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Anti-*Rickettsia rickettsii* antibodies in capybaras (*Hydrochoerus hydrochaeris* Linnaeus, 1766) from an agricultural landscape in Araras, São Paulo, Brazil

Anticorpos anti-*Rickettsia rickettsii* em capivaras (*Hydrochoerus hydrochaeris* Linnaeus, 1766) de uma de uma região agrícola de Araras, São Paulo, Brasil

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Abstract

Spotted fever is a typically endemic infectious disease caused by rickettsiae from the spotted fever group, of which *Rickettsia rickettsii* is the main etiologic agent. It presents high mortality rates in Brazil, with transmission to humans or animals through the bite of infected ticks. The capybara (*Hydrochoerus hydrochaeris*) is an important reservoir for *Rickettsia* spp.; these bacteria can circulate in an infected animal presenting only fever as a clinical sign of the disease, as demonstrated by experimental infection. Considering the high zoonotic potential and the damage caused to human, animal, and environmental health, this study searched for anti-*Rickettsia rickettsii* antibodies in capybaras from an agricultural landscape in the city of Araras, State of São Paulo, Brazil. The indirect immunofluorescence (IFA) technique was used to detect anti-*R. rickettsii* antibodies. From the 28 serum samples tested using IFA, 18 (64.28%) were considered reactive, with antibody titers ranging from 256 to 2048. Seven (38.88%) samples presented titers of 256, three (16.67%) with titers of 512, five (27.78%) with titers of 1024, and three (16.67%) with titers of 2048. However, it was not possible to significantly associate gender to these serologic results. These results demonstrate that at some point during their lives, the studied capybaras were exposed to the etiologic agent, but it is impossible to know when this occurred. Further studies need to be performed to clarify which serological titers ensure an infection in capybaras**,** based on clinical and laboratory assessment of rickettsemia, and to establish the relationship between titers

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and the chronicity of disease. This is necessary owing to the possibility of cross-reactions with other rickettsiae species of the same subgroup, leading to the need for molecular tests to confirm diagnosis.
Key words: Ticks. Serological diagnosis. Spotted Fever. Human. Zoonosis.

Resumo

A febre maculosa é uma doença infecciosa, causada por rickéttsias do Grupo da Febre Maculosa, que geralmente se desenvolve em caráter endêmico e tem como principal agente etiológico *Rickettsia rickettsii*. Apresenta elevadas taxas de letalidade no Brasil, e a transmissão do agente ao homem ou animal ocorre pela picada de carrapatos infectados. A capivara (*Hydrochoerus hydrochaeris*) é um importante reservatório de *Rickettsia* spp., e por meio de infecção experimental demonstrou-se sua capacidade de mantê-la circulante no organismo, sem apresentar sinais clínicos da doença. Considerando o elevado potencial zoonótico e os prejuízos causados na saúde única por esse agente, este trabalho teve o objetivo de detectar anticorpos anti-*Rickettsia rickettsii* em capivara (*Hydrochoerus hydrochaeris*) de um bosque urbano de Araras, São Paulo, Brasil. Foi utilizada a técnica de imunofluorescência indireta (IFA) para detectar anticorpos anti-*R. rickettsii.* Das 28 amostras de soro testadas na IFA, 18 (64,28%) foram consideradas reagentes com títulos de anticorpos variando de 256 a 2048, sendo que sete (38,88%) amostras apresentaram títulos de 256, três (16,67%) títulos de 512, cinco (27,78%) títulos de 1024 e três (16,67%) títulos de 2048 e não foi possível a associação da variável sexo ($p \leq 0.05$) com os resultados sorológicos para *Rickettsia rickettsii*. Outros estudos serão necessários para esclarecer que títulos sorológicos na IFA podem assegurar a positividade da infecção na capivara, a partir de avaliação clínica e laboratorial frente à rickettsemia, e estabelecer a relação entre títulos e a cronicidade da doença. Isso decorre da possibilidade de ocorrência de reações cruzadas com outras espécies de rickéttsias dos mesmos subgrupos, levando à necessidade da realização de testes moleculares para se confirmar o diagnóstico para a enfermidade.
Palavras-chave: Carrapatos. Diagnóstico sorológico. Febre maculosa. Humano. Zoonose.

