Serosurvey of *Rickettsia* spp. in dogs and humans from an endemic area for Brazilian spotted fever in the State of São Paulo, Brazil

Sorologia para *Rickettsia* spp. em cães e humanos de uma área endêmica para febre maculosa brasileira no Estado de São Paulo, Brasil

Adriano Pinter [1,2]
Maurício C. Horta [1]
Richard C. Pacheco [1]
Jonas Moraes-Filho [1]
Marcelo B. Labruna [1]

[1] Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brasil.
[2] Superintendência de Controle de Endemias, São Paulo, Brasil.

Correspondence
M. B. Labruna
Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo.
Av. Prof. Orlando Marques de Paiva 87, São Paulo, SP 05508-270, Brasil.
labruna@usp.br

Abstract

The present study provides a rickettsial serosurvey in 25 dogs and 35 humans in an endemic area for Brazilian spotted fever in the State of São Paulo, where the tick Amblyomma *aureolatum is the main vector. Testing canine and human sera by indirect immunofluorescence against four* Rickettsia *antigens (*R. rickettsii, R. parkeri, R. felis *and* R. bellii*) showed that 16 (64%) of canine sera and 1 (2.8%) of human sera reacted to at least one of these rickettsial antigens with titers ≥ 64. Seven canine sera and the single reactive human serum showed titers to* R. rickettsii *at least four times those of any of the other three antigens. The antibody titers in these 7 animals and 1 human were attributed to stimulation by* R. rickettsii *infection. No positive canine or human serum was attributed to stimulation by* R. parkeri, R. felis, *or* R. bellii. *Our serological results showed that dogs are important sentinels for the presence of* R. rickettsii *in areas where the tick A. aureolatum is the main vector of Brazilian spotted fever.*

Rickettsia*; Spotted Fever; Tick-Borne Diseases; Dogs*

Introduction

Brazilian spotted fever (BSF) is an acute, febrile, tick-borne disease caused by the bacterium *Rickettsia rickettsii*. In the State of São Paulo, Brazil, two tick species have been implicated in the transmission of BSF to humans: *Amblyomma cajennense* in the central part of the State and *A. aureolatum* in the eastern part, where the Atlantic Rainforest is preponderant [1]. *R. rickettsii* is classified in the spotted fever group of Rickettsiae, which includes more than 20 *Rickettsia* species [2]. These include the pathogens *R. parkeri* and *R. felis*, reported infecting ticks and fleas, respectively, in the State of São Paulo [3,4,5]. Besides the spotted fever group of Rickettsiae, *R. bellii* has been reported frequently infecting ticks in the State of São Paulo [3,6,7]. Although strictly associated with ticks, *R. bellii* is not a spotted fever group *Rickettsia*. In addition, the pathogenicity of *R. bellii* in humans and dogs has never been demonstrated [8].

The few available records of natural hosts for sub-adults of *A. aureolatum* have included mostly a few bird and rodent species [9,10,11,12]. Meanwhile, it has been reported that *A. aureolatum* adult ticks feed mainly on dogs in rural areas close to remnants of rainforest [11,13,14]. Additionally, only adult ticks have been collected parasitizing humans [11,14]. Since *A. aureolatum* needs moist habitats like inner rainforests [1], dogs may play an important role in carrying *A. aureolatum*

adult ticks from inside the forest to the human environment. This situation potentially poses an increased risk of dog owners acquiring BSF in areas where *A. aureolatum* is the main vector.

In the pre-antibiotic era, case-fatality rates of BSF among humans were nearly 80% [15]. The case-fatality rate still reaches 40% in the State of São Paulo [16]. In contrast, canine infection by *R. rickettsii* appears to be much less fatal. Nevertheless, *R. rickettsii*-infected dogs develop an anamnestic IgG response, detectable by indirect immunofluorescence [17].

In order to determine which areas are endemic for BSF, it is necessary to detect either a *R. rickettsii*-infected tick population or *R. rickettsii*-seropositive sentinel hosts. The infection rate by *R. rickettsii* within an *A. aureolatum* population has been reported at less than 1% [7], which makes a survey for *R. rickettsii* in a tick population an exhaustive and sometimes fruitless task. Thus, the aim of this study was to use serology to investigate whether dogs are important sentinels for BSF transmitted by *A. aureolatum*. The study was performed in a BSF-endemic area of the State of São Paulo where Pinter & Labruna [7] found dogs parasitized by *R. ricketsii*-infected ticks.

Methods

The study was performed in the rural area of the Taiaçupeba District in the county (municipality) of Mogi das Cruzes, State of São Paulo (23°38'54.9"S, 46°11'0.3"W), a well-known endemic area for Brazilian spotted fever. In Taiaçupeba, several fatal human cases of BSF have been reported in recent years [14]. The canine serosurvey in the present study was conducted during an *A. aureolatum* seasonality study [14] and a *Rickettsia*-infection survey on ticks collected on dogs [7] in the same area.

