BIOLOGICAL PARAMETERS OF TICKS (*Amblyomma cajennense* FABRICIUS, 1787) UNDER FIELD AND LABORATORY CONDITIONS IN PEDRO LEOPOLDO, STATE OF MINAS GERAIS, BRAZIL

CRISTINA M. L. LOPES[1]; PAULO R. OLIVEIRA[2]; JOÃO PAULO HADDAD[2]; LUÍSA N. DOMINGUES[2]; RIZALDO R. PINHEIRO[2]; LÍGIA M. F. BORGES[3]; MARCELO B. LABRUNA[4]; ROMÁRIO C. LEITE[2]

ABSTRACT:- LOPES, C.M.L.; OLIVEIRA, P.R.; HADDAD, J.P.; DOMINGUES, L.N.; PINHEIRO, R. R.; BORGES, L.M.F.; LABRUNA, M.B.; LEITE, R.C. **Biological parameters of ticks (*Amblyomma cajennense* Fabricius, 1787) under field and laboratory conditions in Pedro Leopoldo, State of Minas Gerais, Brazil.** [Parâmetros biológicos de *Amblyomma cajennense* (Fabricius, 1787) sob condições de campo e de laboratório em Pedro Leopoldo, Minas Gerais, Brasil]. *Revista Brasileira de Parasitologia Veterinária*, v. 17, supl. 1, p. 14-17, 2008. [2]Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais. Pampulha, Belo Horizonte, MG E-mail: rcleite@vet.ufmg.br

This study evaluated the biological parameters of the duration of the pre-oviposition and egg incubation periods and maximum survival of unfed larvae, under field and laboratory conditions, with the aim of providing support for more rational measures for controlling *Amblyomma cajennense.* This study was conducted at the Experimental Farm of the Veterinary School of the Federal University of Minas Gerais. Every 14 days, from September 1997 to March 1998 and from October 1998 to April 1999, six netted tubes containing engorged females were kept on pasture of *Brachiaria decumbens* that was up to 60 cm high and another six were kept in a BOD glass chamber (27°C, 80% relative humidity). For both groups, 84 and 72 females were used during the first and second observation periods, respectively. The duration of the pre-oviposition period was similar under both conditions (5-7 days). Significant differences were seen between the duration of the egg incubation period under field conditions (56 to 60 days) and under BOD conditions (34 to 39 days); and between the maximum survival of the unfed larvae under field conditions (14 months) and under BOD conditions (9.5 months). These suggest that behavioral diapause was occurring. The long survival of unfed larvae in pasture needs to be considered when implementing *Amblyomma cajennense* control programs.

KEY WORDS: *Amblyomma cajennense,* unfed larvae, diapause.

RESUMO

Este trabalho avaliou os parâmetros biológicos: período de pré-postura, período de incubação dos ovos e período máximo de sobrevivência das larvas em jejum, sob condições de campo e laboratório, com intenção de criar subsídios para medidas mais racionais de controle de *Amblyomma cajennense*. O estudo foi realizado na fazenda experimental da Escola de Veterinária da UFMG. A cada 14 dias, de setembro/1997 a março/1998 e de outubro/1998 a abril/1999, seis tubos telados contendo fêmeas ingurgitadas foram mantidos em pastos de *Brachiaria decubens,* com mais de 60 cm. de altura e outros seis em estufa BOD (27ºC e 80% UR). Para ambos os grupos, foram utilizadas 84 e 72 fêmeas para o primeiro e segundo período de observação, respectivamente. O período de pré-postura nas duas condições foi similar (5-7 dias). Houve diferença significativa entre: período de incubação dos ovos, grupo mantido no pasto (56-60 dias) e grupo em BOD (34-39 dias) e, período máximo de sobrevivência das larvas em jejum, grupo mantido no pasto (14 meses) e em BOD (9,5 meses), sugerindo a ocorrência de diapausa comportamental. Longo período de sobrevivência das larvas em jejum no pas-

[1] Secretaria Municipal de Saúde de Belo Horizonte, MG.

