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Original Article

Serosurvey of *Rickettsia* spp. in cats from a Brazilian spotted fever-endemic area

Soroprevalência de *Rickettsia* spp. em gatos de uma área endêmica para a Febre Maculosa Brasileira

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Abstract

Rickettsia spp. bacteria are responsible for tick-borne diseases worldwide, mostly maintained by rickettsial amplifiers capybaras in Brazilian endemic areas. The campus of the University of São Paulo, in southeastern Brazil, is an area endemic for Brazilian spotted fever (BSF), with high density of capybaras and *Amblyomma* spp., along with confirmed human cases. Besides capybaras, the university has also an in-campus high population of sheltered and free-roaming cats. Accordingly, the aim of this study was to determine the prevalence and characteristics associated with *Rickettsia rickettsii, Rickettsia parkeri* and *Rickettsia felis* exposure among cats in a BSF-endemic area. Out of 51 cats sampled, 23/35 shelter (65.7%) and 5/16 free-roaming (31.2%) were positive (titers ≥ 64) for at least one *Rickettsia* species. Ticks species were present in 3/16 free-roaming cats (18.8%), consisting of *Amblyomma* spp., nymphs of *Amblyomma sculptum* and adult *Rhipicephalus sanguineus sensu lato*. Despite sharing the capybaras environment, the seropositivity among the free-roaming and shelter cats was lower than owned cats in other endemic areas. Whether equally or less exposed to rickettsial infection, compared with owned cats in endemic areas, free-roaming and shelter cats may be used as environmental sentinels for human exposure to rickettsiae in such areas.

Keywords: *Rickettsia rickettsii*, *Rickettsia parkeri*, brazilian spotted fever.

Resumo

Espécies de *Rickettsia* têm sido responsáveis por doenças transmitidas por carrapatos no mundo, a maioria mantida por hospedeiros amplificadores, como as capivaras em áreas endêmicas no Brasil. A Universidade de São Paulo, em Piracicaba, no sudeste do Brasil, é uma área endêmica para a Febre Maculosa Brasileira (FMB), com alta densidade de capivaras e *Amblyomma* spp., e com casos humanos confirmados. Além de capivaras, a universidade também possui gatos em um abrigo e de vida livre. Assim, o objetivo deste estudo foi determinar a prevalência e as características associadas com exposição à *Rickettsia rickettsii, Rickettsia parkeri* e *Rickettsia felis* em gatos de área endêmica para a FMB. Dos 51 gatos amostrados, 23/35 (65,7%) do abrigo e 5/16 (31,2%) de vida livre foram positivos (títulos ≥ 64) para pelo menos uma *Rickettsia* spp. Carrapatos estiveram presentes em 3/16 (18,8%) gatos de vida livre, representados por *Amblyomma* spp., ninfas de *Amblyomma sculptum* e adultos de *Rhipicephalus sanguineus sensu lato*. Apesar de compartilharem o ambiente com capivaras, os gatos amostrados foram igualmente ou menos expostos à infecção riquetsial do que os gatos com proprietário em outras áreas endêmicas, podendo ser usados como sentinelas para exposição humana à riquétsias nessas áreas.

Palavras-chave: *Rickettsia rickettsii, Rickettsia parkeri,* febre maculosa brasileira.

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Introduction

Rickettsia bacteria are responsible for human and animal diseases worldwide, such as Brazilian spotted fever (BSF) and ixodid ticks are the main vectors of rickettsiae (BENINATI et al., 2005; LABRUNA, 2009). BSF cases in endemic areas of southeastern Brazil have been previously related to two main pathogenic species, namely *Rickettsia rickettsii* and *Rickettsia parkeri* (LABRUNA et al., 2014; NIERI-BASTOS et al., 2018).

Presence of capybaras (*Hydrochoerus hydrochaeris*) has been reportedly correlated with endemic areas for BSF in southeastern Brazil, with capybara population 40 times higher when compared to natural environments, mostly related to extensive sugarcane crops (POLO et al., 2015). Capybaras have been recognized as *Rickettsia rickettsii* amplifiers and as hosts for ticks, especially *Amblyomma sculptum* (LABRUNA, 2009; SOUZA et al., 2009).

