ingurgitated and partially ingurgitated females exposed to *S. carpocapsae*, Santa Rosa strain, were respectively 3 596 and 169 441 and 561 and 2 392 infected juveniles per plate. In the case of tick exposed to *S. carpocapsae*, ALL strain, CL<sub>50</sub> and CL<sub>90</sub> reached 156 J.I. and 5000 J.I. for partially ingurgitated females. Results suggest that entomopathogenic nematodes may have a positive role in the control of cattle tick. However, further studies are needed so that control may be actually effective in field conditions.

**Key words:** *Boophilus microplus*, *Steinernema carpocapsae*, entomopathogenic nematodes, cattle tick, exposition test.

**Action of the  
entomopatogenic nematodes  
*Steinernema glaseri* Santa Rosa  
(Steiner, 1929) and  
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engorged females of *Boophilus  
microplus* (Canestrini, 1887)  
(Acari, Ixodidae)**

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**ABSTRACT:** *Boophilus microplus* (Canestrini, 1887) is a bovine preferential ectoparasitic incident in almost all national territory and it is associate to various diseases that induce to a decrease of the production or even the death of the animal. The main form of control of this ectoparasitic has been the acaricidal treatment, but the resistance generalized dispersion of the tick population associated with the toxic reaction in animals and humans, due to residual chemicals left in the environment, induce us to look for non-chemicals control alternatives which propitiate the reduction of damage caused by the acaricids. The entomopathogenic nematodes have been pointed as excellent candidates at the insects biological control, but due to few studies utilizing these nematodes in the ticks control, the objective was to evaluate the action of the *Steinernema glaseri* (Steiner, 1929) and *Heterorhabditis bacteriophora* Poinar, 1975 in the biological behavior of engorged females of *B. microplus*. The experiment was made from August of 2001 to July of 2002 in the EMBRAPA

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Gado de Leite, Acarology Laboratory – Juiz de Fora (MG). There were applied diferents concentrations of both nematodes species (0, 375, 500, 750, 1500, 2500, 5000 e 25000 infectives juveniles per petri dish) containing five ticks in each petri dish. The engorged females were weighed individually and incubated in a humid chamber (>80% RH) at  $26 \pm 1$  °C. After three days post-inoculation the ticks were transferred to absent petri dishes of nematodes and later were made analysis of biological parametres as well of the females mortality rate. Infectives juveniles of *S. glaseri* and *H. bacteriophora* didn't interfere on the preoviposition and oviposition period, in the eggs eclosion percentual, in the index of reproductive efficiency and index of nutricional efficiency but in some infectives juveniles concentrations of *S. glaseri* occurred reductions in the egg mass mean weight oviposited by engorged females of *B. microplus* that survived to the treatment. About the egg mass total weight, higher concentrations (as 5000 infectives juveniles) of *S. glaseri* reduced approximately 90% the quantity of eggs and in concentrations of 1500 infectives juveniles of *H. bacteriophora* occurred a reduction of approximately 80%. The females mortality rate increased linearly with the increase of concentrations of *S. glaseri*, obtaining 100% in 2,65 days of post-inoculation, before even of egg laying. In the concentrations of *H. bacteriophora*, with 2,30 days post- inoculation, was obtained 80% of mortality. Regarding effectiveness of the treatment with entomopatogenic nematodes, a mean of 80% of efficiency was observed in the concentrations of 1500 infective juveniles of *H. bacteriophora* and more than 90% in the higher concentrations of *S. glaseri*. This way we can discover the susceptibility of engorged females of *B. microplus* to infections of entomopatogenic nematodes in laboratory conditions.

# Effect of immersion of engorged females of *Boophilus microplus* (Canestrini, 1887) (Acari, Ixodidae) in distilled water on the biological parameters of the oviposition

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**ABSTRACT:** *Boophilus microplus* is an ixodid tick which attacks mainly cattle and it might rarely attack other animals like horses and sheep. This species is very important due to the serious damages it has caused to the national cattle breeding like: weight loss, skin lesions which result in poor quality leather leading to secondary infections and the transmission of pathogenic agents. There are a very few studies on the effect of being submerged in water of the engorged females over their oviposition. The present study aims to verify the effect of the submersion time of the engorged *B. microplus* based on the biological parameters related to oviposition. To do so, different periods of time have been used. 60 engorged females were used. The females were weighed on a analytic scale. After being weighed, they were split into 4 groups of 15 females each, having homogenous weight; two were used for control and the others correspond to the treatment of 24, 48 and 72 hours being submerged in distilled water. After the submersion, all of the four groups were kept in a stove at  $27 \pm 1$  °C and > 80% RH. After the beginning of the oviposition, the eggs were collected daily, weighed and placed in one-way plastic syringes, also being kept in a stove. At the end of the oviposition process, all the females of all the