[2] Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais, Pampulha, Belo Horizonte, MG. E-mail: rcleite@vet.ufmg.br

[3] Departamento de Parasitologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO.

[4] Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP.

to deve ser considerado para implantação de um programa de controle de *A. cajennense*.

PALAVRAS-CHAVE: *Amblyomma cajennense*, larvas em jejum, diapausa.

INTRODUCTION

Amblyomma cajennense is a tick species that occurs from the southern United States to northern Argentina (ROBINSON, 1926). It is the main vector of *Rickettsia rickettsii*, the etiological agent of human Brazilian Spotted Fever (FONSECA, 1997), and is related to experimental transmission of *Cowdria ruminantium* and *Ehrlichia bovis* to cattle (MASSARD, 1984; UILEMBERG et al., 1984). Several characteristics differentiate this species from other ixodid ticks, particularly its large geographical distribution, which increases its capacity to transmit diseases to domestic and wild animals, as well as to man. Moreover, *A. cajennense* is a three-host tick, with slow engorgement during all of its parasitic stages. It has a high biotic potential, great longevity in its free-life stages, deep attachment to the host skin and low parasitic specificity, especially regarding the larval and nymphal stages.

In comparison with hematophagous insects such as Diptera, the longevity of ticks is extraordinary, since all their stages can survive for long periods without feeding. Adults of *Ornithodoros papillipes* can survive for 11 years and *Alveonasus lahorensis* for 10 years; larvae and nymphs usually survive for periods ranging from a few weeks to some months (BALASHOV, 1972).

Periods of parasite inactivity, without feeding, have been described for several species of ticks. This phenomenon is known as a diapause, and it favors tick survival when the environmental conditions are inappropriate. Belozerov (1982) classified diapauses into two types: behavioral diapause and morphogenetic diapause. Behavioral diapause is the most common type, and ticks in this physiological condition either are unable to find a host or have suffered delays in engorgement in early stages attached to the host. Occurrences of this type of diapause have been described for 33 species of Ixodidae, in the following genera: *Ixodes, Haemaphysalis, Amblyomma, Hyalomma, Rhipicephalus* and *Dermacentor.* Morphogenetic diapause is characterized by delays in either embryogenesis or ecdysis of immature engorged stages, or additionally, in female oviposition. This kind of diapause, which is less common than the behavioral type, has been described in 23 species, in the genera of *Ixodes, Haemaphysalis, Hyalomma* and *Dermacentor*. Although several studies have been carried out, the factors regulating the beginning and end of diapauses are unclear. It has been considered that photoperiodism may be the dominant environmental stimulus regulating this phenomenon, although low temperatures also play an important secondary role in it (OLIVER JR., 1989; SONENSHINE, 1993).

Several authors have studied some of the biological parameters of *A. cajennense*, such as the maximum survival of unfed larvae (OLIVIERI; SERRA-FREIRE, 1984) and the duration of pre-oviposition, oviposition and egg incubation periods under controlled conditions (ROHR, 1909; DIAMANT; STRICKLAND, 1965; SMITH, 1974, 1975; SERRA-FREIRE; OLIVIERI, 1992). However, little information is available on the biological parameters of *A. cajennense* under natural field conditions (LABRUNA et al., 2003).

The present study had the main objective of evaluating these parameters, both under controlled laboratory and natural field conditions, with the aim to providing support for more rational measures for controlling this tick species in Brazil.

MATERIALS AND METHODS

This experiment was carried out on an extensive horse-rearing farm (Experimental Farm of the Veterinary School of the Federal University of Minas Gerais), in the municipality of Pedro Leopoldo, State of Minas Gerais, Brazil (19°37'S, 42°02'W, altitude 882 m), from September 1997 to February 2000. According to the Köppen climatic classification, this region has the CWA type of climate, with a dry winter and rainy summer. The lowest temperature is less than 18°C and the highest is greater than 22°C (ANTUNES, 1986).

