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Influence of microhabitat use and behavior of *Amblyomma sculptum* and *Amblyomma dubitatum* nymphs (Acari: Ixodidae) on human risk for tick exposure, with notes on *Rickettsia* infection

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ABSTRACT

Brazilian spotted fever (BSF) is a potentially lethal human disease caused by *Rickettsia rickettsii* transmitted by ticks, including *Amblyomma sculptum*. However, in Southeast Brazil, where most BSF cases occur, capybaras are key hosts for both *A. sculptum* and *Amblyomma dubitatum*. We therefore compared the risk of human exposure to these ticks at a non-endemic anthropogenic site in Southeast Brazil where both tick species are maintained by capybaras and occur at high abundance. Cloth dragging, human baits and CO_2 traps were used to assess tick abundance and risk for human exposure. The two tick species displayed profound differences in behavior and microhabitat use. Notably, *A. sculptum* but not *A. dubitatum* quested for hosts openly from vegetation (ambush behavior) and infested human baits. Furthermore, *A. dubitatum* was more aggregated at a specific site whereas *A. sculptum* was more widespread along differing and drier microhabitats. Adults and nymphs of both species were infected with *Rickettsia bellii*. Overall, the results indicate that even though both species co-existed in the same area, *A. sculptum* posed a greater risk for biting humans and thus also for transmitting tick-borne pathogens.

1. Introduction

The main human tick-borne disease in Brazil is spotted fever caused by *Rickettsia rickettsii* (Labruna, 2009). This potentially fatal disease is also known as Brazilian spotted fever (BSF). Two tick species, *Amblyomma sculptum* (referred to as *Amblyomma cajennense* before 2014) and *Amblyomma aureolatum*, are recognized as vectors of the pathogen to humans, although a potential role for other two species, *Amblyomma dubitatum* and *Rhipicephalus sanguineus* sensu lato, cannot be discounted (Szabó et al., 2013a). Most disease cases occur in areas where *A. sculptum* are abundant and feed on capybaras (Labruna, 2009; Szabó et al., 2013a). Nonetheless several features of BSF epidemiology are unknown and it is intriguing that in Southeast Brazil, where most BSF cases occur, capybaras are key hosts for both *A. sculptum* and *A. dubitatum* (Queirogas et al., 2012; Souza et al., 2006). Furthermore, under experimental conditions both tick species were shown to transmit *R. rickettsii* to susceptible hosts (Sakai et al., 2014; Soares et al., 2012). Despite low efficiency of transovarial transmission, tick infection may result from feeding on infectious capybaras, serving as amplifier hosts, and subsequent transstadial passage (Labruna, 2009). Such *R. rickettsii* tick infection rate amplification was experimentally proven for *A. sculptum* (Souza et al., 2009).

One possible explanation for *A. sculptum*, and not *A. dubitatum*, serving as a primary vector for *R. rickettsii* to humans is the high aggressiveness of the former species. In fact, *A. cajennense* (designated as *A. sculptum* after 2014 in southeast and central Brazil) is considered a

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major human biting tick species in Brazil (Guglielmone et al., 2006) but the lack of species identification keys for immatures (a key for nymphs in Brazil was published only in 2010 by Martins and colleagues) may have caused evaluation bias. To date *A. dubitatum* human bites have been recorded but the extent of such parasitism remains unclear (Labruna et al., 2007). Thus, considering that both tick species coexist throughout many capybara territories in southeast Brazil where BSF occurs, the potential for human exposure to both tick species should be examined in greater detail to enhance our knowledge of the risks of tick-borne diseases, particularly of BSF. Therefore, we herein compare risk of human exposure to *A. sculptum* and *A. dubitatum*, and identify their associated *Rickettsia*, at an anthropogenic area that is non-endemic for BSF and where populations of both tick species are maintained by capybaras.

2. Materials and methods

2.1. Study location

The study was conducted on the grounds of a social and sports club (Praia Clube −18°55′49"S; 48°17′ 33"W) in Uberlândia city, Minas Gerais State, Southeast Brazil. Uberlândia is a major town in Southeast Brazil (*circa* 700,000 inhabitants) and is not endemic for Brazilian spotted fever. The town is within the "Cerrado" biome, a savannah, and has a tropical climate with two distinct seasons; a hot and rainy summer and a cool and dry winter. The club is located along, approximately, 1100 m and on both margins of the Uberabinha River. This river flows over several kilometers within the city boundaries and most of its margins are covered by vegetation from a few to several meters wide. Vegetation is heterogeneous, from grassy to several patches of a riparian forest. Several capybara (*Hydrochoerus hydrochaeris*) groups inhabit river margins within the city (Queirogas et al., 2012).