Despite the urban location of the campus of the University of São Paulo in the city of Piracicaba, it is composed of extensive green areas, with native vegetation and natural river courses. This type of terrain has historically been described as capybaras' natural habitat. Because of expansion of sugarcane crop areas surrounding the city, which provide an important food source for capybaras, an increase in the on-campus population of capybaras infested by ticks has been described (GHELER-COSTA et al., 2002). Hence, not surprisingly, the city of Piracicaba alone (among the 650 municipalities in this state) has accounted for 62 (7.6%) out of the total of 814 human BSF cases in the state of São Paulo over the past 12 years, including fatal cases after tick bites that were acquired on campus (SÃO PAULO, 2019). In addition, high seropositive capybara population has overlapped around 250 free-roaming in-campus cats, according to campus administration.

The seroprevalence of *Rickettsia* spp. in cats (*Felis catus*) has been variable in South and North America. In South America, a report on trapped stray cats in four endemic areas in the state of São Paulo (including Piracicaba) found the highest cat seropositivity worldwide, such that 16/19 (84.21%) were seropositive to *R. rickettsii* antigen, 15/19 (78.94%) to *R. parkeri,* and 14/19 (73.68%) to *R. felis*, with highest endpoint titers to *R. rickettsii* (HORTA et al., 2007). In North America, seropositivity of *R. rickettsii* ranged from 9/36 (25.0%) to 22/52 (42.3%) feral cats, 2/20 (10.0%) to 23/29 (79.3%) owned cats and 14/114 (12.3) sheltered cats (CASE et al., 2006; KELLY et al., 2017; STEPHENSON et al., 2017). The tick *A. sculptum* has been found infesting cats in Piracicaba city, suggesting that wild-animal reservoirs and vectors may spread rickettsial pathogens among feral cats and human hosts (HORTA et al., 2007).

Although the impact of capybara populations on human BSF cases has been extensively studied, the impact of overlapping free-roaming cat populations for the tick-borne diseases remains to be established. Accordingly, the aim of this study was to assess the prevalence and characteristics associated with tick-borne diseases, selected by cat exposure of main pathogenic species in BSF-endemic areas overlapping capybaras, reportedly *R. rickettsii* and *R. parkeri*. In addition, serum samples were tested to anti-*R. felis* antibodies.

Methods

Study area

This study was conducted at the Escola Superior de Agricultura "Luiz de Queiroz"

Universidade de São Paulo (ESALQ-USP), in the city of Piracicaba, from July to October 2017. The municipality of Piracicaba has a total area of 1,378.069 km^2; its altitude is 554 meters, its temperatures range is from 16.0 °C to 37.5 °C and its annual rainfall is 123 centimeters. The estimated city population at the time of the survey was 400,100 inhabitants (ranked 61st in size out of the total of 5,570 Brazilian municipalities), with a high human development index (HDI) of 0.785 (ranked 92nd among all Brazilian municipalities) (IBGE, 2017).

The university campus (915 hectares) is located within the urban area of Piracicaba city and is divided between forest remnants of the Atlantic Forest biome and diverse anthropic uses, including landscaped and idle areas, crops and rural facilities, pasture and restored forests (Figure 1). The campus harbors native fauna, mostly consisting of birds, capybaras and opossums, with four kilometers of on-campus river course (ESALQ, 2019).

Animal sampling and testing

It was estimated that around 250 free-roaming resident cats were present on campus at the time of the survey, according to the campus administration, living on offered cat food and hunting birds and rodents. Traps were distributed in several on-campus locations during a four-month trapping period. In addition, 35 cats and 12 dogs from a provisional on-campus animal shelter were sampled.

After trapping and/or physical restraint, blood samples from 35/51 shelter cats (68.6%) and 16/250 free-roaming cats (6.4%) were successfully collected. Because of the death of a shelter feral cat following blood sampling, caused by a preexisting hypertrophic cardiomyopathic condition that was found later at the necropsy, sampling from shelter and free-roaming feral cats was limited by campus mayor to the four-month trapping period only, which limited the number of collected samples.

All the samples were collected in tubes without anticoagulant and were kept at room temperature (25 °C) until visible clot retraction occurred. They were then centrifuged at 1,500 revolutions per minute for five minutes, and the serum was separated and stored at -20 °C until the indirect immunofluorescence assay (IFA) procedure was performed (ZAVALA-VELAZQUEZ et al., 1996). The serum samples were tested by means of IFA to detect IgG antibodies against the *R. rickettsii* strain Taiaçu, and the *R. parkeri* strain At24, which are spotted fever group (SFG) rickettsiae, and additionally tested to *R. felis* strain Pedreira, a transitional group rickettsia. Individual serum samples were initially screened at 1:64 dilutions against each of the rickettsial antigens. A fluorescein isothiocyanate-labeled rabbit anti-cat and anti-dog IgG dilution of 1:1,500 (IgG, Sigma Diagnostics, St. Louis, MO, USA) as conjugate was used for the cat and dog samples.