Engorged females, eggs and larvae were placed in netted tubes (61 mm x 17 mm diameter). Every 14 days, from September 1997 to March 1998 and from October 1998 to April 1999, six tubes with engorged females were kept on pasture of *Brachiaria decumbens* that was up to 60 cm high and another six tubes were kept under BOD incubation (at 27°C, 80% relative humidity). For the first and second periods, the numbers of engorged females studied under field conditions were respectively 84 and 72, and the numbers under BOD incubation were 84 and 72.

The duration of the pre-oviposition and egg incubation periods and the maximum survival of the larvae were recorded for the 312 engorged females studied. The data obtained under field and BOD incubation conditions were compared by means of variance analysis.

RESULTS

The duration of the pre-oviposition periods were similar under both conditions and ranged from 5 to 7 days.

Table 1 shows that there was a significant difference in the duration of the egg incubation period between field conditions (56 to 60 days) and BOD incubation conditions (34 to 39 days). A significant difference in the maximum survival of the larvae under field and controlled conditions was also seen (Table 2).

As shown by Figure 1, the maximum survival of the larvae under BOD incubation ranged from 0.5 to 8.5 months. Around 65% of the larvae kept under incubation survived for 6 to 6.5 months, and approximately 48% of the larvae kept in the field survived for 8.5 to 9.5 months. Over the two years of the experiment, the longest survival of the larvae occurred in December, for females kept under field conditions, and in October, for females kept under BOD incubation (Figure 2).

Table 1. Data on egg incubation periods (IP) for *Amblyomma cajennense*, over two years of experiments under BOD incubation conditions (I1 and I2) and under field conditions (F1 and F2).

Level	N*	Mean (days)	SD
IPF1	74	57.7 [a]	3.4
IPF2	70	57.7 [a]	3.5
IPI1	79	35.5 [a]	1.0
IPI2	71	35.4 [a]	0.8
IPF1	74	57.7 [b]	3.4
IPI1	79	35.5 [a]	1.0
IPF2	70	57.7 [b]	3.5
IPI2	71	34.9 [a]	0.8

[a] Different letters within the same group indicate significant differences ($p < 0.05$).
* Engorged females that produced viable eggs.

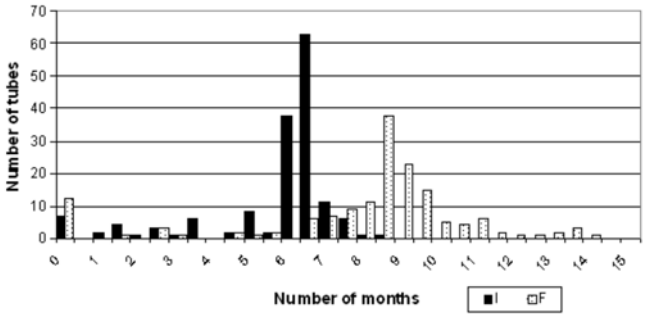

Figure 1. Distribution of tubes containing live unfed larvae of *A. cajennense*, over two years of experiments, under field conditions (F) and under BOD incubation conditions (I) in the municipality of Pedro Leopoldo, State of Minas Gerais, Brazil.

Table 2. Data on the maximum survival of unfed larvae (MS) of *Amblyomma cajennense*, over two years of experiments, under BOD incubation conditions (I1 and I2) and under field conditions (F1 and F2).

Level	N*	Mean (months)	SD
MSF1	84	8.6 [a]	3.3
MSF2	72	8.8 [a]	4.9
MSI1	78	5.9 [a]	2.4
MSI2	71	5.8 [a]	1.6
MSF1	74	8.6 [b]	3.3
MSI1	78	5.9 [a]	2.4
MSF2	70	8.6 [b]	4.9
MSI2	71	5.8 [a]	1.6

[a] Different letters within the same group indicate significant differences ($p < 0.05$)
* Engorged females that had produced viable eggs

DISCUSSION

In the present study, *A. cajennense* presented a duration of pre-oviposition period ranging from five to seven days, for both the field and the BOD incubations. These results differ from those reported by Serra-Freire and Olivieri (1992) in Rio de Janeiro, Smith (1975) in Trinidad and Tobago and Diamant and Strickland (1965) in North America, which were

