Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, v. 27, n. 1, p. 80-85, jan.-mar. 2018
Doi: http://dx.doi.org/10.1590/S1984-29612018003

Original Article

Biological parameters of *Amblyomma coelebs* Neumann, 1906 (Acari: Ixodidae) under experimental conditions

Parâmetros biológicos de *Amblyomma coelebs* Neumann, 1906 (Acari: Ixodidae) em condições experimentais

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Received November 10, 2017
Accepted January 11, 2018

Abstract

One generation of *Amblyomma coelebs* life cycle under experimental conditions was evaluated. Ten tick pairs were allowed to feed on rabbits under laboratory conditions (LC), resulting six engorged females with a mean weight of 1,403.9 mg. Two females were maintained in a forest reserve under natural conditions (NC), and four were maintained in incubators (LC). The engorgement period lasted 10.33 days. Pre-oviposition periods were 10.75 (NC) and 22 days (LC). The mean egg-mass weight was 514.76 mg, and the blood meal conversion index was 36.67% (LC). Incubation period under NC and LC were 91 and 56.33 days and hatching rates were 50% and 28.33%, respectively. Larval engorgement period ranged from 4 to 10 days, with average weight of 1.1 mg. Engorged larvae were incubated under NC and LC, with a premolt period of 27 to 36 days and molting rate of 7.1% and 28.7%, respectively. Nymphal engorgement period ranged from 5 to 7 days, with a mean weight of 18.8 mg and a recovery rate of 54.54%. In LC, the ecdysis mean period was 24.5 days, and molting rate was 44.44%, resulting in 24 adult *A. coelebs*. Our results show a life cycle of 187.45 (NC) and 149 (LC) days.

Keywords: *Amblyomma coelebs*, life cycle, biological parameters, experimental conditions, rabbits.

Resumo

Uma geração do ciclo de vida de *Amblyomma coelebs* em condições laboratoriais foi avaliada. Dez casais de carrapatos foram alimentados em coelhos sob condições laboratoriais (CL), resultando em seis fêmeas ingurgitadas, com um peso médio de 1.403,9 miligramas (mg). Duas fêmeas foram mantidas em uma reserva florestal sob condições naturais (CN), e quatro foram mantidas em incubadoras (CL). O período de ingurgitamento durou 10,33 dias. Períodos de pré-postura foram de 10,75 (CN) e 22 dias (CL). O peso médio das massas de ovos foi de 514,76 mg e o índice de conversão alimentar foi de 36,67% (CL). O período de incubação em CN e CL foi de 91 e 56,33 dias e os percentuais de eclosão foram de 50% e 28,33%, respectivamente. O período de ingurgitamento larval variou de quatro a 10 dias, com peso médio de 1,1 mg. Larvas ingurgitadas foram incubadas em CN e CL, com período de pré-muda de 27 a 36 dias e percentual de ecdise de 7,1% e 28,7%, respectivamente. O período de ingurgitamento das ninfas oscilou de cinco a sete dias, com peso médio de 18,8 mg e uma taxa de recuperação de 54,54%. Em CL, o período médio de ecdise foi de 24,5 dias, e o percentual de muda foi 44,44%, resultando em 24 adultos de *A. coelebs*. Estes resultados demonstram um ciclo de vida de 187,45 (CN) e 149 (CL) dias.

Palavras-chaves: *Amblyomma coelebs*, ciclo de vida, parâmetros biológicos, condições experimentais, coelhos.

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Introduction

Amblyomma coelebs Neumann is one of the 71 species composing the Brazilian tick fauna, which included 46 species from the Ixodidae family and 25 from the Argasidae family (KRAWCZAK et al., 2015; LABRUNA et al., 2016; WOLF et al.; 2016; MUÑOZ-LEAL et al., 2017). *Amblyomma coelebs* is geographically distributed in Neotropical and Nearctic regions (ONOFRIO et al., 2006). In Brazil, it has been reported to occur in the states of São Paulo, Minas Gerais, Mato Grosso do Sul, Paraná, Rondônia, Acre, Mato Grosso, Roraima, Pará and Espírito Santo (GUIMARÃES et al., 2001; LABRUNA et al., 2002b, 2004b; ARZUA et al., 2005; SARAIVA et al., 2012).

