Research Note

Braz. J. Vet. Parasitol., Jaboticabal, v. 24, n. 3, p. 357-360, jul.-set. 2015
ISSN 0103-846X (Print) / ISSN 1984-2961 (Electronic)
Doi: http://dx.doi.org/10.1590/S1984-29612015020

Amblyomma nodosum (Neumann, 1899): observations on life cycle under laboratory conditions

Amblyomma nodosum (Neumann, 1899): observações sobre o ciclo biológico em condições de laboratório

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Received December 8, 2014
Accepted February 13, 2015

Abstract

The natural hosts of *Amblyomma nodosum* in the immature stages are a variety of birds and the anteater in the adult stage. However, so far no data have been published about this tick's life cycle. To fill this gap, a record was made of its development under laboratory conditions. All the procedures were controlled in a BOD chamber set at 27±1 °C and 80±10% relative humidity and scotophase. The parasitic stages were raised on rabbits (*Oryctolagus cuniculus* Linnaeus, 1758), from which more than 50% of larvae and nymphs were recovered, although only a small portion performed ecdysis. The adults did not fixed on the rabbits, which suggests that the experimental conditions were unsuitable for the requirements of this species. The data obtained here indicate that *A. nodosum* is highly dependent on its host and environment whereas under laboratory conditions and host chosen for the study was not obtained satisfactory results and new studies with different hosts and new environmental conditions should be elaborated.

Keywords: Tick, host, biology, Brazil, *Amblyomma nodosum*.

Resumo

Os hospedeiros naturais de *Amblyomma nodosum*, nos estágios imaturos, são uma variedade de pássaros e, na fase adulta, são os tamanduás. No entanto, até agora não há dados publicados sobre o ciclo de vida desse carrapato. Para preencher essa lacuna, um registro foi realizado sobre o desenvolvimento em condições de laboratório. Todos os procedimentos foram controlados em câmaras tipo BOD a 27±1 °C e 80±10% de umidade relativa e escotofase. As fases parasitárias foram desenvolvidas em coelhos (*Oryctolagus cuniculus* Linnaeus, 1758), a partir dos quais mais de 50% das larvas e ninfas foram recuperados, embora apenas uma pequena porção tenha realizado ecdise. Os adultos não se fixaram nos coelhos, o que sugere que as condições experimentais eram inadequadas para os requisitos dessa espécie. Os dados obtidos indicam que *A. nodosum* é altamente dependente de seu hospedeiro e ambiente, enquanto em condições de laboratório e pela escolha do hospedeiro para o estudo não foram obtidos resultados satisfatórios, e novos estudos com diferentes hospedeiros e novas condições ambientais devem ser elaborados.

Palavras-chave: Carrapato, hospedeiro, biologia, Brasil, *Amblyomma nodosum*.

Amblyomma nodosum (Neumann, 1899) is a an ixodid tick restricted to the Neotropical region (GUGLIELMONE et al., 2003; NAVA et al., 2007), whose hosts are the mammals *Tamandua* (Linnaeus, 1758) and *Myrmecophaga* (Linnaeus, 1758) in the adult stage and a variety of birds in the immature stages (JONES et al., 1972; BECHARA et al., 2002; MARTINS et al., 2004; LABRUNA et al., 2007; OGRZEWALSKA et al., 2009; LUZ et al., 2012; PASCOAL et al., 2013; TORGA et al., 2013; GARCIA et al., 2013; MARTINS et al., 2014).

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Although there are no reports of *A. nodosum* acting as a vector of pathogens, isolates of *Rickettsia parkeri* and *Rickettsia bellii* have been obtained from specimens collected in passerine birds caught in the Atlantic Forest, and from *Tamandua tetradactyla* (Linnaeus, 1758) in the Pantanal wetland of southern Mato Grosso (OGRZEWALSKA et al., 2009; ALMEIDA et al., 2013). These findings place *A. nodosum* on the list of species that may be important in the epidemiology of rickettsial diseases that have been little studied.