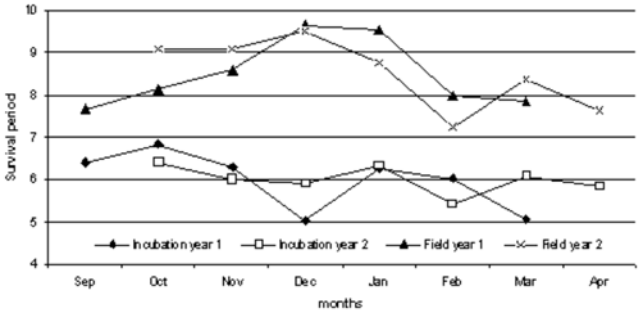

Figure 2. Mean survival of unfed larvae of *A. cajennense* over two years of experiments in the municipality of Pedro Leopoldo, State of Minas Gerais, Brazil.

respectively 4 to 13 days, 7 to 13 days and 9 to 22 days. These differences might be explained by differences between the experimental conditions, geographical locations and tick strains. The results reported by Diamant and Strickland (1965) suggest that longer pre-oviposition periods may be related to lower temperatures at the location of the experiment. The other authors had shorter observation periods and the studies were carried out in regions with higher mean annual temperatures, in South and Central American countries.

Regarding the egg incubation period, the results obtained from BOD incubation (34 to 39 days) are similar to those reported by Olivieri et al. (1985) and Serra-Freire and Olivieri (1992), who found a variation from 30 to 56 days, with a mean of 35.73 days. The hypothesis that the temperature influences this biological parameter was reinforced by the longer egg incubation period (56 to 60 days) that was found under natural field conditions, where, in contrast to BOD, the temperature and relative humidity are extremely variable. Diamant and Strickland (1965) also observed a different egg incubation period for *A. cajennense*, ranging from 37 to 154 days. Rohr (1909) reported different incubation periods when eggs were exposed to different temperatures: at 30°C the incubation period ranged from 61 to 66 days, but few larvae were viable; at 22.5°C the incubation period was 46-50 days, and at 0ºC or 15°C, the larvae were unable to develop.

Analyzing all the data obtained regarding pre-oviposition and egg incubation under field conditions, an intriguing lack of larval infestation activity was noted during the Southern Hemisphere summer and part of the autumn. However, larvae obtained from engorged females collected in September and October had egg eclosion in November and December. In a study on the behavior of *A. cajennense* during its parasitic phase and on pastures, Oliveira (1998) observed that horse infestations occurred only after April and that the maximum larva population on pasture was seen in May. This phenomenon can be named “autumn rise”, similarly to the “spring rise”, observed for some monoxenic tick species. Thus, a three to four-month period of larval parasitic inactivity is seen. All this information suggests that a behavioral diapause was occurring during this period. Outside of their predominance period, these larvae were able to infest different hosts in a pre-experiment carried out by Lopes et al. (1998). The

occurrence of a diapause for this tick species should be further investigated. In the present study region, this phenomenon is possible mediated by temperature and photoperiod, which stimulate larvae to hide in the soil until the environmental conditions become more favorable, usually after March, when temperatures are milder and animal infestation is visible.

Regarding the maximum survival of unfed larvae, a significant difference was also observed between field conditions (up to 14 months) and BOD incubation conditions (up to 9.5 months). These data differ from those reported by Olivieri and Serra-Freire (1984) and Serra-Freire and Olivieri (1992), who found that *A. cajennense* larvae can survive without feeding, while maintaining their infestation capacity, for up to five months. However, it should be stressed that in the present study, the viability of these long-surviving larvae could not be tested, since they were in low numbers (0.5% of the total number of viable eggs). The longest mean survival periods in the field were seen in December and January, while under BOD incubation conditions, they were seen in October. Long survival periods for unfed larvae were observed throughout the year, thus demonstrating that, although *A. cajennense* ticks have a single generation per year, pastures could be infested by two different generations of larvae, in the same way as has been described for other tick species (BALASHOV, 1972).

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