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Life-cycle of *Amblyomma oblongoguttatum* (Acari: Ixodidae) under laboratory conditions

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Abstract This study evaluated for the first time the life cycle of *Amblyomma oblongoguttatum* in the laboratory. For this purpose, larvae and nymphs were exposed to *Gallus gallus* (chicks), *Rattus norvegicus* (wistar rat), *Calomys callosus* (vesper mouse), *Oryctolagus cuniculus* (domestic rabbit), *Cavia porcellus* (guinea pig), and *Didelphis albiventris* (white-eared opossum). Nymphs were exposed to *G. gallus*, *C. callosus*, *C. porcellus, O. cuniculus, R. norvegicus,* and *Nectomys squamipes* (water rat). Adult ticks were exposed to domestic dogs. The life-cycle of *A. oblongoguttatum* in the laboratory could be completed in an average period of 188 days, considering prefeeding periods of 25 days for each of the parasitic stages. Under laboratory conditions, none of the host species was highly suitable for *A. oblongoguttatum* larvae, since the recovery rates of engorged larvae were always <15%, or most of the times ≤5%. Similar results were obtained for nymphs, with recovery rates of engorged nymphs always <6%. Our results, coupled with literature data, suggest that small mammals, especially small rodents, do not have an important role in the life-cycle of *A. oblongoguttatum* under field conditions. Domestic dogs showed to be highly suitable for the adult stage of *A. oblongoguttatum*, in agreement with literature data that have appointed dogs as important hosts for the adult stage of *A. oblongoguttatum* in South America.

Keywords *Amblyomma oblongoguttatum* · Ixodidae · Tick · Biology

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Introduction

Amblyomma oblongoguttatum Koch is a Neotropical tick distributed from Mexico to Brazil, with the northern limit of its distribution reaching the Nearctic Region (Guglielmone et al. 2003, 2014; Barros-Battesti et al. 2006). In South America, *A. oblongoguttatum* has been recorded from the Amazon biome within Colombia, Surinam, Guyana, French Guiana, Venezuela, Peru, Bolivia and northern Brazil (Guglielmone et al. 2003, 2014; Mendoza-Uribe and Chávez-Chorocco 2004; Labruna et al. 2005b, 2010); however, there are also established populations within the Atlantic forest biome in southeastern Brazil (Aragão 1936; Ogrzewalska et al. 2007).

Adults of *A. oblongoguttatum* have been reported on a variety of wild and domestic animals, with most of the records on Artiodactyla (Tayassuidae, Suidae, Cervidae), Perissodactyla (Tapiridae and Equidae) and Carnivora (mostly Canidae and Felidae) (Aragão 1936; Fairchild et al. 1966; Jones et al. 1972; Labruna et al. 2005a, b, 2010; Soares et al. 2015). Overall, the most commonly reported host species for *A. oblongoguttaum* adult ticks is the domestic dog; however this condition has been indeed biased to the much higher sampling of domestic dogs, when compared to any other host species or group (Labruna et al. 2000a, 2005a). In addition, *A. oblongoguttatum* is reported as one of most frequent human-biting adult tick in the Amazon biome (Jones et al. 1972; Guglielmone et al. 2006; Labruna et al. 2005a; Ogrzewalska et al. 2007).

In contrast to the adult stage, host records for nymphs of *A. oblongoguttatum* have been limited to the following host species: deer (*Odocoleus* sp.) (Nuttall 1912) and two large bird species, *Catharista urubu* (Nuttall 1912) and *Crax rubra* (Fairchild et al. 1966), in Panama; domestic dogs (Martins et al. 2009; Soares et al. 2014), peccary (*Tayassu pecari*), coati (*Nasua nasua*) (Acosta et al. 2016), agouti (*Dasyprocta azarae*) (Witter et al. 2016) and humans in Brazil (Labruna et al. 2005a; Ogrzewalska et al. 2007); and human in Surinam (Santos Dias 1986). This relatively low number of nymphal records, hitherto, suggests low host specificity, similarly to the adult stage. Host specificity of the *A. oblongoguttatum* larval stage remains obscure, since there is only one host record on deer (*Odocoleus* sp.) in Panama (Nuttall 1912).

