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ARTÍCULO ORIGINAL

Rickettsial infection in capybaras (*Hydrochoerus hydrochaeris*) from São Paulo, Brazil: serological evidence for infection by *Rickettsia bellii* and *Rickettsia parkeri*

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Introduction. In Brazil, capybaras (*Hydrochoerus hydrochaeris*) are important hosts for *Amblyomma* ticks, which in turn can transmit rickettsiae to humans and animals. Therefore, capybaras are potential sentinels for rickettsial infection.
Objective. The present study evaluated rickettsial infection in capybaras in different areas of the state of São Paulo, where rickettsiosis has never been reported.
Materials and methods. Blood sera from 73 capybaras from six localities in São Paulo were tested by indirect immunofluorescence assay using *Rickettsia rickettsii, Rickettsia parkeri*, and *Rickettsia bellii* antigens. Capybara spleens were tested by PCR, targeting a fragment of the rickettsial *gltA* gene. Ticks were collected from each capybara sample and taxonomically identified to species.
Results. A total of 94 positively reacting capybara samples, 19 (26.0%), 25 (34.2%), and 50 (68.5%) capybara sera reacted to *R. rickettsii, R. parkeri,* and *R. bellii,* respectively. Twenty-five capybara sera showed titers to *R. bellii* at least four-fold higher than to any of the other two antigens. These sera were considered homologous to *R. bellii*. Using the same criteria, 3 capybara sera were considered homologous to *R. parkeri.* No sera were be considered homologous to *R. rickettsii.* No rickettsial DNA was detected in capybara spleen samples. Ticks collected on capybaras were *Amblyomma dubitatum* and *Amblyomma cajennense.*
Conclusions. The first evidence is reported of *R. bellii* natural infection in vertebrate hosts, and the first evidence of *R. parkeri* infection in capybaras. While *R. parkeri* is known to infect and cause disease in humans, no similar evidence for human infection has been indicated by *R. bellii.*

Key words: *Rickettsia*, rickettsia infections/diagnosis, rodentia, serology, zoonoses, Brazil

Infección por rickettsia en capibaras (*Hydrochoerus hydrochaeris*) de São Paulo, Brasil: evidencia serológica de infección por *Rickettsia bellii* y *Rickettsia parkeri*

Introducción. En Brasil, los capibaras (*Hydrochoerus hydrochaeris*) son importantes huéspedes para garrapatas del género *Amblyomma*, las cuales transmiten rickettsiosis a humanos y animales. Por lo tanto, estos roedores pueden ser potenciales centinelas para detectar infección por rickettsia.
Objetivos. Este trabajo evaluó la infección por rickettsia en capibaras de diferentes regiones del estado de São Paulo, donde las rickettsiosis nunca han sido reportadas.
Materiales y métodos. Se examinaron los sueros de 73 capibaras de seis localidades en São Paulo con la prueba de inmunofluorescencia indirecta con antígenos de *Rickettsia rickettsii, Rickettsia parkeri* y *Rickettsia bellii.* Los bazos de los capibaras se extrajeron y se analizaron por reacción en cadena de la polimerasa para un fragmento del gene *gltA* de rickettsia. Las garrapatas se recolectaron de los capibaras y se identificaron hasta especie.
Resultados. Diecinueve (26,0%), 25 (34,2%) y 50 (68,5%) sueros de los capibaras reaccionaron con *R. rickettsii, R. parkeri* y *R. bellii,* respectivamente. De los 50 sueros que reaccionaron con

antígenos de *R. bellii*, 25 presentaron títulos, por lo menos, cuatro veces mayores que los otros dos antígenos. Estos sueros fueron considerados homólogos de *R. bellii*. Usando el mismo criterio, tres sueros de los capibaras se consideraron homólogos de *R. parkeri.* Ningún suero se consideró homólogo de *R. rickettsii.* No se detectó ADN de rickettsia en bazo. Las garrapatas recolectadas de los capibaras fueron identificadas como *Amblyomma dubitatum* y *Amblyomma cajennense.*

Conclusiones. Este trabajo reporta la primera evidencia de infección natural por *R. bellii* en vertebrados y, también, la primera evidencia de infección por *R. parkeri* en capibaras. Se sabe que *R. parkeri* infecta y produce enfermedad en humanos; sin embargo, no hay evidencia de infección humana por *R. bellii.*