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groups were weighed again to obtain information about weight loss during the oviposition process. Six out of the females which had been submerged in water for 24 hours died without carrying out the oviposition. The same happened to ten females of the group of the 48 hours and to all the females submerged for 72 hours. Just one of the five ovipositions of treatment submerged for 48 hours was fertile. The parameters were evaluated: periods of the preoviposition, oviposition and incubation, total weight of eggs, Indexes of Reproductive Efficiency (IRE) and Nutritional (INE), the percentage of hatching and the periods of hatching and the larvae survival. Among the groups there was a significant difference just in the period of preoviposition, the total weight of eggs and the Index of Reproductive Efficiency (IRE). After 24 hours being submerged in water, the preoviposition became longer, and after 48 hours there was a negative effect over the weight of the eggs and also over the IRE.

# Moluscicidal and repellent activity of three *Euphorbia* (Euphorbiaceae) species on *Leptinaria unilamellata* d'Orbigny, 1835 (Gastropoda, Subulinidae)

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**ABSTRACT:** Aquatic and terrestrial pulmonate molluscs (snails and slugs) can act as intermediary hosts of helminths. Due to their herbivorous habit, the terrestrial mollusks can provoke considerable damage in cultivated plants and, because of this many species are considered agricultural plagues. The first records on the chemical control of snails date from the beginning of the 20<sup>th</sup> century and the substances used were nonspecific toxins already employed in the control of other plagues. Just around the middle of the 20<sup>th</sup> century substances were developed specifically for the mollusks control. However, synthetic molluscicides have technical limitations that stimulated the search of natural products with similar activity. In the past years there have been discovered several plant species with molluscicidal activity. Among these we have *Euphorbia cotinifolia* L., *Euphorbia milii* des Moul. var. *splendens* (Bojer ex Hook) Ursch & Leandri and *Euphorbia tirucalli* L. which are worthy of mention due to the excellent results obtained in molluscicidal activity on aquatic snails. On the other hand, studies about the molluscicidal activity of plants against terrestrial snails are rare, despite their great parasitological and agricultural importance. *Leptinaria unilamellata* d'Orbigny, 1835, for example, is a terrestrial snail

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described as intermediary host of digenetic trematodes that affect domestic animals. The anatomical and physiologic similarities shared by the aquatic and terrestrial mollusks suggest that strategies of chemical control can have similar efficiency for both groups. Therefore, the present work had as a goal to evaluate the molluscicidal activity of the latex of *E. cotinifolia*, *E. millii* var. *splendens* and *E. tirucalli*. Due to the possibility of a toxic compound affecting the behavior of the mollusks, which can reduce the efficiency of the chemical control, in this present study it was also evaluated the repellent activity of the latex of the tested plant species. With the purpose of foreseeing the possible impact of these latex on the environment tests of toxic activity against *Artemia salina* Leach, 1812 were carried out. *E. millii* var. *splendens* showed a great molluscicidal effect on *L. unilamellata*. The latex of this plant was 100% lethal up to a dilution of 1:800 in water, and the effect on the mollusks had already been observed in the first minutes after the application. Although they are also cited in the literature as toxic for aquatic mollusks, *E. cotinifolia* and *E. tirucalli* did not exhibit molluscicidal activity on *L. unilamellata*. On the other hand, these two species showed the biggest impact on the behavior of this snail. In general, diluted latex of the three plant species provoked only a small increase in the horizontal dislocation of the animals, and just *E. cotinifolia* stimulated the appearance of vertical dislocation, an atypical behavioral act for *L. unilamellata*. All tested plants were toxic for *A. salina*, which showed mortality around 100% when treated with diluted latex to 1:10 and 1:100. In this experiment it was observed an abrupt reduction of lethality of *E. millii* and *E. tirucalli* latex in dilutions lower than 1:100, and *E. millii* was innocuous in the dilutions lower than 1:10.000. LD50 values for *A. salina* allow classification of these plants in a decreasing order of toxicity: *E. cotinifolia* (0.04 ?l/ml) > *E. tirucalli* (0.15 ?l/ml) > *E. millii* (0.66 ?l/ml). The results of this study indicate the latex of *E. millii* var. *splendens* as a promising source of watersoluble molluscicidal compounds with a reduced impact on the environment. The use of the latex of this plant can also constitute an economically viable strategy for the chemical control of terrestrial mollusks.