Introduction

Capybaras (*Hydrochoerus hydrochaeris*) are large, semi-aquatic, herbivorous rodents that live in flocks found all over South America. They are social and gregarious animals that occupy diverse habitats including riparian forests, seasonally flooded savannas, mangroves, wetlands, water bodies, pastures, and bushes, where they protect themselves from predators and natural phenomena (GARCIAS; BAGER, 2009).

The species is currently taking advantage of the conditions offered by certain environments modified and occupied by humans, where there are no natural predators. This allows capybaras to develop large populations that become problematic in both rural and urban areas, especially public parks (SHARMA et al., 2004). In at least two Brazilian states, São Paulo and Paraná, there has been serological evidence that capybaras were exposed to tick-borne rickettsiae of the spotted fever group (SOUZA et al., 2004; FORTES et al., 2011; KRAWCZAK et al., 2014).

Spotted fever is an infectious disease caused by rickettsiae of the spotted fever group (SFG). The disease is usually endemic, presenting high fatality rates in Brazil, and *Rickettsia rickettsii* is the main etiologic agent (NASSER et al., 2015), being transmitted through the bite of infected ticks. In these invertebrate hosts, rickettsiae multiply in multiple organs, including the hemolymph, salivary glands, and ovaries, enabling transovarial transmission between tick generations (QUINN et al., 2005). While there is transstadial perpetuation of rickettsiae among tick developmental stages, rickettsial infection can sometimes be lethal for ticks, or can promote reduction in tick fertility (QUINN et al., 2005). In vertebrates, SFG rickettsiae

multiply mainly within endothelial cells (QUINN et al., 2005; RIBEIRO et al., 2013).

There are approximately 60 species of ticks in Brazil, with the largest number belonging to the *Amblyomma* genus. This genus also has the greatest medical importance since it includes the main species that parasitize humans (BRASIL, 2002). Among *Amblyomma* species responsible for Brazilian spotted fever (BSF) in humans, *A. sculptum* (formerly known as *A. cajennense*) and *A. aureolatum* are considered the most important for the enzootic maintenance and transmission of BSF (PACHECO et al., 2007; RIBEIRO et al., 2013).

According to data from the Centro de Vigilância Epidemiológica (CVE), there were 17 reported cases of BSF in the city of Araras between 2007 and 2015. Campinas, which is situated approximately 80 km southeast of Araras, had the largest number of registered cases in São Paulo during this period at 60 in total, characterizing itself as an endemic region (SÃO PAULO, 2015).

The capybara (*Hydrochoerus hydrochaeris*) is an important amplifier host of *R. rickettsii* and its ability to maintain circulating rickettsiae while presenting with only fever has been demonstrated through experimental infection (SOUZA et al., 2009). Considering the high zoonotic potential and the potential risks of this disease to human, animal, and environmental health, this study searched for anti-*Rickettsia rickettsii* antibodies in capybaras from an agricultural landscape in the city of Araras, State of São Paulo, Brazil.

Materials and Methods

Ethical Aspects

This project was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under protocol no. 37227-1 and by the Ethics Committee in Research involving Animal Experimentation (*Comitê de Ética em Pesquisa Envolvendo Experimentação Animal* - CEPEEA) from the Universidade Paranaense (UNIPAR) under protocol no. 26226/2014.

Study area

This study was performed in an area that belongs to a college campus located in the city of Araras (Latitude: 22° 21 ‘ 25 “S; Longitude: 47° 23 ‘ 03 “W) in the State of São Paulo, Brazil, with a total area of 226 hectares and containing 12.7 hectares of semi-deciduous seasonal agricultural landscape surrounded by sugarcane plantations. There are two lakes, supporting a capybara population of approximately 56 individuals.