Adults of this species prefer parasitizing tapirs (*Tapirus terrestris*) (LABRUNA & GUGLIELMONE, 2009; GUGLIELMONE et al., 2014) but can also be found parasitizing equines (BELDOMÉNICO et al., 2003). They have also been found parasitizing other mammals and birds (OGRZEWALSKA et al., 2010; NAVA et al., 2017; LOPES et al., 2016). Nymphs appear to be more generalists and exploit a larger host range that includes carnivores, marsupials, rodents, birds and, occasionally, humans (BELDOMÉNICO et al., 2003; SPONCHIADO et al., 2015; GARCIA et al., 2015; OGRZEWALSKA & PINTER, 2016; OGRZEWALSKA et al., 2009; 2010; ITO et al., 2017).

The importance of this tick as a pathogen vector has been described by Labruna et al. (2004b), who in a study of ixodid species with *Rickettsia* infections found that *A. coelebs* may be a carrier of *Rickettsia amblyommatis*, formerly known as "*Candidatus* Rickettsia amblyommii" (KARPATHY et al., 2016), as also reported by Silveira et al. (2015). The pathogenicity of this *Rickettsia* species is still uncertain (NAVA et al., 2017), but findings from these studies suggest that *A. coelebs* is a possible vector and may be a carrier of other agents with zoonotic potential for animals and humans.

In view of this information, there is a need for a better understanding of the biological aspects of this species under experimental conditions and its relationships with its hosts.

Materials and Methods

Study location and animal use

The study was carried out in the Tick Biology Laboratory (Laboratório de Biologia do Carrapato) in the Animal Health Department of Embrapa Gado de Corte (20°25'03" / 54°42'20"), Campo Grande, Mato Grosso do Sul, Brazil.

The Ethics Commission on the Use of Animals (CEUA) at the Federal University of Mato Grosso do Sul (UFMS) approved this study under protocol number 699/2015.

Tick collection

Twelve adult *A. coelebs* (four female and eight male) were captured using dry ice traps (CO_2) as described by Oliveira et al. (2000), and fourteen ticks (seven female and seven male) were captured using the cloth dragging technique (RECHAV, 1982). These free-living ticks were captured in a forest reserve belonging to the Embrapa Gado de Corte experimental farm in the municipality of Terenos (20°33'11.7" S / 54°48'49.0" W), Mato Grosso do Sul, Brazil.

After capture, the ticks were placed in tubes with perforated tops, transported to the Tick Biology Laboratory and identified according to the dichotomous key proposed by Barros-Battesti et al. (2006).

Experimental infestation with adult A. coelebs

Six New Zealand rabbits (*Oryctolagus cuniculus*) with no previous tick contact and of undetermined sex were used for infestation. Rabbits were chosen as the experimental host because they are easy to acquire and to handle in the laboratory. In addition, juvenile instars of various tick species are known to parasitize small mammals (TATCHELL, 1987). Tick feeding chambers were used following the technique previously described by Szabó et al. (1995). Two rabbits were infested with *A. coelebs*. Five pairs of adult ticks were placed in separate feeding chambers on each rabbit.

Recovery of engorged females

Six engorged female ticks were collected after completing their blood meal. They were placed into individual tubes with perforated tops, weighed individually and divided into two groups:

Group 1: Four engorged females were maintained in B.O.D. incubators at 28°C (82.4°F) and 80% humidity in individual tubes with perforated tops (laboratory conditions; LC).

Group 2: Two engorged females were packed into cylindrical stainless steel wire tubes (60 mesh/cm^2, 61 mm length, 37 mm diameter) that were sealed with rubber stoppers. The females were taken to a riparian forest reserve located within the Embrapa Gado de Corte in Campo Grande in July (07/16/2016). The tubes were placed horizontally on the soil surface along the roots of vegetation. The area was on the edge of a stream under dense vegetation and had a high humidity (natural conditions; NC). The temperature and humidity at the site were measured daily using a thermo-hygrometer (Highmed) to record maximum and minimum values. The thermos-hydrometer was placed in contact with the ground.