# Embryo development in the oviduct microenvironment of rats (*Rattus norvegicus* Berkenhout, 1769) treateds with *Hypericum perforatum* Linnaeus, 1753

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**ABSTRACT:** The understanding of the reproductive process of mammals is of great importance to the comprehension of the factors which may affect an animal's normal development. The animal's gestation occurs in specific environments, such as the macroenvironment (external), matronenvironment (maternal) and microenvironment (embryo/fetus), where a perfect integration between them is necessary for the development of the conceptus. Outside factors that interfere with these environments may produce lesions which could kill conceptus or cause malformation or growth retardation of the fetus. The embryonic developmental stage that precedes implantation has not been studied in regard to the search of toxic agents which might damage this stage because of the idea that if the fetus was injured, it would either recover or die. However, some studies have demonstrated that the embryo could be injured but not die from the injures and the resulting fetus could show malformations or growth retardation. Considering this information, the present work was aimed at verifying the effect of the administration of *Hypericum perforatum* extract, known as "Jarsin" extract, on the embryonic development before

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implantation in Wistar rats. This extract is being used specially by fertile women as an efficient alternative of the synthetic antidepressants. Female Wistar rats obtained from the animal house of the Biotério do Centro de Biologia da Reprodução -UFJF – were mated with males of proven fertility. After the insemination, the female rats were randomly distributed in groups of 10 in the following experiments: I (treatment on the first day of gestation); II (treatment on the second day of gestation); III (treatment on the third day of gestation); IV (treatment on the fourth day of gestation) and V (treatment from the first to the fourth day of gestation). The experiments were carried out with the administration of 0.5 ml of *H. perforatum* extract, by oral gavage, to a group of female rats in the morning and in the afternoon (36mg of *H. perforatum* / 1 ml of distilled water) and of 0.5 ml of distilled water to another group of female rats also by oral gavage and at the same time. The animals were killed on the fifth day of gestation and at this point the collection, counting and evaluation of the morphologic development of the embryos in the pre-implantation stage were performed. To dissociate maternal toxic effect from effect on the embryonic development, maternal body weight at the beginning and at the end of treatment and on sacrifice day; maternal food consumption during the experimental procedure, ovary weight and the number of corpora lutea, the number of deaths and the clinical signs of physical alterations, indicative of maternal intoxication, were measured. The data were analyzed by the Student's t test and the Chi-square test (level of significance  $\alpha = 0,05$ ). The results showed that no alterations were observed in the embryonic development before implantation, suggesting that *H. perforatum* doesn't seem to interfere with the matronenvironment or the oviduct microenvironment.

# Influence of substrate on the life cycle and behaviour of *Subulina octona* (Brugüière, 1789) (Mollusca, Subulinidae), under laboratorial conditions

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**ABSTRACT:** The influence of substrate on growth, reproduction and behaviour of *Subulina octona*, reared in sand, clay and humus, was investigated under laboratorial conditions. There were significant differences in growth, onset of sexual maturity, egg production and aging in each reproductive event, total number of eggs produced and total number of reproductive events. The individuals reared in humus, showed smaller shell lengths at the ages of 15 days and 30 days, and at the first, second and third reproductive events. They spent more time to reach sexual maturity, as well as to accomplish the subsequent reproductive events. Moreover, produced fewer eggs in the second, third and fourth ovipositions and exhibited a longer interval between the first and second reproductive events. After 120 days of experiment such individuals had produced fewer eggs, and performed fewer reproductive events, when compared with those kept in sand and clay. There was a progressive decline in growth rate of shell length and in egg number increase with time. This fact was observed for the individuals kept in the three kinds of substrate, demonstrating that the indeterminate growth, with a progressive decline in growth after maturity, is the strategy exhibited by *S. octona*. In this study was also observed that *S. octona* of different developmental stages fed on substrate and the molluscs reared in humus consumed less food. This is probably due to the fact

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that humus is more likely to be take as food resourse. So that, the snais reared in humus tend to consume more substrate and less ration, obtaining less proteins, carbohydrates and calcium than those snails reared in sand and clay. This low intaken of ration mighth have negative effects over reproduction of the snails. In the second part of this work the behavioural repertory and activity schedule of *Subulina octona*, reared in three different substrates were observed and described, and the existence of differences in behaviour of young and mature individuals was verified. For this, two experimental groups were maintained and observed under laboratorial conditions. Direct observations were made through the "scan" method for behaviour record. The group one was composed by 15 newly hatched and 15 mature snails, mantained in two boxes respectively. Ninety individuals, distributed in three different boxes, composed the group two. The box one contained sand, box two, clay and box three, humus. As soon as the individuals completed 45 days old, a 24-hours observational session was realized. Ninety-six hours of observation was totalized. The behavioral acts observed were resting, locomotion, feeding, eate substrate, interaction, digging, an emerging. The Youngs exhibited three activity periods: between 12:05am and 3:00pm; 05:00pm and 02:20am; 03:40am and 10:00am. The adults exhibited two periods of greater activity: between 08:50pm and 00:00; 01:15am and 04:20am. Positive correlation between activity of young and adult snails was obtained. This synchronism may have been occurred because the existence of a circadian rhythm or in response to temperature flutuations. No differences in behavioural repertories and activity schedules were observed among the snails reared in different sustrates. There was concurrence between the activities peaks and activities schedules, reinforcing the idea that the activity rythm of this species is governed by endogenous factors.