Although there have been reports in Brazil of *A. oblongoguttaum* infected by *Rickettsia bellii,* a rickettsial agent of unknown pathogenicity (Labruna et al. 2004), nothing is known of the role of *A. oblongoguttatum* in the transmission of pathogens to humans or animals. Because of the likely medical and veterinary importance of *A. oblongoguttatum,* associated to the lack of knowledge of its life-cycle, this study reports for the first time the life-cycle of *A. oblongoguttatum* under laboratory conditions.

Materials and methods

The ticks used in this study were the progeny of engorged females collected on domestic dogs in the Municipality of Monte Negro, State of Rondônia, Brazil (latitude −10.25; longitude −63.31). The engorged females were taken to laboratory and maintained in an incubator at 27 ± 1 °C and 95–100% RH, under 24 h darkness for egg laying. The hatched larvae were used to start a laboratory colony that was used to evaluate the experimental life-cycle of *A. oblongoguttatum.* Voucher specimens (unfed larvae, nymphs and adults) were deposited in the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" of the University of São Paulo, under accession numbers CNC-634, 1477, 3477.

Infestations were performed with larvae, nymphs and adults of approximately 25 days old from two consecutive generations in the laboratory. For larval infestations, we used a total of six individuals of each of the species *Gallus gallus* (chicks), *Rattus norvegicus* (wistar rat), *Calomys callosus* (vesper mouse), *Oryctolagus cuniculus* (domestic rabbit), *Cavia porcellus* (guinea pig), and five individuals of *Didelphis albiventris* (white-eared opossum). For nymphal infestations, we used six individuals of *G. gallus*, *C. callosus*, *C. porcellus,* and three individuals of *O. cuniculus, R. norvegicus,* and *Nectomys squamipes* (water rat). All larval and nymphal infestations were performed with $\approx$1000 larvae or 60 nymphs per host. Infestations with adult ticks were performed on two crossbred dogs, each receiving 15 pairs of ticks. With the exception of dogs, all individual hosts were tick-naïve before used in each of the infestations of this study. All infestations consisted of placing the unfed ticks (larvae, nymphs or adults) directly on the animal nape and dorsum, and nothing was done to prevent host grooming. Each infested animal was kept in a suspended wire cage large enough to allow for host grooming. Water and commercial appropriate food were offered ad libitum. Each cage was kept inside a plastic box two times wider than the cage, with a double face foam adhesive tape (19 mm wide, 3 M) attached to the box walls to prevent engorged ticks from escaping. Feeding period was individually annotated as the number of days from placing the ticks on host to the day that each engorged tick naturally detached from the host.

Detached engorged larvae, nymphs or adult females were immediately transferred to the same incubator cited above, where the following biological parameters were daily evaluated: premolt period (number of days from detachment to ecdysis), preoviposition period (number of days from detachment to the beginning of oviposition), and incubation period (number of days from the beginning of oviposition to the hatching of the first larva). Each engorged female was weighed on the day of detachment, and later, its total egg mass using an electronic balance with a 0.1 mg precision. The index of egg production efficiency (EPE) was calculated in accordance with Bennett (1974): EPE = (weight of egg mass/initial weight of engorged female) $\times$ 100. Percentage of hatching for each female egg mass was visually estimated according to Labruna et al. (2000b).

Tick feeding and premolt periods were compared between host species or tick sex using the non-parametric Mann–Whitney test for comparison of pairs. The numbers of ticks that engorged or successfully molted, after exposed to different host species were analyzed by the Chi-square or Fischer test.

In order to generate sufficient number of adult ticks to be used in canine infestations, we performed controlled infestations by nymphs on five tick-naive rabbits. In this case, the back of each host was shaved and a cotton sleeve (15 by 15 cm) was glued to the skin as described (Pinter et al. 2002). An Elizabethan collar was used to prevent host grooming. These rabbits were maintained in the same room of the other hosts, where they were each infested with 200 nymphs from the same cohorts used in the above-mentioned nymphal infestations. Each cotton sleeve was opened daily, when detached engorged nymphs were collected and taken to the incubator mentioned above.