Infestation with A. coelebs larvae and nymphs

Approximately 2,200 larvae were used to infest two rabbits in individual feeding chambers. All larvae used in experimental infestations were between 15 and 25 days old. After their blood meal, engorged larvae were collected daily from the chambers, counted and weighed. Engorged larvae were separated into two lots: Lot 1 was composed of 113 larvae that were taken to the forest reserve (NC) in November (11/21/2016); and Lot 2 was composed of 317 larvae that were maintained in B.O.D. incubators (LC) (28°C, 80% relative humidity, 12:12 light: dark) until ecdysis.

A total of 99 nymphs developed from the larvae maintained in the laboratory and in the forest reserve, which were used to infests two rabbits. The chambers were monitored daily, and

engorged nymphs were collected, individually weighed, placed in vials and taken to the laboratory, where they were held in B.O.D incubators for posterior ecdysis.

Biological parameters evaluated

The recovery rate, engorgement period, engorged female weight, pre-oviposition period, egg-mass weight, blood meal conversion index (BENNETT, 1974), egg incubation period and hatching rate, determined by visual estimation (LABRUNA et al., 2000), were evaluated. The egg-mass weight and blood meal conversion index were not determined in the engorged female ticks allocated to the NC treatment because we decided not to manipulate them to prevent stress, death and egg desiccation.

For larval and nymphal instars, the following parameters were measured following the methods described by Sanches et al. (2008) and Szabó et al. (2009): period of engorgement, weight, molting duration, recovery rate and molting success rate.

Results

The biological parameters measured in *A. coelebs* are presented in Tables 1, 2 and 3.

The mean engorgement period of *A. coelebs* females was 10.33 days; after the blood meal, six engorged ticks were recovered (60% recovery rate), with an average weight of 1,403.91 mg. The pre-oviposition periods of engorged females under natural (NC) and laboratory (LC) conditions were 22 and 10.75 days, respectively. The mean egg-mass weight was 514.76 mg, and the blood meal conversion index was 36.67% under LC. Incubation periods for the eggs maintained in NC and LC were 91 and 56.33 days, respectively, and the larval hatching rates were 50% and 28.33%, respectively (Table 1).

Only one engorged female tick recovered and maintained under NC oviposited, and larvae were subsequently obtained from these eggs. Only one engorged female tick maintained under LC died after initiating oviposition, while the others successfully completed oviposition (Table 1).

The larval engorgement period ranged from 4 to 10 days. The mean weight of larvae was 1.1 mg, and the recovery rate was 19.54%. The molting success rates for nymphs were 7.1% and 28.7%, under NC and LC, respectively. Biological parameters for larvae are shown in Table 2.

A total of 99 nymphs were obtained from engorged larvae maintained under NC (8) and LC (91). Fed nymphs showed an engorgement period ranging from 5 to 7 days, a mean weight of 18.8 mg and a recovery rate of 54.54% (54 engorged nymphs). All nymphs were maintained under LC. The duration of the molting process was 24.5 days, and the molting success rate was 44.44%, resulting in 24 adult ticks (Table 3).

Discussion

We present, for the first time, the biological parameters of the life cycle of *A. coelebs* when allowed to engage in natural feeding behaviors on laboratory hosts (New Zealand rabbits) and maintained under experimental conditions (NC and LC).

The adult phase of *A. coelebs* was able to exploit rabbits as an experimental host, and the ticks were able to successfully engorge, as demonstrated by the recovery rate of engorged females, their mean weight and the mean egg-mass weight. However, some

Table 1. Mean, Standard Deviation and Amplitude (in parentheses) related to the biological parameters of *Amblyomma coelebs* female ticks in laboratory condition (LC) and natural condition (NC) in Campo Grande, MS, Brazil.

Biological parameters	E.P.* (days)	E.F.W.* (mg)	P.O.P (days)	E.M.W. (mg)	F.C.R. (%)	Inc.P. (days)	L.H.R. (%)
Laboratory (LC)	10.33±1.36 (8-12)	1403.91± 574.72 (544-2146.9)	10.75±1.70 (9-13)	514.76± 364.9 (175-905.5)	36,67± 9.35 (32.2-50)	56.33±11.37 (47-69)	28.33± 36.17 (5-70)
Natural (NC)			22±0 (22)	-	-	91±0 (91)	50±0 (50)

- Values not obtained; E.P. = Engorgement Period (days); E.F.W . = Engorged Female Weight (mg); P.O.P = Pre-oviposition Period (days); E.M.W.= Egg Mass Weight (mg); F.C.R. = Feed Covertion Ratio (%); Inc.P. = Incubation period (days); L.H.R. = Larvae Hatching Rate (%). *Parameteres "E.P." and "E.F.W." both performed only in laboratory conditions.