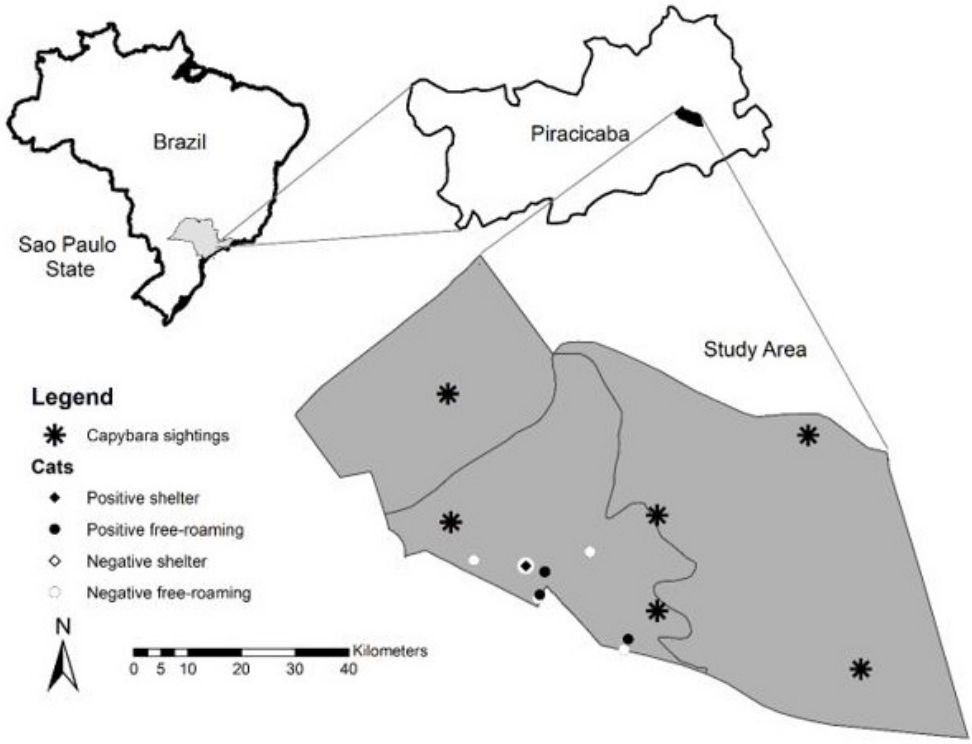

Figure 1. Spatial location of capybara sightings and locations of shelter and free-roaming cats, sampled at the "Luiz de Queiroz" School of Agriculture (ESALQ), University of São Paulo (USP), in the city of Piracicaba, between July and October 2017.

Tick collection and testing

After blood samples had been taken, the cats were carefully examined for the presence of ticks. All the ticks obtained were collected and preserved in isopropyl alcohol until analysis at the laboratory for taxonomic identification, which was performed following standard morphological keys, as previously described (ARAGÃO & FONSECA, 1961; BARROS-BATTESTI et al., 2006; MARTINS et al., 2010).

Amblyomma sculptum tick nymphs were individually subjected to DNA extraction using the guanidine isothiocyanate technique (SANGIONI et al., 2005) and were individually tested by means of standard polymerase chain reaction (PCR) for tick mitochondrial 16S rRNA (MANGOLD et al., 1998) and the rickettsial gene gltA (LABRUNA et al., 2004). For each PCR run, a negative control (water) and a positive control (*Rickettsia vini* DNA) were also included, as previously described (NOVAKOVA et al., 2016).

Epidemiological data and statistical analysis

Individual information and epidemiological data were obtained and analyzed. Statistical analysis was performed by means of the Epi Info statistical software, version 3.5.2 to determine the association between *Rickettsia* sp. antibodies and the variables (age, sex and sterilization). The data were evaluated by determining the odds ratios and 95% confidence intervals. A nonparametric test (Fisher's exact test) was used for univariate analysis and the significance level was determined as $p < 0.05$.

A map showing spatial locations of capybara sightings and locations of shelter and free-roaming cats was designed using the ArcGIS software, version 10.0 (Figure 1).