**Key Words:** Mollusca; *Subulina octona*; reproduction; growth; behaviuor; substrate.

# Moluscicidal and fago-inhibitory activity of caffeine and thymol on three species of terrestrial gastropod mollusks in laboratory conditions

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**ABSTRACT:** Pulmonate terrestrial mollusks has parasitological importance once that can act as intermediary hosts of parasite helminths of domestic animals and of the man. Moreover, terrestrial mollusks often are herbivorous and some species are cited as agricultural plagues. In spite of your economic importance, there are few studies on the control of these snails, especially about use of molluscicidal natural products. Caffeine and thymol are pointed as potentially toxic substances for aquatic and terrestrial mollusks. Thymol also is described as a chemical signal that, due to your repellent activity, give plants resistance to mollusks attack. At the present work are evaluated the molluscicidal activity of the caffeine and thymol on *Bradybaena similaris* Férussac, 1821; *Leptinaria unilamellata* d'Orbygni, 1835 and *Subulina octona* Bruguière, 1789. Moreover, it was verified the deterrence of the caffeine and of thymol on *B. similaris*. Both tested compounds showed molluscicidal activity on *B. similaris*, *L. unilamellata* e *S. octona*, but these snails have a clear difference in susceptibility to the toxic effects of these compounds. While caffeine was toxicer for *L. unilamellata* ( $CL_{50} = 0,17g/l$ ), thymol provoked the biggest mortality for *S. octona* ( $CL_{50} =$

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0,17g/l). In a general way *B. similaris* was more resistant to the toxic effects of the caffeine and thymol, which exhibited elevated deterrence for this mollusk. The results obtained at present work supply favorable indications to the use of caffeine and thymol in the chemical control of terrestrial snails that act as intermediary hosts of parasites and as agricultural plagues.

Biological parameters of the parasitary phasis and of free life of engorged females of *Amblyomma cajennense* (Fabricius, 1787) (Acari, Ixodidae) in experimental infestation of capybaras (*Hydrochaeris hydrochaeris* L.).

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**ABSTRACT:** *Amblyomma cajennense* (Fabricius, 1787) is a tick of three hosts, which possesses little parasitary speciality, but in its adult phase sponges preferably on equines, being habitual reports of occurrence in other domestic and sylvan species, amongst these, capybaras. The objective of the actual study was to evaluate capybaras as hosts of the adult phases of *A. cajennense*. For this purpose artificial infestations have been done in two capybaras using 30 couples each animal. The artificial infestations have been done in the right costal arch of two capybaras, adapting the NEITZ technic *et al.* (1971). These infestations have been accomplished in October 2000. Thirty-two females have been gathered and conveyed to the Laboratory of Biology and Ecology of Ticks of the Post-Graduation Course in Biological Sciences – Animal Behaviour and Ecology, Federal University of Juiz de Fora. In the laboratory the females have been weighted, individually arranged in Petri plates and maintained in acclimatized environment, adjusted to  $27 \pm 1$  °C and relative humidity over 80 %. The parasitical period varied from 8 to 12 days ( $10,03 \pm 1,2$  days) and the percentage of recovery was 53,3 %, having been

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discovered escapes of parasites through the container apparatus.

Three of the females died before completing the cycle. The middle-weight of the ingorged females was  $865,16 \pm 106,76$  mg; the mid-period of pre-laying of eggs and laying of the 29 females which took part on the process, was  $5,75 \pm 0,78$  and  $17,48 \pm 5,22$  days respectively; the middle-weight of the egg masses was  $442,87 \pm 168,41$  mg.

The indexes of reproductive and nutritional efficiency displayed  $52,04 \pm 8,56$  % and  $69,47 \pm 17,35$  %, respectively; the mid-periods of hatching and eclosion amounted to  $36,33 \pm 1,24$  days and  $11,09 \pm 1,33$  days, respectively, and the percentage of eclosion was  $63,57 \pm 23,77$  %.

The longevity of the larval was observed during a period of seven month.

Bigger ingorged females with a larger volume of egg mass and larval with also longer survival, associated with other parameters near to those observed in equines, show that capybaras may to serve as efficient hosts for adults *A. cajennense* and be of service as diffusion agents of these parasites.