Table 2. Mean, Standard Deviation and Amplitude (in parentheses) related to the biological parameters of *Amblyomma coelebs* larvae fed on rabbit and maintained in laboratory condition (LC) and natural condition (NC) in Campo Grande, MS, Brazil.

Biological parameters	E.P.* (days)	Weight (mg)*	P.M.P. (days)	M.S. (%)
Laboratory (LC)	5.62±1.62 (4-10)	1.11±0.17 (0.7-1.5)	36	28.7
Natural (NC)			28±1 (27-29)	7.1

E.P. = Engorgement Period; P.M.P. = Premolt period; M.S. = molting success. *Parameteres "E.P." and "Weight" both performed only in laboratory conditions.

Table 3. Mean, Standard Deviation and Amplitude (in parentheses) related to the biological parameters of *Amblyomma coelebs* nymphs in laboratory conditions, Campo Grande, MS, Brazil.

Biological Parameters					
Number of exposed nymphs	Number of nymphs that engorged	Engorgement Period (days)	Weight (mg)	Pre-molt period (days)	Molting success (%)
99	54 (54.54%)	6±1.41 (5 -7)	18.8±10.2 (11,6 – 26,03)	24.5±2.12 (23 – 26)	44.44

difficulties were observed in the subsequent developmental stages of the life cycle of this species.

Difficulties have also been reported by other authors for several tick species, which has required the use of hosts either similar to or different than the natural hosts to facilitate the successful rearing of ticks and maintenance of colonies; this can alter the duration of the life cycle in some species (e.g., in Argasidae and Ixodidae) (RODRIGUES et al., 2002; LABRUNA et al., 2002b; SANCHES et al., 2008; OLEGÁRIO et al., 2011).

The use of rabbits as alternative hosts is a well-established method for the maintenance of colonies of diverse tick species of the genera *Amblyomma*, *Rhipicephalus*, *Ixodes*, *Dermacentor*, and *Haemaphysalis*. Rabbits are easy to maintain and handle in the laboratory, and it is easy for juvenile instars of certain tick species to successfully parasitize them (GUGLIELMONE et al., 1991; MANGOLD & GUGLIELMONE, 1993; BECHARA et al., 1995; TROUGHTON & LEVIN, 2007; RODRIGUES et al., 2017).

In this study, we attributed some of the difficulties in perpetuating several generations of *A. coelebs* to issues related to egg incubation, the low rate of recovery for engorged larvae and the death of larva. Most of the tick eggs became desiccated, preventing larva from hatching. A considerable number of engorged larvae died under conditions where the temperature and humidity were controlled and in the natural environment. Considering the results presented here, the abiotic conditions were not ideal for some stages of the tick life cycle; however, the conditions were satisfactory for the analysis of the biological parameters we assessed. The engorged larvae allocated to the NC were introduced in the transition period between spring and summer, which is a period with hot and humid days and high light levels, which is theoretically unfavorable for this instar stage: according to Labruna et al. (2002a, 2003a), from studies on the population dynamics of *Amblyomma sculptum* (published as *Amblyomma cajennense*), conditions corresponding to those that occur in November are only favorable for adult ticks of the genus *Amblyomma* in Brazil.

These observations corroborate the data presented by Szabó et al. (2009) in a study of the life cycle of *A. incisum*. These authors changed their protocol to include a natural reserve area in the study in an attempt to observe some of the biological parameters of this tick species. In the current study, due to the difficulties of observing some of the biological parameters of the tick free-living stage, we attempted to maintain *A. coelebs* in a natural environment as described by Szabó et al. (2009).