Palabras clave: *Rickettsia*, rickettsiosis/diagnóstico, roedores, serología, zoonosis, Brasil

Rickettsia are obligate intracellular bacteria, many of which cause zoonotic diseases in different parts of the world. *Rickettsia* species not associated with specific diseases are considered as having an unknown pathogenicity, or are symbionts of invertebrate organisms (1). Pathogenic rickettsiae have been divided into two groups: (i) the typhus group (TG), composed by *Rickettsia prowazekii* and *Rickettsia typhi,* transmitted primarily by lice and fleas, respectively; (ii) and the spotted fever group (SFG), including *Rickettsia rickettsii* and *Rickettsia parkeri* (2) transmitted mostly by ticks,

Rickettsia rickettsii is the etiological agent of the rickettsial disease with the greatest mortality and is known in different parts of its range as Brazilian spotted fever (BSF, Brazil), Rocky Mountain spotted fever (United States), and Tobia fever (Colombia). In the pre-antibiotic era, mortality rates of BSF were nearly 80% (3). Currently, in the Brazilian state of São Paulo, mortality rates can reach 40% (4). In addition to *R. rickettsii,* another pathogenic tick-borne rickettsia occurring in Brazil is *R. parkeri.* It is the etiological agent of a recently recognized spotted fever rickettsiosis in the United States and Uruguay, where it is transmitted by the ticks *Amblyomma maculatum* and *A. triste,* respectively (5,6). Spotted fever caused by *R. parkeri* seems to have milder clinical manifestations than the classical BSF, with no fatal cases yet reported (5,6). In Brazil, human cases due to *R. parkeri* have not been reported; however the infection by *R. parkeri* and a close-related genotype (strain COOPERI) have been reported in the ticks *A. triste* and *Amblyomma dubitatum* (=*Amblyomma cooperi*), respectively, in the state of São Paulo (7,8). In addition, serological evidence of *R. parkeri* infection has been reported in dogs, horses, and opossums in the state of São Paulo (9, Horta MC, Labruna MB, Pinter A, Linardi PM, Schumaker TT. *Rickettsia* infection in five areas of the State of Sao Paulo, Brazil. Mem Inst Oswaldo Cruz. Submitted).

Other tick-associated rickettsiae of unrecognized pathogenicity for humans have been reported recently in Brazil. These include the SFG species *R. amblyommii* in the state of Rondônia (10) and *R. rhipicephali* in Rondônia and São Paulo (11,12). *R. bellii,* a species classified neither in the TG or SFG, has also been reported in Rondônia and São Paulo (7,10,12). While serological evidence of canine infection due to *R. amblyommii* and *R. rhipicephali* has been reported in the state of Rondônia (13) there has been no evidence for *R. bellii* natural infection in vertebrate hosts.

The indirect immunofluorescence assay (IFA) is currently the test of choice for serologic diagnosis of rickettsial infection in humans and animals (14,15). However, cross-reactive antibodies between *Rickettsia* species are often observed, rendering difficult the serologic identification of the *Rickettsia* species involved in an infection. The geographic origin of the infection has been one of the best indicators of species identity. Testing a

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clinical serum against the possible *Rickettsia* species known to occur in a given area is ideal, because often homologous antibody titers are higher than heterologous antibody titers. In some cases, the differences in titers may be great enough to differentiate among the rickettsial species potentially stimulating the immune response (14). Using this criteria, serological evidence for human and animal infection due to *R. rickettsii,* and animal infection due to *R. parkeri, R. amblyommii,* and *R. rhipicephali* have been reported in the states of São Paulo and Rondônia (9,13, Horta MC, Labruna MB, Pinter A, Linardi PM, Schumaker TT. *Rickettsia* infection in five areas of the State of Sao Paulo, Brazil. Mem Inst Oswaldo Cruz. Submitted).

In the state of São Paulo, capybaras (*Hydrochoerus hydrochaeris*) are incriminated as primary hosts for the ticks *Amblyomma cajennense* and *A. dubitatum* (16). Since *A. cajennense* is incriminated as a vector of *R. rickettsii* (17), and *A. dubitatum* as a potential vector of *R. parkeri* (7), capybaras are potential sentinel hosts for rickettsial infection. The current study evaluated rickettsial infections in capybaras in several areas of the state of São Paulo, where BSF has not been reported previously.