Capture of animals

In order to capture the animals, which took place between 2013 and 2014, the animals were attracted with baits and later contained in galvanized wire traps (squeeze chutes) measuring 4 x 3 m, fitted with guillotine-type gates. The wired walls of these traps were covered with black plastic canvas to prevent the animals from hurting themselves while restrained. Corn, banana leaves, and salt were used as bait to lure the animals into the trap and a 4-month adaptation period was defined, during which the gates were held open for animals to become accustomed to the device. After that, the capybaras were captured directly inside the trap and physically restrained with nets while covering their eyes with fabric towels to reduce stress. In this way, it was possible handle the animals and collect blood.

Animal identification

The animals were sexed and classified into three age groups based on estimated body weight: infants (animals weighing up to 10 kg), juveniles (10 to 30 kg), and adults (over 30 kg), according to criteria established by Ojasti (1973). Following that, they were identified and marked with numbered carbon steel rings, applied to the right outer ear. Finally,

after blood collection, the animals were released in the same location.

Sample collection

A sample of approximately 10 mL of blood was collected from each animal by puncturing a saphenous vein, using individual and disposable syringes and needles. The blood was placed in a test tube and kept at room temperature until clot retraction. The serum obtained from each sample was then collected into a sterile vial, which was labeled and stored at -20°C until completion of serological diagnosis.

Serological diagnosis

Diagnosis was performed by indirect immunofluorescence assay (IFA) for detection of anti-*R. rickettsii* antibodies. Each capybara serum sample was individually tested using *R. rickettsii* antigen (SOUZA et al., 2009). For this purpose, a 2-fold dilution series in phosphate-buffered saline was prepared from the serum, starting from the 1:64 dilution. A fluorescein isothiocyanate-labeled sheep anti-capybara IgG (CCZ, São Paulo/SP, Brazil) was used as conjugate. For each sample, the endpoint IgG titer reacting with *R. rickettsii* strain Taiaçu crude antigen was determined. In each slide, a serum sample previously shown to be nonreactive (negative control) and another known as being reactive (positive control), both from the study by Souza et al. (2009), were tested at the 1:64 dilution.

Statistical Analysis

To verify the association of serologic results with gender or age variables in the studied capybaras, Fischer's exact test was performed using BioEstat 5.0 (AYRES et al., 2007). Results were considered significant at a significance level of 0.05.

Results

A total of 28 animals were captured, comprising five males (17.85%) and 23 females (82.15%), which were further categorized as six infants (21.43%), 12 juveniles (42.86%), and 10 adults (35.71%). Of the 28 serum samples tested by IFA, 18 (64.28%) were reactive to *R. rickettsii* antigens, with endpoint titers ranging from 256 to 2048. Seven (38.88%) samples presented titers of 256, three (16.67%) of 512, five (27.78%) of 1024, and three (16.67%) of 2048 (Table 1).

Table 1. Frequency of antibody titers detected using indirect immunofluorescence assay (IFA) for *Rickettsia rickettsii* in 18 capybara serum samples (*Hydrochoerus hydrochaeris*) from an agricultural landscape in the city of Araras, São Paulo, Brazil, 2013-2014.

Antibody Titer	Frequency		Total (%)
	Female	Male	
256	05	02	07 (38%)
512	03	-	03 (16%)
1024	03	02	07 (38%)
4096	03	-	03 (16%)
Total (%)	14 (77.78%)	04 (22.22%)	18 (100%)

Of the 18 seroreactive samples, four (22.23%) were from males and 14 (77.77%) from females; four (22.22%) from infants, seven (38.89%) from juveniles, and seven (38.89%) from adults (Table 1). No significant association with gender or age variables could be established for these serologic results (Table 2).