Ethical approval

The present study was approved by the Ethics Committee for Animal Use of the Setor de Ciências Agrárias da Universidade Federal do Paraná (protocol no. 103/2017).

Results

A total of 28/51 cats (54.9%) were seropositive for at least one of the three *Rickettsia* species tested: 23/35 (65.7%) of the shelter cats and 5/16 (31.2%) of the free-roaming cats.

Among the free-roaming cats, 3/16 (18.8%) reacted to *R. rickettsii* and 5/16 (31.2%) reacted to *R. parkeri*, and the IFA endpoint titers ranged from 64 to 1024 for *R. rickettsii* and 64 to 256 for *R. parkeri*. Among the shelter cats, 16/35 (45.7%) reacted to *R. rickettsii* and 17/35 (48.6%) reacted to *R. parkeri*, and the IFA endpoint titers ranged from 64 to 512 for *R. rickettsii* and 64 to 1024 for *R. parkeri.* Only 3/51 (5.8%) sheltered cats were seropositive for *R. felis* with titer of 64; these three cats were also seropositive for *R. rickettsii* and *R. parkeri* (Table 1). No statistically significant differences were found between the groups (shelter and free-roaming) regarding the frequencies of occurrence of *R. rickettsii* (OR 0.2; 95% CI 0.07-1.13; p =0.12) and *R. parkeri* (OR 0.5; 95% CI 0.14-1.68; p = 0.36). Because

Table 1. Seropositive cats and dogs to *Rickettsia* spp. sampled at the University of São Paulo at Piracicaba city, "Luiz de Queiroz" School of Agriculture (ESALQ-USP) from July to October 2017.

Sample identification	Habitat	Age (months)	Sex	Titers			PAIHR*	Presence of ticks
				R. rickettsii	*R. parkeri*	*R. felis*		
Cats								
12	Shelter	7	Sterilized male	64	NR	64		
14	Shelter	7	Female	128	512	NR		
15	Shelter	12	Sterilized male	256	NR	NR		
17	Shelter	7	Sterilized female	128	128	NR		
19	Shelter	7	Female	128	NR	NR		
21	Shelter	9	Sterilized male	64	64	NR		
22	Shelter	12	Sterilized male	NR	64	NR		
23	Shelter	7	Female	256	128	NR		
25	Shelter	7	Sterilized male	NR	128	NR		
26	Shelter	7	Sterilized male	64	NR	NR		
27	Shelter	60	Sterilized male	512	64	NR	*R. rickettsii*	
28	Shelter	60	Sterilized female	NR	64	NR		
29	Shelter	7	Sterilized female	128	NR	NR		
31	Shelter	7	Female	NR	64	NR		
33	Shelter	7	Sterilized female	256	512	64		
34	Shelter	48	Sterilized female	NR	256	NR		
35	Shelter	48	Sterilized female	NR	512	NR		
36	Shelter	7	Sterilized male	128	NR	NR		
37	Shelter	48	Sterilized female	256	512	NR		
38	Shelter	36	Sterilized female	512	1024	NR		
39	Shelter	24	Sterilized female	128	64	NR		
41	Shelter	3	Male	NR	64	NR		
42	Shelter	3	Male	256	128	64		
06	Free-roaming	36	Male	128	256	NR		
07	Free-roaming	-	Female	64	64	NR		
08	Free-roaming	-	Male	NR	64	NR		
49	Free-roaming	36	Male	1024	64	NR	*R. rickettsii*	4 *Amblyomma* sp., 5 *Amblyomma sculptum* and 1 *Rhipicephalus sanguineus* sensu lato
50	Free-roaming	8	Sterilized male	NR	128	NR		
	Yes/ Total (%)			19/28 (67.8%)	22/28 (78.6%)	3/28 (10.7%)		
	Yes/ Overall Total (%)				28/51 (54.9%)			1/28 (0.03%)
Dogs								
5	Shelter	96	Female	512	512	NR		
6	Shelter	60	Female	NR	128	NR		
7	Shelter	48	Female	64	64	NR		
8	Shelter	24	Male	NR	128	NR		
10	Shelter	108	Male	1024	1024	NR		
13	Shelter	36	Female	NR	128	NR		
	Yes/ Total (%)			3/6 (50.0%)	6/6 (100.0%)	0/6 (0.0%)		
	Yes/ Overall Total (%)				6/12 (50.0%)			0/12 (0.0%)

*PAIHR: probable antigen involved in homologous reaction.

of the low number of *R. felis*-seropositive cats, they were included in the statistical analyses.