Materials and methods

Capybaras were sampled from six different localities in the state of São Paulo (Figure 1): São Paulo city on the banks of Tietê River (23°32'S, 46°37'W), Cordeirópolis (22°28'S, 47°27'W), Bonfim Paulista (21°09'S, 47°49'W), Andradina (20°53'S, 51°22'W), Valparaíso (21°13'S, 50°51'W), and Cosmorama (20°28'S, 49°46'W). Capture of

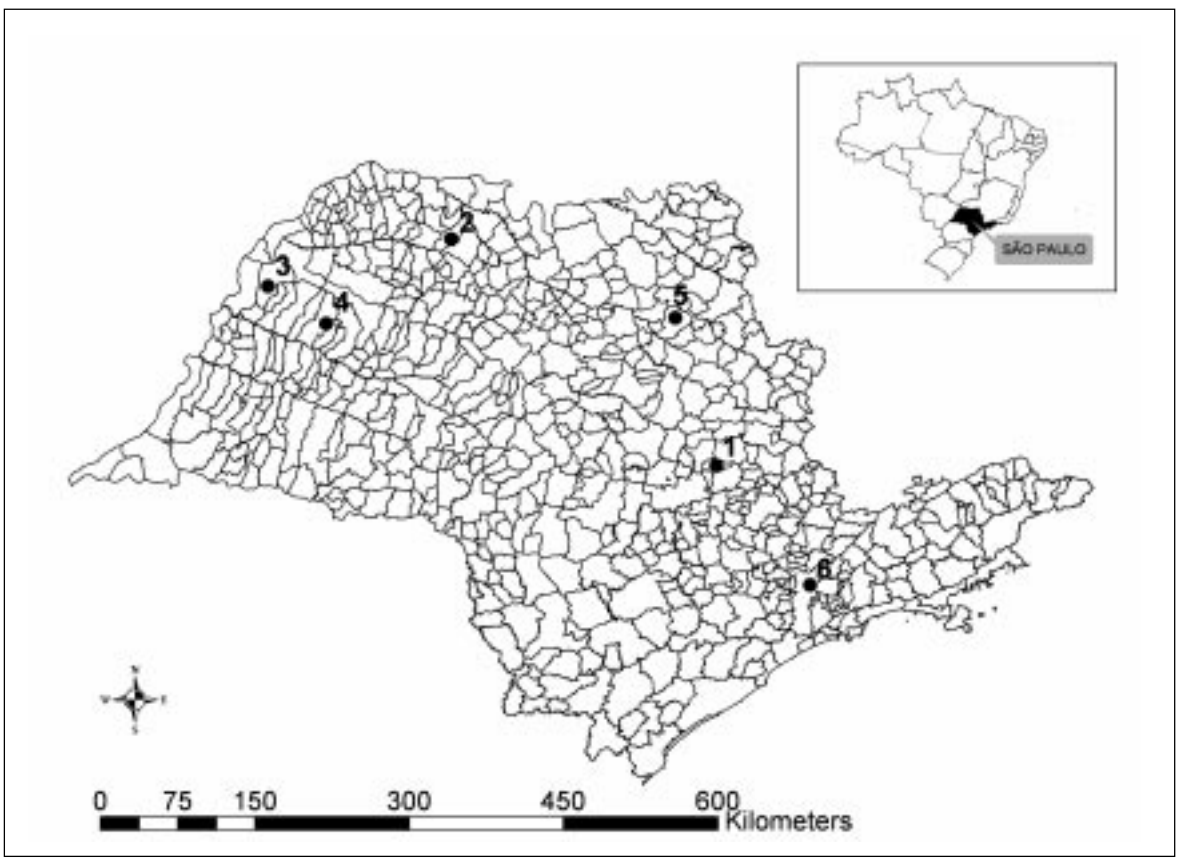

Figure 1. Map of the state of São Paulo, Brazil, showing the six localities where capybaras were sampled in the present study: 1-Cordeirópolis; 2-Cosmorama; 3-Andradina; 4-Valparaíso; 5-Bonfim Paulista; 6-São Paulo.

capybara samples have been authorized by the state office of the "Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis" (IBAMA). Samples were collected during slaughtering in an official wildlife abattoir in Iguape Municipality. Serum, spleen, and ticks were collected from each capybara.

A 3-min time regime was adopted for tick evaluation on each capybara; attached ticks were collected as they were encountered and stored within plastic tubes. Tick samples were available for 3 localities: São Paulo city, Cordeirópolis, and Bonfim Paulista. Ticks were brought to the laboratory, where they were taxonomically identified according to Barros-Battesti *et al*. (18).