Information on *A. nodosum* in the scientific literature covers the morphology, taxonomy and records of surveys on fauna of ixodid ticks (SERRA-FREIRE et al., 1993; AMORIM & SERRA-FREIRE, 1994; BITENCOURTH et al., 2007), but data on its biological cycle have yet to be reported.

Biological data on Neotropical ticks is usually difficult to obtain because most of the species are heteroxenous, their hosts are wild animals, and they live in ecosystems in which the microclimates required for their development are also often unknown. In view of this shortcoming, a record was made of its development under laboratory conditions.

An engorged female of *A. nodosum* was collected from a giant anteater (*Myrmecophaga tridactyla*) at the Wild Animal Screening Center (CETAS- IBAMA), which receives animals captured in the metropolitan region of Rio de Janeiro. The specimen was sent to Laboratory of Ixodology at the Federal Rural University of Rio de Janeiro, where it was washed with water and hypochlorite, blotted dry, identified following Onofrio et al. (2006) and placed in a Petri dish, and fixed in the supine position using adhesive tape.

During the life cycle, both the female *A. nodosum* and the other non-parasitic stages were kept in controlled laboratory conditions in a acclimatized BOD (Biochemical Oxygen Demand) chamber, set at a temperature of 27±1 °C, 80±10% relative humidity and scotophase. The parasitic stages were raised on rabbits (*Oryctolagus cuniculus* Linnaeus, 1758) without prior contact with ticks or acaroids. Notes were taken daily during all the experimental steps. Infestations were recorded according to the method described by Neitz et al. (1971).

The total egg mass of the female *A. nodosum* was collected and placed in 10 ml disposable plastic syringes, which were cut off close to the plunger, closed with cotton wool, and again placed in the BOD chamber. Larval hatching was recorded, and 15 to 20-day-old larvae were raised on the rabbits. After dropping off the hosts spontaneously, the engorged larvae were collected, treated in the same way as the eggs, and kept in an incubator until the nymphs finished molting.

Fifteen to 20-day post-ecdysial nymphs were raised on rabbits and, after dropping off spontaneously, were stored in syringes and placed in the BOD chamber. Adult ecdysis was recorded and at 20 to 25 days the adults were placed on the host's back (NEITZ et al., 1971). The biological parameters evaluated here were the ones assessed by Chacón et al. (2003) and Bellato & Daemon (1997).

The present study was approved by the Ethics Committee on the Use of Animals at the Federal Rural University of Rio de Janeiro/COMEP, under process number 160/2011. The rabbits were given water and commercial feed pellets *ad libitum*.

The total weight of the egg mass produced by the female *A. nodosum* was 593.10 mg, from which 792 (256.3 mg) larvae hatched. After feeding on the rabbits, 413 (52.1%) engorged larvae were recovered. Twenty-five nymphs molted (6.05%), from which 14 (56%) engorged nymphs (89.7 mg) were recovered after infestation and spontaneous dropping off the hosts. Seven adult *A. nodosum* emerged, two females and five males, representing a total molt rate of 50%. The adults did not fixed on the rabbits (Table 1).

The life cycle of *A. nodosum* in the laboratory did not complete one generation, presenting difficulties in its evolution, which suggests that the experimental conditions were unsuitable for the requirements of this species.