Among the shelter dogs, 6/12 (50.0%) were seropositive for at least one of the two *Rickettsia* species tested: 3/6 (50.0%) reacted to *R. rickettsii* and 6/6 (100.0%) reacted to *R. parkeri*. No dog serum reacted to *R. felis*. The IFA endpoint titers ranged from 64 to 1024 for *R. rickettsii* and *R. parkeri*, and 64 for *R. felis* (Table 1).

Four larvae of *Amblyomma* spp., five nymphs of *Amblyomma sculptum* and one adult of *Rhipicephalus sanguineus sensu lato* were collected from the free-roaming cats: 1/5 (20%) of the seropositive cats had ticks and 2/11 (18.2%) of the seronegative cats had ticks (Table 1). No ticks were found on the dogs. No statistically significant association was found between the presence of ticks and rickettsiae seropositivity (OR 1.1; 95% CI 0.08-16.31; p = 1.00). Three *A. sculptum* tick nymphs collected from 3/16 (18.75%) of the free-roaming cats were randomly selected for detection of SFG rickettsial DNA by means of PCR. No rickettsial DNA was detected in these ticks, even though amplicons targeting tick 16S rRNA gene were produced through PCR to ensure successful extraction.

The frequencies and associations between individuals, and the epidemiological data, are shown in Table 2. There was a statistically significant association between *Rickettsia* seropositivity and age (p = 0.04), but not with sex (p = 1.00) or sterilization (p = 0.77). The difference in seroprevalence between shelter and free-roaming cats was also statistically significant (p = 0.03). Animals under 12 months old had a 4.3 times (95% CI 1.22 – 15.54) higher chance of being exposed to SFG rickettsiae than animals over 12 months old. This association was independent of the environment, since age was not significant when the cats were divided between a shelter group (p =0.16) and a free-roaming group (p =0.45) (Table 2).

Table 2. Risk factors association among living status, age, sex and sterilization with *Rickettsia* seropositivity frequency of animals and groups (shelter and free-roaming), sampled at the University of São Paulo at Piracicaba city, "Luiz de Queiroz" School of Agriculture (ESALQ-USP) from July to October 2017.

Cats	*Rickettsia* frequency (%)		Odds Ratio	p
	Positive	Total		
Living status				
Free-roaming	5 (31.2)	16 (100.0)	0.2 (0.07-0.84)	0.03
Shelter	23 (65.7)	35 (100.0)		
Age				
Up to 12 months	15 (75.0)	20 (100.0)	4.3 (1.22-15.54)	0.04
Over 12 months	11 (40.7)	27 (100.0)		
Sex				
Male	14 (56.0)	25 (100.0)	1.1 (0.36-3.29)	1.00
Female	14 (53.8)	26 (100.0)		
Sterilization				
Intact	10 (50.0)	20 (100.0)	0.72 (0.23-2.24)	0.77
Neutered	18 (58.1)	31 (100.0)		
Groups	***Rickettsia* frequency (%)**		**Odds Ratio**	**p**
	Positive	**Total**		
Age				
Shelter				
Up to 12 months	14 (77.8)	18 (100.0)	3.11 (0.72-13.44)	0.16
Over 12 months	9 (52.9)	17 (100.0)		
Free-roaming				
Up to 12 months	1 (50.0)	2 (100.0)	4.00 (0.16-95.76)	0.45
Over 12 months	2 (20.0)	10 (100.0)		
Sex				
Shelter				
Male	10 (66.7)	15 (100.0)	1.08 (0.26-4.42)	1.00
Female	13 (65.0)	20 (100.0)		
Free-roaming				
Male	4 (40.0)	10 (100.0)	3.33 (0.28-40.29)	0.58
Female	1 (16.7)	6 (100.0)		
Sterilization				
Shelter				
Intact	6 (75.0)	8 (100.0)	1.76 (0.30-10.47)	0.68
Neutered	17 (63.0)	27 (100.0)		
Free-roaming				
Intact	4 (33.3)	12 (100.0)	1.50 (0.12-19.44)	1.00
Neutered	1 (25.0)	4 (100.0)		

Discussion

To the authors' knowledge, the present study was the first concomitant report of overlapping free-roaming cat and capybara populations in an area endemic for BSF. Although antibodies against both *Rickettsia rickettsii* and *Rickettsia parkeri* have previously been reported in cats and human cases from areas of the state of São Paulo that are endemic or nonendemic for BSF (HORTA et al., 2007), only stray or owned cats were surveyed. *R. rickettsii* is the most important tick-borne zoonotic agent, and it is primarily transmitted to humans and other vertebrates by *A. sculptum* and *Amblyomma aureolatum* (Pallas) (LABRUNA et al., 2011, 2017).