The spleens were stored in individual sealed plastic bags, placed immediately on dry ice, brought to the laboratory, and refrigerated at -80°C freezer until use. Spleens were not available from the Valparaiso capybaras from Valparaíso and half of those from São Paulo. Each spleen was subjected to DNA extraction using the DNeasy Tissue Kit (Qiagen, Chatsworth, CA) following the manufacturer's protocol, and tested by PCR using the primers CS-5 (forward) and CS-6 (reverse) that amplify a 147 bp fragment of the citrate synthase gene (*gltA*). These primers have a sensitivity for detection of a single copy of *R. rickettsii* DNA and 10 copies of *R. bellii* (7). PCR conditions were as described previously (17). For each reaction, three negative controls (water) and a positive control [300 ng of DNA of *R. parkeri*-infected *A. cajennense* ticks (19)] were included.

Blood samples were transported to the laboratory at room temperature where samples were centrifuged (1,500x*g* for 10 minutes), aliquoted into labeled microtubes and stored at -20°C. The indirect immunofluorescence assay (IFA) used crude antigens derived from three *Rickettsia* isolates from Brazil: *R. rickettsii* strain Taiaçu, *R. parkeri* strain At24, and *R. bellii* strain Mogi. These three rickettsiae comprise the *Rickettsia* species known to infect *Amblyomma* ticks that infest capybaras in Brazil (7,17).

For antigen preparation, each *Rickettsia* was cultivated in Vero cells and harvested when the 100% infection level was approached. The infected cells were centrifuged at 12,000x*g* for 10 min and the pellet was washed in 0.1 M phosphate buffered saline (PBS, pH 7.4), centrifuged again, and resuspended in PBS containing 1% bovine calf serum and 0.1% sodium azide. Ten microliters of rickettsiae-infected cells were applied onto each well of 12-antigen slides. The antigens on the slides were air-dried and then fixed in acetone for 10 min. Slides were held at –80°C until inspection.

Capybara sera were diluted in two-fold increments with PBS starting from a 1:64 dilution. Ten microliters of diluted sera were added to each well of the antigen slides. The slides were incubated at 37°C for 30 min in a humidity box, rinsed once, and washed twice for 15 min per wash in PBS. The slides were incubated with fluorescein isothiocyanate-labeled sheep anti-capybara IgG (produced by the Centro de Controle de Zoonoses, São Paulo city) and washed as described above. The slides were prepared for inspection with buffered glycerin under cover slips. The slides were read using an ultraviolet microscope (Olympus BX60, Japan) at 400X magnification. For each sample, the endpoint titer reacting with each of the three *Rickettsia* antigens was determined. Sera showing at least four-fold higher titer for a given *Rickettsia* species than the other *Ricketttsia* species was considered homologous to the first *Rickettsia* species or to a very closely related genotype (9,13).

All reactions were performed with the same preparation lot of reagents; this including the secondary antibody, which was always used at the 1:400 dilution, as previously titrated in our laboratory. All slides were read by each of the three authors. Reactive sera were tested in two or three replications before determining the endpoint titer. In each slide, a serum previously shown to be non-reactive (negative control) and a known reactive serum (positive control) were tested at the 1:64 dilution. This positive control serum was previously shown to react with *R. rickettsii, R. parkeri*, and *R. bellii* at the endpoint titers 1:1024, 1:512, and 1:128, respectively.

Results

Of the 73 capybaras tested by IFA, 54 (74.0%) reacted positively to at least one *Rickettsia* species.

A total of 19 (26.0%), 25 (34.2%), and 50 (68.5%) capybara sera reacted to *R. rickettsii, R. parkeri,* and *R. bellii,* respectively. In five localities (São Paulo, Cordeirópolis, Bonfim Paulista, Andradina, and Cosmorama), *R. bellii* was the antigen that elicited the highest frequency of seropositive capybaras, with values ranging from 23.1 to 100% (Table 1). In contrast, capybaras from Valparaíso showed highest seropositive frequency for *R. parkeri* (100%), followed by *R. bellii* and *R. rickettsii* at equal frequencies (71.4%).