Results

Biological data for two consecutive generations of *A. oblongoguttatum* in the laboratory were grouped and are presented in Tables 1, 2 and 3. Among six different host species, *C. porcellus* yielded the highest recovery ($P < 0.05$) of engorged larvae (14.2%),

Table 1 Feeding periods of *Amblyomma oblongoguttatum* larvae on different host species and premolt periods under 27 °C and 95–100% RH

Host species (number of individuals)	Number of exposed ticks	Number of ticks that engorged (%)	Mean number of recovered ticks per host (range)	Feeding period (days)+	Number of engorged ticks that molted (%)	Premolt period (days)+
Cavia porcellus (6)	6000	842 (14.0)a	140.3 (34–286)	5.7 ± 1.0 (4–10)a	551 (65.4)a	15.7 ± 1.5 (13–23)a
Gallus gallus (6)	6000	295 (5.0)b	49.1 (10–120)	4.4 ± 0.7 (3–9)b	198 (67.1)a	14.6 ± 1.5 (12–20)b
Oryctolagus cuniculus (6)	6000	284 (4.7)b	47.3 (15–94)	6.6 ± 1.2 (4–11)c	89 (31.3)b	15.7 ± 2.1 (12–21)a
Calomys callosus (6)	6000	234 (3.9)c	39 (5–133)	5.9 ± 1.0 (4–11)a	142 (60.7)a	16.1 ± 1.6 (13–21)a
Rattus rattus (6)	6000	38 (0.6)d	6.3 (2–13)	5.6 ± 1.1 (4–9)a	23 (60.5)a	16.0 ± 1.3 (13–19)a
Didelphis albiventris (5)	5000	6 (0.1)e	1.2 (0–3)	5.5 ± 1.6 (4–7)	0 (0)	0 (0)

+ Mean ± SE (range in parentheses)

Values in the same column followed by different letters are significantly different (Mann-Whitney test, $P < 0.05$)

Table 2 Feeding periods of *Amblyomma oblongoguttatum* nymphs on different host species and premolt periods under 27 °C and 95–100% RH

Host species (number of individuals)	Number of exposed ticks	Number of ticks that engorged (%)	Mean number of recovered ticks per host (range)	Feeding period (days)+	Number of engorged ticks that molted (number per sex) (%)	Premolt period (days)+
Cavia porcellus (6)	360	19 (5.2)a	3.1 (1–7)	6.0 ± 1.5 (4–9)	16 [15♂–1♀] (84.2)	17.5 ± 1.0 (17–20)
Gallus gallus (6)	360	6 (1.6)b	1.0 (0–6)	5.0 ± 0.9 (4–6)	2 [1♂–1♀] (33.3)	17.5 ± 0.7 (17–18)
Oryctolagus cuniculus (3)	180	2 (1.1)b,c	0.6 (1–1)	4.0 ± 0.0 (4–4)	2 [2♂] (100)	17.5 ± 0.7 (17–18)
Rattus rattus (3)	180	2 (1.1)b,c	0.6 (0–2)	7.5 ± 0.7 (7–8)	2 [2♂] (100)	17.0 ± 1.4 (16–18)
Calomys callosus (6)	360	0 (0.0)c	0 (0.0)			
Nectomys squamipes (3)	180	0 (0.0)c	0 (0.0)			

+ Mean ± SE (range in parentheses)

Values in the same column followed by different letters are significantly different (Mann-Whitney test, $P < 0.05$)

Table 3 Feeding periods of *Amblyomma oblongoguttatum* female ticks on dogs and reproductive data under 27 °C and 95–100% RH in the laboratory

Biological data	Infested dogs		Total
	Dog 1	Dog 2	
No. exposed females	15	15	30
No. females that engorged (%)	12 (80.0)	3 (20.0)	15 (50.0)
No. females that oviposited (%)	11 (73.3)	3 (100)	14 (93.3)
Feeding period (days)+	12.5 ± 1.3 (11–15)	12 ± 0.0 (12)	12.5 ± 1.3 (11–15)
Engorged female weight (mg)+	478.5 ± 102.4 (319.8–665.3)	522.1 ± 191.1 (304.1–660.6)	487.9 ± 118.4 (304.1–665.3)
Pre-oviposition period (days)+	7.2 ± 0.6 (6–8)	8.3 ± 0.6 (8–9)	7.4 ± 0.8 (6–9)
Egg mass weight (mg)+	251.0 ± 62.7 (163.5–382.8)	275.6 ± 95.0 (166.3–338.1)	256.3 ± 67.2 (163.5–338.1)
Egg incubation period (days)+	47.1 ± 1.6 (44–50)	55.3 ± 0.9 (54–57)	48.2 ± 3.2 (44–56)
% egg mass hatching+	87.7 ± 6.8 (75–95)	90.0 ± 5.0 (85–95)	88.2 ± 6.2 (75–95)
Egg production efficiency (EPE)+	52.2 ± 3.4 (47.1–57.5)	53.2 ± 4.0 (48.8–56.2)	52.4 ± 3.2 (47.1–57.5)

