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Molecular screening of *Bartonella* in free-ranging capybaras (*Hydrochoerus hydrochaeris*) from Paraná State, Southern Brazil

Avaliação molecular de *Bartonella* em capivaras (*Hydrochoerus hydrochaeris*) de vida livre do Estado do Paraná, Brasil

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Highlights

Wild capybaras not infected by *Bartonella* sp.
Capybaras exclusively ectoparasitized by *Amblyomma dubitatum* ticks.
Salivary glands of *Amblyomma dubitatum* ticks not presented *Bartonella* sp DNA.

Abstract

Bartonella is an emerging group of facultative intracellular bacteria causing circulatory and systemic disorders. Hosts for *Bartonella* are mostly mammals, specifically rodents, having a growing number of *Bartonella* species related to their infection. Capybaras (*Hydrochoerus hydrochaeris*) are abundant native rodents of Brazil, commonly found in urban parks. In the present study, we aimed to perform molecular screening of capybaras for *Bartonella* spp. Blood samples were collected from 17 free-ranging animals captured in Paraná State, Southern Brazil. None of the collected samples tested positive for the *Bartonella*-*nuo*G gene by quantitative polymerase chain reaction (qPCR), although all of them successfully amplified

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the mammal endogenous glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene. Additionally, all animals were infested exclusively by *Amblyomma dubitatum* ticks at the time of sampling. This study was part of an active surveillance program, which is critical for monitoring animal health status, particularly in capybaras.

Key words: *Amblyomma dubitatum.* Bartonellosis. Wild rodents.

Resumo

Bartonella é um grupo emergente de bactérias intracelulares facultativas que causam doenças circulatórias e sistêmicas. Mamíferos são os principais hospedeiros das bartonelas, especificamente roedores, com crescente número de espécies de *Bartonella* relacionadas a estes animais. Capivaras (*Hydrochoerus hydrochaeris*) são abundantes roedores do Brasil, comumente encontrados em parques urbanos. Nosso objetivo no presente estudo foi realizar uma triagem molecular das capivaras para *Bartonella* spp. Amostras de sangue foram coletadas de 17 animais de vida livre capturados no estado do Paraná, Sul do Brasil. Nenhuma das amostras coletadas apresentou resultado positivo para o gene *nuoG* de *Bartonella* pela reação em cadeia da polimerase quantitativa (qPCR), apesar de todas elas amplificarem com sucesso o gene endógeno de mamíferos gliceraldeído-3-fosfato desidrogenase (*gapdh*). Adicionalmente, todos os animais estavam infestados exclusivamente por carrapatos *Amblyomma dubitatum* no momento da coleta. Este estudo foi parte do programa de vigilância ativa, que é importante para monitorar a condição de saúde dos animais, particularmente em capivaras.

Palavras-chave: *Amblyomma dubitatum.* Bartonelose. Roedores silvestres.

The *Bartonella* genus is composed of emerging, facultative intracellular, Gram-negative bacteria showing fastidious growth in the culture medium. The infection by these agents causes long-lasting erythrocytic bacteremia (Dehio, 2004). While infecting a mammalian host, it can invade erythrocytes and endothelial cells, a strategy to evade the immune system of the host (Breitschwerdt & Kordick, 2000). Their transmission among animals is carried out mainly through bloodsucking vectors. These abilities contribute to the successful propagation of *Bartonella* worldwide, especially in many rodent species (Gutiérrez et al., 2015). When the agent is inoculated in any host, the latter can develop variable disease, acute or chronic, or even no perceptible in reservoir hosts depending on the degree of adaptation (Breitschwerdt & Kordick, 2000).

Despite many rodents have been identified as reservoirs for *Bartonella* spp. (Gutiérrez et al., 2015), the role of capybaras (*Hydrochoerus hydrochaeris*), the biggest rodent in the world, have been scarcely investigated (Gonçalves et al., 2020). This mammal species inhabits wetlands from South America in conserved environments to urban parks, where they coexist with humans (Queirogas, Del Claro, Nascimento, & Szabó, 2012). Accordingly, the present study aimed to screen free-ranging capybaras for the presence of *Bartonella* spp.

This study was approved by the Ethics Committee in Animal Experimentation and Animal Welfare at the Universidade Federal do Paraná (UFPR) (protocol number 45/16). Animal and laboratory procedures were approved and performed under the regulations of the Chico Mendes Institute for Biodiversity Conservation (ICMBio, protocol number 69426-1).

A total of 17 capybara DNA samples, 10 females and 7 males among youth and adults, from a previous study were retrieved and used, animals from municipality of Pinhais, Paraná State, Southern Brazil (49º7'41.897" W 25º23'12.278" S) (Vieira et al., 2021). To evaluate the presence of *Bartonella* DNA in the samples of capybaras, quantitative PCR (qPCR) targeting the nicotinamide adenine dinucleotide dehydrogenase gamma subunit (*nuoG*) was used, as described elsewhere (André et al., 2015).