Table 2. Relationship between gender and anti-*Rickettsia rickettsii* antibody detection in 18 capybaras (*Hydrochoerus hydrochaeris*) from an agricultural landscape in the city of Araras, São Paulo, Brazil, 2013-2014.

Gender	Antibody Reactivity	P*	OR
Male	04/05 (80.00%)	0.6264	0.3889
Female	14/23 (60.27%)		

P = probability; * Fischer Exact Test; OR = Odds Ratio.

Discussion

Several studies have demonstrated the presence of antibodies against *Toxoplasma gondii, Neospora caninum, Leptospira* spp.*, Trypanosoma cruzi,* and *Sarcocystis neurona* in capybaras in the state of São Paulo, Brazil (VALADAS et al., 2010; CHIACCHIO et al., 2014), emphasizing the importance of this species as a potential transmitter of infectious parasitic diseases to humans and other animals.

The search for anti-*R. rickettsii* antibodies in capybaras is justified by the fact that this vertebrate species, besides being a primary host for *A. sculptum* (a vector for BSF), has been incriminated as an efficient amplifier host of *R. rickettsii,* which is important for the creation of new lines of *R. rickettsii*-infected *A. sculptum* ticks (SOUZA et al., 2009).

This study detected that 64.28% of capybaras presented anti-*R. rickettsii* antibodies, which is more than the 47.62% and 32.2% reported by Souza et al. (2004, 2009), respectively, when assessing samples from capybaras in the endemic region of Campinas, State of São Paulo, Brazil. In contrast, Chiacchio et al. (2014) also studied the Campinas region, but did not find any reactive samples in their research. The high prevalence detected in this study may be explained by data reported by Ribeiro et al. (2013) that points to higher rates of spotted fever in the most densely occupied regions of each state. The authors found greater incidence in cities with smaller forest areas and larger farming areas, where a higher percentage of the population lives in rural regions, concluding that these factors lead to a greater potential for BSF infection.

The life cycles of vectors, reservoirs, and hosts participating in the spotted fever transmission chain are strongly related to the environmental dynamics of the ecosystems these organisms inhabit, with proximity to cities and increased by human interference causing imbalance and facilitating spread of the disease (DEL FIOL et al., 2010). Thus, ecological and climatic factors that influence the life cycle of vectors, reservoirs, and hosts may, in turn, influence the spatial and temporal distribution of the disease (PACHECO et al., 2007).

In this study, no significant difference in prevalence was observed between males and females. A census performed in the State of Rio Grande do Sul by Garcias and Bager (2009) reported a 6.6 ± 0.1 female per male ratio for capybaras. The same study stated that capybaras are animals that live in groups ranging from two to 40 individuals and each flock has a dominant male that establishes a harem. Thus, the gender variable does not affect the prevalence of disease; this result was already expected, since there are no reports showing that

rickettsial infection has a gender bias.

Diagnosis of BSF is difficult both in humans and in animals, especially in the initial phase. As a multisystem disease, it may present variable clinical characteristics, ranging from the classic macular form to atypical forms without exanthema. Additionally, when not properly treated, lethality can reach 80%. Quick laboratory diagnosis is necessary, making IFA the test of choice for diagnosis in both humans and animals (SOUZA et al., 2004; PACHECO et al., 2007).

In this study, of the 18 (64.28%) samples considered reactive, seven (38.88%) had titers of 256, three (16.67%) of 512, five (27.78%) of 1024, and three (16.67%) of 2048. These results demonstrate that at some point during their lives, the studied capybaras were exposed to the etiologic agent, but it is impossible to know when this occurred. Further studies need to be performed to clarify which serological titers ensure an infection in capybaras**,** based on clinical and laboratory assessment of rickettsemia, and to establish the relationship between titers and the chronicity of disease. This is necessary due to the possibility of cross-reactions with other Rickettsiae species of the same subgroup, leading to the need for molecular tests to confirm diagnosis (KRAWCZAK et al., 2014).

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