+ Mean ± SE (range in parentheses)

followed by *G. gallus* (5.0%), *O. cuniculus* (4.7%) and *C. callosus* (3.9%) of 6000 exposed larvae. The remaining two host species, *R. rattus* and *D. albiventris*, were much less suitable, since <1% of the 6000 and 5000 exposed larvae, respectively, successfully fed on them (Table 1). The proportion of engorged larvae that successfully molted to nymphs after feeding on *C. porcellus, G. gallus, C. callosus,* and *R. rattus* were similar ($P > 0.05$), varying from 60.5 to 67.1%. On the other hand, a significantly lower ($P < 0.05$) molting success (31.3%) was observed for engorged larvae that fed on *O. cuniculus*.

Feeding success rates of nymphs were generally very low, with 0–1.6% recovery of engorged nymphs from all host species, except for *C. porcellus,* on which 5.2% of the 360 exposed nymphs successfully fed ($P < 0.05$). The proportion of engorged nymphs that successfully molted to adults after feeding on different host species varied from 33.3 to 100%; however, these proportions were not compared statistically due to the low number of recovered ticks (Table 2). In order to obtain sufficient number of adult ticks to continue our study, we performed controlled nymphal infestations inside cotton sleeves glued to rabbit skin, where ticks were completely restrained and protected from host grooming. In this case, the recovery of engorged nymphs was 80.3% (803 from 1000 exposed nymphs). The mean number of engorged nymphs recovered from each of the five rabbits was 160.6 (range: 100–178). A total of 629 engorged nymphs completed molting to adults (78.3% molting success), which were 313 males and 316 females (sex ratio 1: 1.01).

Mean feeding periods of larvae varied from 4.4 days on *G. gallus* to 6.6 days on *O. cuniculus*. Larvae exposed to *G. gallus* showed the shortest feeding periods ($P < 0.05$), which varied from 3 to 9 days (mode: 4). When larvae were exposed to mammals, feeding period varied from 4 to 11 days (mode: 5 or 6), with the longest feeding periods ($P < 0.05$) on *O. cuniculus*. Larval premolt periods were generally similar among ticks fed on different host species, ranging from 12 to 23 days, and the mean values varying from 14.6 (for larvae fed on *G. gallus*) to 16.1 days (for larvae fed on *C. callosus*). The only statistical difference between these premolt periods were for the larvae fed on *G. gallus,* which were significantly shorter ($P < 0.05$) (Table 1). Nymphal feeding periods varied from 4 to 8 days when ticks were released freely on the host and only a total of 29 nymphs were recovered from the six host species (Table 2). Mean premolt periods of these nymphs were quite similar, with mean periods varying from 17.0 to 17.9 days. The 629 engorged nymphs recovered from controlled infestations on rabbits had a mean feeding period of 5.6 days (range 4–10) and mean premolt period of 17.9 days (range 15–28). Premolt periods of male nymphs (mean: 17.7 days) were significantly shorter ($P < 0.05$) than premolt periods of female nymphs (18.2 days).

A total 15 engorged females out of 30 exposed females were recovered from the two infested dogs, resulting in a 50% feeding success. These females fed from 11 to 15 days (mean: 12.5), reaching a mean weight of 487.9 mg (range: 304.1–665.3 mg). All but one engorged females oviposited viable egg masses (mean weight: 253.3 mg) with an 88.2 mean% hatching (range: 75–95%).