Dogs were sampled in 8 small family farms (< 10ha) located in an area originally consisting of Atlantic rainforest, at 800m altitude on the Serra do Mar mountain chain, next to the Jundiai River Dam. The agriculture was mostly vegetable farming, with no livestock. Most of the farm families owned one or more domestic dogs, mostly raised unrestrained with free access to forest areas. All the dogs were sampled, except for a minority that were raised completely restrained on the farms. Blood samples were drawn from the dogs on two visits to the farms, in August 2001 and April 2002. A total of 19 and 19 dogs were sampled during the first and second visits, respectively. Since only 13 individual dogs were sampled on both visits, 6 dogs were sampled on the first visit only and another 6 dogs were sampled on the second visit only. Thus, a total of 25 dogs were sampled in the study. The age of each dog was noted. Each dog sample was individually identified with a capital letter (referring to the farm where the dog lived) followed by an Arabic numeral (referring to the individual dog present on that farm) (Table 1). A total of 35 humans living on the farms, in direct contact with the dogs, had blood samples drawn. Eighteen humans were sampled on both visits, while 7 and 10 were sampled only on the first and second visits, respectively.

The blood samples were taken to the laboratory at room temperature and were centrifuged (1,500g for 10 minutes), and serum aliquots were stored at -20ºC until tested. Serum samples were processed by indirect immunofluorescence as described elsewhere [8,18], using crude antigens derived from four *Rickettsia* isolates from Brazil: *R. rickettsii* strain Taiaçu, *R. bellii* strain Mogi das Cruzes, *R. felis* strain Pedreira, and *R. parkeri* strain At24. While the first three *Rickettsia* species constitute the Rickettsiae known to occur in the study site [4,7], the latter species is known to occur in other parts of the State of São Paulo [3,5]. Human and canine sera were diluted in twofold increments with PBS starting from a 1:64 dilution. Serum was considered to contain antibodies against the Rickettsiae if it displayed a reaction at 1:64. Endpoint titers against each *Rickettsia* strain were determined by testing serial twofold serum dilutions. Reactive sera were tested in two or three replications before determining the endpoint titer. On each slide, a serum previously shown to be non-reactive (negative control) and a known reactive serum (positive control) were tested. A serum showing a titer for a *Rickettsia* species at least fourfold that observed for any other *Ricketttsia* species was considered homologous for the first *Rickettsia* species or a very closely related genotype [8,19].

During a two-year study (December 2000 to November 2002) of rickettsial infection in *A. aureolatum* ticks collected on some of the dogs sampled in the present study [7], *R. rickettsii* and *R. bellii* were found infecting 0.9 and 1.5% of the *A. aureolatum* ticks, respectively. Since our blood samples were drawn on dates (August 2001 and April 2002) within this two-year period, we compared the data of infected ticks reported by Pinter & Labruna [7] with the serological results of the present study.

Before starting, the present study was submitted to and approved by the Research Ethics Committee of the Universidade de São Paulo [University of São Paulo].

Table 1

Endpoint indirect immunofluorescence titers for four *Rickettsia* species in 16 seropositive dogs from the Taiaçupeba District, municipality of Mogi das Cruzes, State of São Paulo, Brazil.

Dog identification	Age (months) *	Indirect immunofluorescence titers for the following Rickettsia antigens								PAIHR
		R. rickettsii		*R. bellii*		*R. felis*		*R. parkeri*		
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	
A3	18	256	256	NR	NR	NR	NR	64	NR	*R. rickettsii*
B1	18	128	**	NR	**	NR	**	NR	**	*R. rickettsii*
C1	96	64	256	NR	NR	NR	NR	NR	NR	*R. rickettsii*
C2	84	512	512	NR	NR	NR	NR	512	512	-
C3	18	512	4,096	NR	NR	NR	NR	NR	1,024	*R. rickettsii*
D1	96	512	512	NR	NR	128	NR	512	512	-
D2	72	2,048	2,048	NR	NR	1,024	1,024	512	512	-
E1	36	512	**	NR	**	NR	**	128	**	*R. rickettsii*
E2	48	8,192	**	NR	**	128	**	512	**	*R. rickettsii*
F1	12	1,024	**	256	**	128	**	1,024	**	-
F2	36	4,096	2,048	256	NR	512	NR	2,048	1,024	-
F3	24	**	512	**	NR	**	NR	**	64	*R. rickettsii*
G1	8	1,024	1,024	1,024	1,024	1,024	1,024	1,024	1,024	-
G2	8	1,024	~	NR	**	NR	**	1,024	**	-
H1	96	**	512	**	NR	**	NR	**	512	-
H2	48	**	1,024	**	NR	**	NR	**	512	-

Note: serum samples were collected from some dogs on two occasions, August 2001 (1st) and April 2002 (2nd).