As for the immature stages of *A. nodosum*, it was possible to recover more than 50% of the specimens, which is comparable to the range of recovery of other Neotropical species of *Amblyomma* raised in the laboratory under similar conditions (SANAVRIA & PRATA, 1996; LABRUNA et al., 2002a, 2004; PINTER et al., 2004; FACCINI et al., 2010; MARTINS et al., 2012; GERARDI et al., 2013). The immature stages of *A. nodosum* parasitize several species of birds, showing low specificity (LABRUNA et al., 2007; OGRZEWALSKA et al., 2009, LUZ et al., 2012; PASCOAL et al., 2013; TORGA et al., 2013; NAVA & GUGLIELMONE, 2013). Rabbits are routinely used as experimental hosts to raise the different stages of several tick species (SANAVRIA & PRATA, 1996; PRATA et al., 1998; FACCINI et al., 2010; PINHEIRO et al., 2013). Although more than half of the larvae were recovered, only a small portion (6%) molted. This may be attributed to incomplete and improper feeding by the tick because rabbits are not natural hosts, or because the climatic parameters did not favor the continuity of the cycle. As most ticks spend the best part of their life away from the host, climate conditions such as temperature and humidity are important factors for the success of the cycle. Randolph (2004) pointed out that these factors and others such as seasonality, search time to find a host, and mortality, influence the life cycle. In this study, a temperature of 27±1 °C and relative humidity of 80±10% were used to raise *A. nodosum*, since these values are used to breed most Neotropical ticks (*Amblyomma parvum* Aragão 1908 – GUGLIELMONE et al., 1991; OLEGÁRIO et al., 2011; *Amblyomma aureolatum* – RODRIGUES et al., 2002; *Amblyomma tigrinum* Koch 1844 – LABRUNA et al., 2002b; CARDOSO et al., 2008; *Amblyomma triste* – LABRUNA et al., 2003; *Amblyomma cooperi* Nuttal; Warburton, 1908 – LABRUNA et al., 2004; *Amblyomma incisum* Neumann, 1906 – SZABÓ et al., 2009;

Table 1. Biological parameters of *Amblyomma nodosum* ticks raised on rabbits in the laboratory (27± 1°C, 80% RH and scotophase).

Biological parameters	Larvae	Nymphs	Adults
Pre-oviposition period (days) period (days)+(dias)	-	-	3
Oviposition period (days)	-	-	18
Egg hatching period (days) hatching(days)	11 ± 1.73 (10-13)	-	-
Feeding period (days)	6.5 ± 0.70 (6-7)	10.33 ± 2.08 (8-12)	-
Ecdysis period (days)	10.66 ± 1.15(10-12)	22.62 ± 10.69 (11-32)	-
Ticks recovered (%)	36.67	56	-

Values are presented as mean ± SE (range in parentheses).

Amblyomma auricularium – FACCINI et al., 2010; and *Amblyomma ovale* – MARTINS et al., 2012). However, other values for these variables may be tested to determine the best conditions for the development of this species.

Rabbits were not good hosts for the adult ticks, since in these conditions no individual of *A. nodosum* showed any stimulus or action to feed. The host species for this stage appear to be restricted to those within the superorder Xenarthra, particularly Myrmecophagidae (JONES et al., 1972; BECHARA et al., 2002; MARTINS et al., 2004, 2014; GARCIA et al., 2013). Occasional records of hosts such as the six-banded armadillo *Euphractus sexcinctus* (Linnaeus, 1758) (BECHARA et al., 2002) and dogs can be considered accidental (MAZIOLI et al., 2012). Nava & Guglielmone (2013) consider that ticks do not generally show high host specificity, and that ecological factors such as habitat specificity, time elapsed between generations, the free-living stage, and the type of cycle are more important than host selectivity. Therefore, *A. nodosum*, a characteristic tick of the Cerrado biome, probably prefers drier and warmer environments (OGRZEWALSKA et al., 2009). However, there are no studies that address the climate requirements of free-living *A. nodosum*.

The data obtained here indicate that the *A. nodosum* life cycle is highly dependent on its host and environment whereas under laboratory conditions and host chosen for the study was not obtained satisfactory results. Although it was not possible to complete the cycle of *A. nodosum* due to the low number of adults and their non-attachment on the rabbits, the data may contribute to the body of knowledge about its biology and to new attempts to raise it in the laboratory. While not representative of the reality of the *A. nodosum* life cycle in natural conditions, data obtained in the laboratory are one of the most common ways to make inferences about tick biology, particularly those that parasitize wild animals, and serve to underpin predictive studies.

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