2.2. Site and time of tick sampling

Tick sampling occurred twice, a preliminary one, in March 2015, and the other in August 2015. The preliminary one, in summer, evaluated infestation sites along the riverside within the club and determined the main tick species. Adult stages of *Amblyomma* prevail in this season in Brazil (Labruna et al., 2009) and therefore species identification is easier. This sampling occurred along the full extension of the club on both sides of the fence that separates the club from the river. The second sampling focused on evaluating human tick-bite risk and was conducted on a single morning in August 2015. This sampling occurred at the most heavily infested site of the Uberabinha River as determined by the preliminary sampling. August is in the middle of winter in Brazil, when nymphs of *A. sculptum* are found in high numbers. This tick species and life stage commonly infest humans (Ramos et al., 2014) and is associated with spotted-fever occurrence (Labruna, 2009). This second sampling, occurred parallel to the river, on both sides of a 1.3 m high and ivy-covered fence that separates the club from the river margin and along, approximately, 130 m (Fig. 1). Along the studied path, the river margin was high precluding flooding in the wet season, 6–10 m wide until the fence, and had several high and dense bamboo tussocks that provided a constant shadow precluding the growth of other vegetation. Thus, the ground was mostly devoid of vegetation and partially covered with bamboo leaves. A group of capybaras frequently used this shadowed area to rest and was restricted to the river side by the fence. The other side of the fence (the club side) had a four-meter-wide grass area from the fence until a concrete path (two meter-wide), and then an additional 14 m-wide grass area until a jogging path and a sports field. This whole area by the club side was fully exposed to the sun light and capybaras could not access it.

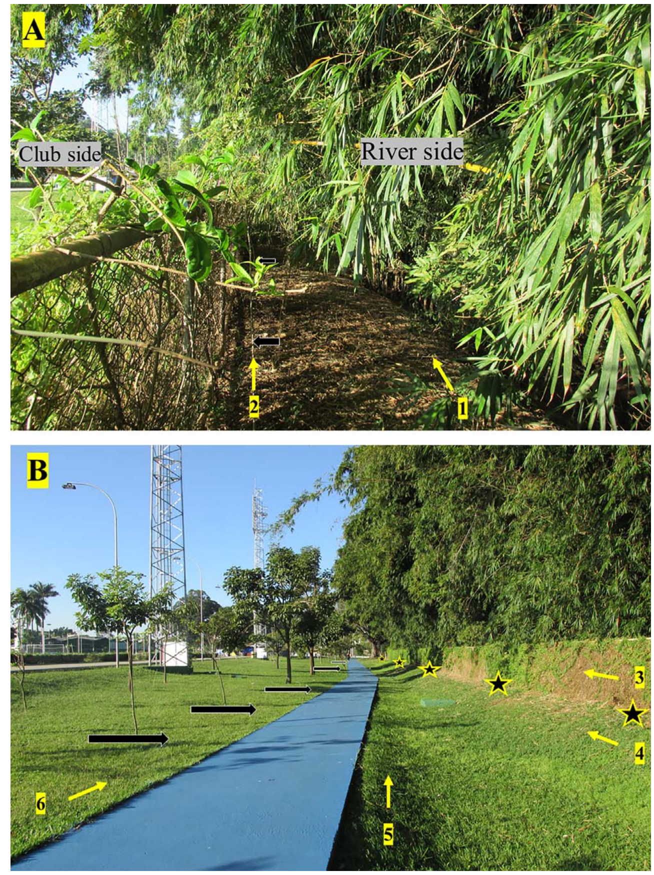

Fig. 1. Locations of tick sampling. (A) River side with several high and dense bamboo tussocks: (1) 1.5 m from the fence and (2) immediately besides the fence; (B) Club side with 4 m wide grass area from the fence to a blue concrete path, and additional 14 m wide grass area until a jogging path: (3) the ivy on the fence, (4) beside the fence, (5) 4 m from the fence and (6) 6 m from the fence after the concrete walking path. Direction of cloth dragging is indicated by yellow arrows. Black arrows show the direction of CO_2 traps. Human bait locations are indicated by stars. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Tick sampling methods

Three sampling methods, cloth dragging, human bait and CO_2 traps were used. Cloth dragging is used to sample ticks questing on the tips of vegetation and trying to maximize contact with hosts that are passing by. For our sampling, a one-meter-wide and two-meter-long white cotton flannel was pulled over the ground vegetation or over the ivy-covered fence and examined every 10–20 m of dragging to collect ticks. The second technique, human bait, was used to evaluate attraction of ticks specifically to this host when stationary in one spot. Such human behavior is common during sunbathing, fishing, hunting, picnicking and others. Human baits consisted of five of the authors each sitting on a white flannel (50 cm × 100 cm) for approximately 30 min, and collecting ticks crawling towards them on the flannel. The last technique used, CO_2 trapping, was used to provide the actual tick population size since both targeted tick species are much attracted by such a trap (Szabó et al., 2007; Szabó et al., 2009). CO_2 traps attracts both hunter ticks that are hiding but also those questing from the vegetation. Briefly, each CO_2 trap consisted of a piece of white square flannel (50 × 50 cm) with 200 g of dry ice in the middle allowed to sublimate for 90 min. Ticks were collected with fine forceps and stored in ethyl alcohol-containing vials until identification in laboratory.

2.4. Preliminary sampling in March

36 CO_2 traps were set up along both sides of the fence that separates

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