Three, 8, 3, 10, and 1 capybara sera from São Paulo, Cordeirópolis, Andradina, Cosmorama, and Valparaíso, respectively, showed titers to *R. bellii* at least four-fold higher than to any of the other two antigens (Table 2). These sera were considered homologous to *R. bellii* or a very closely related genotype. Using the same criteria, 3 sera from Valparaíso were considered homologous to *R. parkeri* (Table 2). No sera were found homologous to *R. rickettsii.*

Adult ticks from the three localities were identified as *A. cajennense* (63 specimens) and *A. dubitatum* (443 specimens). In São Paulo and Cordeirópolis, most of the ticks (80.6 and 98.9%, respectively) were *A. dubitatum,* whereas *A. cajennense* comprised 75% of the ticks collected on capybaras from Bonfim Paulista. A total of 57 capybara spleens were tested by PCR; none showed to contain rickettsial DNA.

Discussion

The current study tested capybara sera against all known *Rickettsia* species found in ticks that infest capybaras in São Paulo state. In the six localities tested, serological evidence for rickettsial infection one or more of the capybaras sampled. In five localities (São Paulo, Cordeirópolis, Bonfim Paulista, Andradina, and Cosmorama), more animals were seroreactive to *R. bellii* than to *R. rickettsii* or *R. parkeri.* In addition, we present serological evidence for *R. bellii* infection in at least 25 capybaras (Table 2). *R. bellii* has been reported infecting *A. dubitatum* ticks from several parts of the state of São Paulo (7, Horta MC, Pinter A, Souza CE, Neto EJ, Souza SS, Soares RM *et al*. Ocorrência de Rickettsia bellii em Carrapatos colhidos nos Municípios de Piracicaba, Pedreira, Campinas, Itu e Cordeirópolis, Estado de São Paulo,. In: XIII Congresso Brasileiro de Parasitologia Veterinária e I Simpósio Latino-Americano de Rickettsioses, 2004, Ouro Preto-MG). Since *R. bellii* is found in the *A. dubitatum* hemolymph (7), this agent possibly can infect tick salivary glands and subsequently be transmitted via tick feeding. Therefore, *A. dubitatum* may have transmitted *R. bellii* to the seropositive capybaras in the current study, since capybaras are primary hosts for all parasitic stages of *A. dubitatum* (20. This hypothesis is corroborated by the tick infestation data (Table 1). In São Paulo and Cordeirópolis, where >80%

Table 1. Results of indirect immunofluorescence assay (IFA) for antibodies to *Rickettsia* spp. (titers ≥64) in capybara sera and tick species found on the capybaras from six localities of the state of São Paulo.

Localities	Number of capybaras	Number of reactive serum by IFA using the following *Rickettsia* antigens (%)			Total number of ticks collected on the capybaras	
		R. rickettsii	*R. parkeri*	*R. bellii*	*Amblyomma cajennense*	*Amblyomma dubitatum*
São Paulo	19	1 (5.2)	3 (15.7)	10 (52.6)	20	83
Cordeirópolis	9	1 (11.1)	3 (33.3)	9 (100)	4	347
Bonfim Paulista	13	2 (15.3)	2 (15.3)	3 (23.1)	39	13
Andradina	10	4 (40.0)	5 (50.0)	9 (90.0)	NE	NE
Valparaíso	7	5 (71.4)	7 (100)	5 (71.4)	NE	NE
Cosmorana	15	6 (40.0)	5 (33.3)	14 (93.3)	NE	NE
Total	73	19 (26.0)	25 (34.2)	50 (68.5)	63	443

NE: not evaluated for ticks

Table 2. Endpoint titers of indirect immunofluorescence assay (IFA) for three *Rickettsia* species in capybaras from the state of São Paulo.