Maintaining abiotic factors such as temperature, humidity and photoperiod within acceptable ranges is fundamental for the maintenance of tick colonies. Most studies concerning the maintenance of ticks under controlled conditions report temperatures varying from 27 to 29°C and a range of relative humidity values between 70 and 90% (BECHARA et al., 1995; RODRIGUES et al., 2002; LABRUNA et al., 2002a, 2003a; SZABÓ et al., 2009; OLEGÁRIO et al., 2011), which is in agreement with the methodology of the current study as we maintained a temperature of 28°C and a relative humidity of least 80% for ticks raised under LC.

The average ambient temperatures for engorged female ticks and engorged larvae under NC (forest) were 25°C and 28°C, respectively. However, because the area is near a stream, the relative humidity for the two periods ranged from 75 to 90%. It is important to keep in mind that the experimental ticks were, at least theoretically, in a favorable microenvironment, with suitable temperatures and humidity due to the abiotic effects of the forest and river. This area is also a natural habitat and part of the ecosystem of the tick's main host, the tapir (unpublished data).

Life cycles, under laboratory conditions, of other tick species which parasite tapirs were already described, such as *Amblyomma ovale* (MARTINS et al., 2012), *Amblyomma dubitatum* (published as *Amblyomma cooperi* – LABRUNA et al., 2004a), *Amblyomma triste* (LABRUNA et al., 2003b) and *Amblyomma oblongoguttatum* (MARTINS et al., 2017). When fed on rabbits, larvae of *A. triste*, *A. ovale* and *A. oblongoguttatum* present a recovery rate lower than 20%, a value similar to the one obtained on this study. For nymphs, on the same host species, recovery rates described for these tick species is also lower than 20% (LABRUNA et al., 2003b; MARTINS et al., 2012; 2017). Nymphs of *A. dubitatum*, when submitted to feeding on *Cavia porcellus,* present a recovery rate of 36% (LABRUNA et al., 2004a). This differed from results of the present study, which demonstrated a recovery rate higher than 50%. Regarding engorging periods of larvae and nymphs of different cited tick species, results are similar amongst each other (LABRUNA et al., 2003b; 2004a; MARTINS et al., 2017) and corroborate with values obtained on this experiment.

The abovementioned colony management practices together with the use of rabbits as hosts for the larval stage may have caused death, especially for eggs and engorged larvae, because little is known about the dynamics of larval parasitism in this species. The low rate of recovery of engorged larvae demonstrated in the present study is also worth mentioning as it suggests that the rabbits were not adequate hosts for this instar stage. Data from Ogrzewalska et al. (2009, 2010) show that birds can serve as hosts but that this is not common, and only a few individuals of this tick species have been found parasitizing birds in two different study areas, including the Amazon in northern Brazil and the Atlantic rain forest region.

The nymphal stage was the only stage that was amenable to both the experimental host and the controlled temperature and humidity (LC), and this stage showed a high recovery rate in which the majority of individuals became engorged and later molted to adults. However, it is known that this instar stage shows non-selective parasitism and exploits a wide variety of hosts, including humans, even if only occasionally (BELDOMÉNICO et al., 2003; LABRUNA et al., 2005; SARAIVA et al., 2012; SPONCHIADO et al., 2015; GARCIA et al., 2015; OGRZEWALSKA & PINTER, 2016; OGRZEWALSKA et al., 2009, 2010; ITO et al., 2017).

It is worth mentioning that despite the difficulties encountered in this study, the results are consistent and highly informative because until now nothing was known about the biological parameters related to the life cycle of *A. coelebs*. There has only been one previously published study regarding adult and nymphs ticks of this species parasitizing humans (BELDOMÉNICO et al., 2003; GARCIA et al., 2015).

On the basis of the biological parameters evaluated in this study, we can infer that the life cycle of *A. coelebs* maintained under partially NC and under LC can be completed in 187.45 and 149.53 days, respectively. Part of the knowledge gap concerning the biological

parameters of this tick species under experimental conditions has been filled here; however, there is still a need for additional studies to better understand the influences of abiotic factors and host-related factors on the life cycle of this tick species.

Acknowledgements

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (Fundect, MS) - Governo do Estado de Mato Grosso do Sul and Embrapa Gado de Corte. We also thank the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Rondônia (Fapero-RO) - Governo do Estado de Rondônia and the Fundação Oswaldo Cruz/Fiocruz, Rondônia.

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