All 17 capybaras tested negative for *Bartonella* spp. by qPCR, although all samples have consistently amplified the *gapdh* gene. Additionally, we found at the time of sampling that each of the animals was infested by ticks (adults, nymphs, and larvae) (infestation rate, 9.82), all morphologically identified as *Amblyomma dubitatum* (*A. dubitatum*) (Barros-Battesti et al., 2006).

Although the present study failed to detect *Bartonella* DNA in capybaras, several studies have reported an increasing number of wild rodent-associated *Bartonella* infections in different Brazilian biomes. In the Pantanal region, midwestern Brazil, 110 small rodents of three species, namely 77 *Thrichomys fosteri,* 25 *Oecomys mamorae,* and eight *Clyomys laticeps* were molecularly tested for the bacteria. Thirty-five animals (31.8%) belonging to *Thrichomys fosteri* and *Oecomys mamorae* species, infested by fleas and ticks, were positive for *Bartonella* spp. (Sousa et al., 2018). In Mato Grosso do Sul State, midwestern Brazil, seven out of eight rodent species collected from farm fields [*Callomys callosus* (4/19), *Cerradomys maracajuensis* (4/4), *Hylaeamus megacephalus* (1/1), *Necromys lasiurus* (6/13), *Nectomys squamipes* (1/1), *Oecomys catherinae* (1/2), *Oxymycterus delator* (1/1), and *Thrichomys fosteri* (0/1)] presented 42.9% positivity for *Bartonella.* In the state of Rio de Janeiro, southeastern Brazil, 23/131 small rodents belonging to 18 different species presented 17.6% positivity to Bartonella spp. (Favacho et al., 2015; Rozental et al., 2017). In five Brazilian biomes, 117/457 (25.6%) small rodents from 52 different species were positive for the bacteria (Gonçalves et al., 2016).

A recent study demonstrated that rodent populations that live far from the coast showed a lower infection rate for *Bartonella* spp. than those near coastline (Gonçalves et al., 2020). In that study, capybaras from Campo Grande municipality, Mato Grosso do Sul State, midwestern Brazil, were infested with ticks (*Amblyomma* sp. larvae, *A. dubitatum,* and *Amblyomma sculptum*), and all animals and ticks tested negative for *Bartonella* spp. by the same qPCR targeting the *nuoG* gene used herein. Capybaras are favored for their amphibian lifestyle, which blocks parasitism by ectoparasites that are not adapted to semi-aquatic habitats, such as fleas and lice, commonly found in other wild rodents species (Lord, 1994). This adaptation may make the infestation of capybaras by fleas, the main vector of bartonellae among rodents, unsuccessful. As a consequence, the chances of infection by *Bartonella* spp. are likely diminished.

Although there is evidence of the vector competence of the tick *Ixodes ricinus* to transmit *Bartonella* spp. (Reis et al., 2011), to date these arthropods are likely not epidemiologically important to the epidemiology of bartonellae among rodents. In Northeastern Brazil, a study focusing on *Bartonella* and its possible vectors for free-ranging and domesticated animals was conducted. In that study, all ticks (*A. dubitatum, Amblyomma auricularium, Rhipicephalus sanguineus* sensu lato, and Argasidae) were PCR-negative for *Bartonella,* while flea and lice species (*Pulex irritans, Ctenocephalides felis,* and *Polyplax* spp.) have tested PCR-positive for *Bartonella* (Fontalvo et al., 2017). Similarly, ticks (*A. dubitatum, Amblyomma sculptum,* and *Amblyomma* sp. larvae) parasitizing *Mus musculus, Rattus rattus,* capybaras, and opossums (*Didelphis albiventris*), also tested negative for *Bartonella* in Midwestern Brazil (Gonçalves et al., 2020). It is a notorious evolutionary adaptation that some bacterial agents are developed as tick-borne pathogens, like *Ehrlichia, Anaplasma,* and *Rickettsia* (Mccoy, Léger, & Dietrich, 2013). However, rodent-associated *Bartonella* appears to be less adapted to ticks than other hematophagous ectoparasites. Rodents are known to exhibit greater diversity of *Bartonella* genotypes among mammals, with this phenomenon being related to their fleas parasitism (Gutiérrez et al., 2015).

In the present study, tick-infested capybaras were negative for *Bartonella* spp. At this moment, it may be premature to state that capybaras do not harbor *Bartonella* spp. Further studies, involving capybaras from different biomes, are needed to confirm our hypothesis. Due to the close contact with human beings, we suggest monitoring this species.

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