The seroconversion of *Rickettsia bellii* has been reported in capybaras of southern Brazil (FORTES et al., 2011). Nonetheless, a previous study conducted in the same area of the present study, even with several positive sera of opossums, dogs, cats, horses, and humans to *R. bellii* and *R. felis*, no antibody response has been observed to these agents, probably due to similar or low titers in comparison with titers to *R. rickettsii* or *R. parkeri* (HORTA et al., 2007). These results may be explained by the low frequency of *Amblyomma dubitatum* and consequently *R. bellii* in the same area (HORTA et al., 2004), and the unconfirmed vector competence of fleas for *Rickettsia felis* transmission (WEDINCAMP & FOIL, 2000).

Only two cats presented any antigen that was possibly involved in a homologous reaction (PAIHR) for *R. rickettsii*. As previously described in IFA tests, *R rickettsii* and *R. parkeri* showed considerable cross-reactivity (BEATI et al., 1994). Nonetheless, seropositivity among free-roaming cats in an area with overlapping capybara distribution may suggest that cats have a role as sentinels in relation to human exposure to SFG rickettsiae, as previously observed among owned cats (HORTA et al., 2007; MATTHEWMAN et al., 1997). In the present study, *R. parkeri* strain At24, mentioned as *sensu stricto* and isolated from *Amblyomma triste* ticks Koch in Paulicéia County, state of São Paulo-Brazil, has been used as the only crude antigen of *R. parkeri* to IFA (SILVEIRA et al., 2007). Even so, a previous study has already shown that the serological response of dogs to *R. parkeri sensu stricto* (strain At24) and *R. parkeri* strain Atlantic rainforest were the same (SZABÓ et al., 2013). Thus, differentiation between *R. parkeri sensu stricto* and the rest of strains mentioned as *R. parkeri sensu lato* may not be possible using solely IFA with crude antigens. Finally, the low endpoint titers (64) to *R. felis* in three cats of the present study is likely to be a result of cross-reaction with SFG rickettsiae, as previously demonstrated for *R. rickettsii*-infected dogs (PIRANDA et al., 2008).

Stray cats trapped in forest edges from Piracicaba city were all (7/7; 100%) found to be seropositive for *R. rickettsii* and mostly (6/7; 85.7%) seropositive for *R. parkeri* (HORTA et al., 2007). These frequencies were higher than in the present serosurvey on both free-roaming and shelter cats (Table 3). These differences were probably due to the presence of infected capybara ticks where cats were sampled in previous study.

The IFA endpoint titers for *Rickettsia* spp. ranged from 64 to 1024 in both groups. Similar outcomes for both antigens have previously been reported, but a few cats have presented higher titers of 2048 for both antigens (HORTA, 2006). Contrary to previous studies that did not shown any significant association between seropositivity for rickettsiae and habitat or outdoor activities (CASE et al., 2006; SEGURA et al., 2014), the present study showed that free-roaming cats had 80% lower likelihood of exposure to *Rickettsia* than shelter cats. We hypothesize that an outbreak of rickettsiae prior to cat rescue or while at the shelter may have occurred and may have led to seroconversion among the shelter cats.

Cats younger than 12 months had 4.3 times higher chance of seropositivity than older cats in the present study (Table 2). Although evidence of increasing exposure to *R. rickettsii* as a function of cat age was found in a previous study (CASE et al., 2006); other studies have not found any association between age and seropositivity for rickettsiae (SOLANO-GALLEGO et al., 2006; SEGURA et al., 2014). Younger cats have been found to

Table 3. Cat worldwide seroprevalence against *Rickettsia rickettsii* and *R. parkeri*, according to available literature.