NR: non-reactive at titer ≥ 1:64; PAIHR: possible antigen involved in a homologous reaction (a serum with a titer for one *Rickettsia* at least four times that observed for any other *Ricketttsia* species was considered homologous for the first species).

* Age in months when dogs had first blood sample drawn;

** Serum sample was not drawn.

Results

Indirect immunofluorescence detected antibodies reactive to *R. rickettsii* (titer ≥ 64) in 16 (64%) out of 25 dogs. Of those, 14 (56%) also reacted to *R. parkeri*, 6 (24%) to *R. felis,* and 3 (12%) to *R. bellii*. No serum reacted to any of these three Rickettsiae without reacting to *R. rickettsii*. The serum endpoint titers ranged from 64 to 8,192 for *R. rickettsii*, 64 to 2,048 for *R. parkeri*, 64 to 1,024 for *R. felis*, and 256 to 1,024 for *R. bellii* (Table 1). Seven canine sera (A3, B1, C1, C3, E1, E2, and F3) showed titers to *R. rickettsii* at least four times higher than those of any of the other three antigens. The antibody titers in these 7 animals were attributed to stimulation by *R. rickettsii* infection. For another 9 animals, it was not possible to determine whether *R. rickettsii* had been the infecting agent, since they also showed high titers for *R. parkeri* (C2, D1, F1, F2, G2, H1, and H2) or *R. felis* (D2), or showed the same titer for the four antigens (G1) (Table1).

The overall proportion of dogs that were reactive to *R. rickettsii* was 64% (16/25). However, this frequency increased to 77.7% (14/18) for dogs older than 12 months and 100% (9/9) for those older than 36 months.

Canine blood samples were collected twice (August 2001 and April 2002), i.e., with an 8-month interval. Among the 13 dogs sampled on both occasions, 8 sera were reactive to *R. rickettsii*. Of these, 5 sera (A3, C2, D1, D2, G1) showed stable titers, whereas 2 dogs (C1, C3) showed ≥ fourfold rise and 1 serum (F2) showed a twofold decrease in *R. rickettsii* antibody titers (Table 1).

According to data reported by Pinter & Labruna [7] in January 2001, one *R. rickettsii*-infected tick and one *R. bellii*-infected tick were taken from dog E2. Seven months later (August 2001), this dog had a titer of 8,192 for *R. rickettsii* and was not reactive to *R. bellii* (Table 1). Dog E2 was not sampled on the second occasion, in April 2002. In June 2001, a *R. bellii*-infected tick was taken from dog C4 [7]. Two months later (August 2001), this dog was not reactive to any of the four rickettsial antigens, a condition detected again in April 2002, when blood was drawn the second time (Table 1). The remaining *R. rickettsii* or *R. bel-*

lii-infected ticks reported by Pinter & Labruna [7] were collected from other dogs not sampled in the present study, or were collected from our sampled dogs at dates after our second samples.

Only 1 (2.8%) of 35 humans showed antibodies to *R. rickettsii* (titer: 256). No human serum reacted to *R. parkeri*, *R. felis,* or *R. bellii*. This single reactive serum was collected from the only person with a previous history of BSF, which was confirmed by checking the files of the São Paulo State Health Secretariat.

Discussion

Indirect immunofluorescence is currently the test of choice for serological diagnosis of rickettsial infection in humans and animals [17,20,21]. However, cross-reactive antibodies between *Rickettsia* species are often observed, thus hindering the serological identification of the *Rickettsia* species involved in an infection. Testing a clinical serum against the possible *Rickettsia* species known to occur in a given area is ideal, because homologous antibody titers are often higher than heterologous antibody titers. In some cases, the differences in titers may be great enough to differentiate the rickettsial species potentially stimulating the immune response [20,21]. Since our study tested serum samples against the *Rickettsia* antigens known to occur in the study area (*R. rickettsii*, *R. bellii*, and *R. felis*) plus one species yet to be reported (*R. parkeri*), we can technically assume that *R. rickettsii* was the *Rickettsia* species responsible for infection in seven dogs. In addition, we present no serological evidence of canine infection due to *R. felis*, *R. bellii*, or *R. parkeri* in the study area.