The life-cycle of *A. oblongoguttatum* (larvae to larvae) under laboratory conditions was completed in 188 days, considering the mean feeding and premolt periods of larvae exposed to *C. porcellus* (Table 1) and nymphs exposed to *O. cuniculus* (controlled infestations), the mean feeding period and reproductive data of females exposed to dogs (Table 3), and the pre-feeding periods of 25 days for each of the three parasitic stages.

Discussion

The present study evaluated the suitability of five different host species for the immature stages of *A. oblongoguttatum.* For this purpose, we used the same methodology that has been used in our laboratory for studying the life-cycle of other neotropical tick species, namely *A. tigrinum* (Labruna et al. 2002), *A. triste* (Labruna et al. 2003), *A. dubitatum* (reported as *A. cooperi*) (Labruna et al. 2004), *A. aureolatum* (Pinter et al. 2004), and *A. ovale* (Martins et al. 2012). This methodology consisted of releasing known number of ticks on host body with no interference on host grooming, with the intention to simulate a tick-host contact under natural conditions. Low tick feeding and molting success rates for some host species would be an indication that they could not serve as suitable hosts under natural conditions, while high feeding and molting success rates could indicate host suitability in nature. Obviously, in this later case, other factors are also involved, as for example, the host-seeking strategy of the tick. For example, small rodents would not be suitable hosts for ticks that quest on vegetation at heights above 10 cm. Finally, all these experimental results need to be confirmed by field data, represented by the number of records that a given tick stage has been recorded from different host taxa.

Considering the above criteria, Labruna et al. (2003) suggested that birds and small rodents could be important hosts for immature stages of *A. triste,* since these host groups yielded up to 40–65% recovery rates for engorged larvae and nymphs in the laboratory. Several field works have confirmed that immature stages of *A. triste* feed preferably on small rodents and birds (Venzal et al. 2008; Nava et al. 2011). Similarly, Martins et al. (2012) observed highest recovery rates of engorged larvae and nymphs of *A. ovale* from small rodents, and suggested that these animals would be important hosts for *A. ovale* larvae and nymphs in nature, a condition that has been confirmed by field works (Szabó et al. 2013; Martins et al. 2016). In the present study, no host species was highly suitable for *A. oblongoguttatum* larvae, since the recovery rates of engorged larvae were always <15%, or most of the times ≤5%. Similar results were obtained for nymphs, with recovery rates of engorged nymphs always <6%. Contrastingly, when we used nymphs from the same experimental batches to feed inside cotton sleeves glued to rabbit skin, the recovery rate of engorged nymphs was 80.3%. This later recovery rate indicate that the nymphs used in the present study were highly viable; therefore, the low recovery rates observed when ticks were released freely on host dorsum were indeed indicative that these host species were not suitable for *A. oblongoguttatum.*

Since we used five different species of small mammals during larval and nymphal infestations, our results led us to speculate that small mammals, especially small rodents, do not have an important role in the life-cycle of *A. oblongoguttatum* under field conditions. Interestingly, a field study in two Amazon forest areas showed that *A. oblongoguttaum* nymphs were efficiently collected by dragging on dense vegetation (Labruna et al. 2009), an indication that these nymphs host quest on vegetation above ground level. This condition, coupled with the results of the present study, reinforce our suggestion that small mammals are not important hosts for *A. oblongoguttatum.* This statement is corroborated by the few host-record data available for *A. oblongoguttatum* immature ticks, which were mostly on medium-sized mammals, such as deer, dogs, peccary, coati, agouti, and humans (Nuttall 1912; Santos Dias 1986; Labruna et al. 2005a; Ogrzewalska et al. 2007; Martins et al. 2009; Soares et al. 2014; Acosta et al. 2016; Witter et al. 2016). Interestingly, Labruna et al. (2000c) reported a case of gynandromorphism in *A. oblongoguttatum* specimen collected on dog in the Brazilian Amazon; on the other hand, none gynandromorphy specimen was observed in the present study during two consecutive generations in the laboratory. Finally, our results of infestation of adult ticks indicate dogs as suitable hosts for *A. oblongoguttatum* females, corroborating field studies that have appointed dogs as important hosts for the adult stage of *A. oblongoguttatum* (Labruna et al. 2000a, 2005a; Bermúdez et al. 2011).

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