Locality (capybara serum number)	IFA titers			PAIHR
	R. rickettsii	*R. parkeri*	*R. bellii*	
São Paulo (2)	NR	NR	128	*R. bellii*
São Paulo (3)	NR	NR	128	*R. bellii*
São Paulo (19)	NR	NR	256	*R. bellii*
Cordeiróplis (1)	NR	64	256	*R. bellii*
Cordeiróplis (3)	NR	NR	128	*R. bellii*
Cordeiróplis (4)	NR	NR	128	*R. bellii*
Cordeiróplis (5)	NR	NR	256	*R. bellii*
Cordeiróplis (6)	NR	NR	128	*R. bellii*
Cordeiróplis (7)	NR	NR	128	*R. bellii*
Cordeiróplis (8)	NR	64	256	*R. bellii*
Cordeiróplis (9)	NR	64	256	*R. bellii*
Andradina (3)	NR	NR	128	*R. bellii*
Andradina (4)	NR	NR	64	*R. bellii*
Andradina (5)	NR	NR	64	*R. bellii*
Valparaíso (1)	NR	128	NR	*R. parkeri*
Valparaíso (5)	128	512	64	*R. parkeri*
Valparaíso (6)	64	256	64	*R. parkeri*
Valparaíso (7)	NR	64	256	*R. bellii*
Cosmorama (1)	NR	NR	128	*R. bellii*
Cosmorama (3)	NR	NR	128	*R. bellii*
Cosmorama (4)	NR	NR	256	*R. bellii*
Cosmorama (5)	NR	NR	512	*R. bellii*
Cosmorama (6)	NR	NR	256	*R. bellii*
Cosmorama (7)	64	64	256	*R. bellii*
Cosmorama (8)	64	NR	512	*R. bellii*
Cosmorama (9)	512	256	2048	*R. bellii*
Cosmorama (10)	64	64	512	*R. bellii*
Cosmorama (13)	NR	NR	128	*R. bellii*

PAIHR: possible antigen involved in a homologous reaction (serum showing for a *Rickettsia* species titer at least four-fold higher than that observed for any other *Ricketttsia* species was considered homologous to the first *Rickettsia* species)
NR: non reactive at titer 64 or higher.

of the ticks collected on capybaras were *A. dubitatum,* 52.6 to 100% of the capybaras were seropositive to *R. bellii.* On the other hand, in Bonfim Paulista, where only 25% of the ticks were *A. dubitatum,* only 23.1% of the capybaras were seropositive for *R. bellii.*

In Valparaíso, substantial evidence for *R. parkeri* infection were observed in at least three capybaras (Table 2), although ticks were not collected from these capybaras. Nonetheless, capybaras are frequently found to be parasitized by *A. dubitatum* ticks in the state of São Paulo (20, Pacheco RC, Pinter A, Ferreira PM, Ferreira F, Labruna MB. Carrapatos infestando capivaras em cinco áreas do estado de São Paulo. In: XIII Congresso Brasileiro de Parasitologia Veterinária e I Simpósio Latino-Americano de Rickettsioses, 2004, Ouro Preto, MG. Revista Brasileira de Parasitologia Veterinária 2004;13:315). Since *R. parkeri* (strain COOPERI) has been reported infecting *A. dubitatum* in the state of São Paulo (7), the *A. dubitatum* population from Valparaíso were probably infected with *R. parkeri.* This finding indicates the possibility of clinical cases of human infection by *R. parkeri,* since it is a recognized agent of spotted fever.

No serological evidence for the presence of *R. rickettsii* infection was recovered from the capybaras sampled. This result was not surprising since the samples were from areas where BSF has never been reported. Capybaras are also the primary hosts of *A. cajennense,* one of the vectors of BSF in Brazil (16). Consequently, the serological results indicated that *R. rickettsii* was

not circulating in *A. cajennense* populations currently sampled.

Rickettsial DNA were not found in any of the capybara spleen samples. Spleen is one of the organs of choice for rickettsial infection surveillance (14). However, detection of rickettsia in vertebrate animals is usually a rare event. Once infected, vertebrates display a rickettsemia for only a few days or weeks, and thereafter no rickettsia is found in the organism (21).

The current study reports the first evidence of *R. bellii* natural infection in vertebrate hosts, and also the first evidence of *R. parkeri* infection in capybaras. While *R. parkeri* is known to infect and cause disease in humans (5), there has been no evidence of human infection by *R. bellii.* In fact, previous studies in areas of occurrence of *R. bellii*-infected ticks failed to demonstrate serological evidence of *R. bellii* infection among dogs, horses, cats, opossums, or humans (9,13, Horta MC, Labruna MB, Pinter A, Linardi PM, Schumaker TT. *Rickettsia* infection in five areas of the State of Sao Paulo, Brazil. Mem Inst Oswaldo Cruz. Submitted). If capybaras are more susceptible to *R. bellii* infection than other vertebrates, this has yet to be demonstrated. Nevertheless, the possible role of *R. bellii* causing infection in vertebrate hosts, including humans, is worthy of further investigation.

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Conflict of interests

We declare that there is no conflict of interests on the results presented in this article.

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