No dog appeared to have been infected by *R. felis*, even though natural *R. felis*-infected fleas were found parasitizing dogs in the study area [4]. Beyond the widespread occurrence of *R. felis*-infected fleas in the world, there has been no evidence for the vectorial competence of fleas for *R. felis*. Similarly, no dog appeared to have been infected by *R. bellii* in the present study, although 1.5% of the *A. aureolatum* ticks collected on dogs in the study area were shown to be infected by *R. bellii* [7]. For instance, interestingly, a *R. bellii*-infected tick was removed from dog C4 in June 2001 [7], but this dog was serologically negative for *Rickettsia* in August 2001 and April 2002 (Table 1). These results corroborate a recent study in Northern Brazil in which no dog was shown to have *R. bellii* antigen-stimulating antibodies, despite the high frequency of *R. bellii*-infected ticks parasitizing the dogs [8]. Thus, these results indicate that either *R. bellii* is not infective for dogs or that *R. bellii*-infected ticks are not able to transmit the agent to dogs.

The proportion of seropositive dogs increased with age (100% of dogs > 36 months of age were *R. rickettsii*-seropositive). Since puppies (< 6 months of age) are not likely to be physically capable of circulating extensively inside the forest, they are probably much less exposed to *A. aureolatum* ticks. Thus, the older the dog the higher the odds that the animal has been parasitized at least once by a *R. rickettsii*-infected tick.

Interestingly, dog C3 appeared to have been infected by *R. rickettsii* at least twice: the first contact with *R. rickettsii* is believed to have occurred before 18 months of age, when the first blood sample was drawn and the dog had an IFA titer of 1:512 for *R. rickettsii*. When the same animal was 26 months old it had a 1:4,096 titer for *R. rickettsii* (Table 1). This supposed re-infection is corroborated by laboratory data on experimental infection of dogs with *R. rickettsii* [17], showing that once inoculated with the agent, dogs elicit indirect immunofluorescence peak titers (between 2,048 and 4,096) around 3 weeks after inoculation, after which they gradually decreased in the following weeks reaching 128 to 512 at around 7 months post-inoculation. A similar supposition of re-infection can be inferred for dog C1, which showed titers of 64 and 256 for the first and second samples, respectively (Table 1).

Sangioni et al. [22] proposed surveys of horse sera as a useful method for BSF surveillance in areas where humans are exposed to *A. cajennense* ticks. This procedure was based on the fact that horses are primary hosts for *A. cajennense*, and thus that the seroprevalence of *R. rickettsii*-reactive horses in BSF-endemic areas has varied from 57.1% to 90% [19,23]. In contrast, the following seroprevalence values of *R. rickettsii*-reactive dogs have been reported in areas where *A. cajennense* is the vector: 8% [24], 13.7% [25], 31.2% [19], and 36.4% [23]. These lower values for dogs are due to the fact that unlike horses, dogs are not primary hosts for *A. cajennense* (they are merely accidental hosts). Meanwhile, the present study showed an overall seroprevalence of 64% of *R. rickettsii*-reactive dogs in a BSF-endemic area, where the *A. aureolatum* tick is the vector. This higher value is due to the fact that dogs are primary hosts for the adult stage of *A. aureolatum*. We thus recommend surveys of dog sera as a useful method for Brazilian spotted fever surveillance in areas where humans are potentially exposed to *A. aureolatum* ticks. This procedure would be much more useful and productive than a direct tick assay targeting *R. rickettsii*. For a survey of dog sera, the sample to be tested should include mainly dogs older than 36 months, born and raised in the target region.

Resumo

*Este estudo avaliou a ocorrência de anticorpos anti-*Rickettsia *em 25 cães e 35 humanos, em uma área endêmica para a febre maculosa brasileira no Estado de São Paulo, onde o principal vetor é o carrapato* Amblyomma aureolatum. *Soros dos cães e humanos foram testados pela técnica de imunofluorescência indireta contra quatro antígenos de riquétsias (*R. rickettsii, R. parkeri, R. felis, R. bellii*), mostrando que soros de 16 (64%) cães e 1 (2,8%) humano reagiram com títulos ≥ 64 para pelo menos um dos antígenos de riquétsias. Sete soros caninos e o único soro humano reativo demonstraram títulos para* R. rickettsii *no mínimo quatro vezes maior do que aqueles para os outros antígenos de riquétsias. Os títulos de anticorpos nesses cães e um humano foram considerados homólogos a* R. rickettsii, *enquanto que nenhum soro de cão ou humano foi considerado reativamente homólogo para* R. parkeri, R. felis *ou* R. bellii. *Os resultados sorológicos mostraram que cães são importantes sentinelas para a presença da bactéria* R. rickettsii *em áreas onde o carrapato A. aureolatum é o principal vetor da febre maculosa brasileira.*

Rickettsia*; Febre Maculosa; Doenças Transmitidas por Carrapatos; Cães*

Contributors

A. Pinter participated in the field collection of biological material, laboratory tests, and elaboration of the manuscript. M. C. Horta, R. C. Pacheco, and J. Moraes-Filho contributed to the field collection of biological material, laboratory tests, analysis and interpretation of data, and critical revision and approval of the manuscript. M. B. Labruna oriented the research work, contributed to the elaboration of the manuscript, and final revision of the text.

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