Location	Cat population	*Rickettsia* spp. and seropositivity (%)	Reference
28 states, USA	Paired samples from febrile and non-febrile owned cats.	Overall 8/182 (4.4) for *R. rickettsii,* comprising 6/91 (6.6) febrile and 2/91 (2.2) non-febrile cats.	(BAYLISS et al., 2009)
California and Wisconsin, USA	Owned, shelter and feral cats.	29/170 (17.05) for *R. rickettsii*, comprising 2/20 (10.0) owned, 14/114 (12.3) shelter and 9/36 (25.0) feral cats.	(CASE et al., 2006)
State of São Paulo, Brazil	Cats from four endemic areas (trapped stray cats).	Overall 16/19 (84.2) for *R. rickettsii* and 15/19 (78.9) for *R. parkeri*. In city of Piracicaba, 7/7 (100%) for *R. rickettsii* and 6/7 (85.7%) for *R. parkeri*. In addition, 6/7 (85.7%) were seropositive to *R. felis*.	(HORTA et al., 2007)
St Kitts, West Indies	Feral cats	22/52 (42.3) *R. rickettsii*	(KELLY et al., 2017)
Northern California, USA	Owned cats in rural communities	23/29 (79.3) *R. rickettsii*	(STEPHENSON et al., 2017)

be statistically more susceptible to rickettsial infection, possibly due to less efficient self-cleaning habits, particularly in relation to ixodid ticks, compared with adult cats. This greater susceptibility may lead to greater potential for outbreaks of disease in situations of infestation with infected ticks. Likewise, infestation with cat fleas (*Ctenocephalides felis*) is greater among younger cats than among older cats, as a direct relationship between the host's ability to groom and ectoparasite intensity (HINKLE et al., 1998). Nevertheless, a previous study in the same endemic area has shown only *R. rickettsii* and *R. parkeri* as "possible antigen involved in a homologous reaction (PAIHR)", with no cats likely infected by *Rickettsia felis* (HORTA et al., 2007). Moreover, cats experimentally and continuously exposed to *R. felis*-infected fleas have failed to seroconvert to *R. felis* within the first few months following infestation; actually, some cats have never seroconverted (WEDINCAMP & FOIL, 2000). Thus, exposure to *R. felis*-infected fleas may not be a risk factor for *R. felis* seroconversion (BAYLISS et al., 2009; HORTA et al., 2010). In addition, the pathogenic role of *R. felis* has been still under debate worldwide (LABRUNA & WALKER, 2014). Hence, it is reasonable to speculate that the young cat seropositivity could be associated with tick infestation.

Rickettsia spp. usually infect and remain inside host endothelial cells, and molecular detection has failed when blood samples are investigated (LA SCOLA & RAOULT, 1997). Under experimental conditions of infestation with *R. rickettsii*-infected ticks, rickettsial DNA was detected through PCR in only one out of 32 blood samples (3.1%) from rickettsemic capybaras. The rickettsemia of these PCR-negative capybaras was demonstrated by intraperintoneal inoculation of capybara blood samples into guinea pigs, which developed severe spotted fever that resulted in death or seroconversion. Thus, in the present study, no molecular investigation was made on the blood samples from these free-roaming and shelter cats.

Previous studies in the same area of Piracicaba showed that the frequencies of seropositivity for *Rickettsia* spp. among cats concomitantly parasitized by nymphs of *Amblyomma* spp. were 3/7 (42.8%) (HORTA et al., 2007) and 6/7 (85.7%) (PEREZ et al., 2008). Molecular testing on the ticks was not conducted in either of these previous studies, but the testing using PCR in the present study did not detect any *Rickettsia* spp. in *A. sculptum* ticks.

In the present study, 6/12 shelter dogs (50.0%) were seropositive for *Rickettsia* spp., and among the seropositive dogs, 3/6 (50.0%) showed titers for both *R. rickettsii* and *R. parkeri*. All of these dogs had been rescued within the campus limits but they originated from outside the campus. They were caught because of a policy of suppression of on-campus roaming of dogs.

Lastly, although feral (free-roaming) cat movement was not estimated in the present study, previous studies has been shown to undergo periodic shifts in home ranges, with mean distances of 1.2 km between the centers of these cats' successive home ranges (EDWARDS et al., 2001). Even with relatively low frequencies of ixodid tick infestation and cat seropositivity were found in the present study, the overlapping with capybaras' natural habitat sites may make cats potential sources for infected ticks. Whether equally or less exposed to rickettsial infection, compared with owned cats in endemic areas, free-roaming and shelter cats may be used as environmental sentinels for human exposure to SFG rickettsiae in such areas.

Conclusions

To the authors' knowledge, the present study was the first concomitant report on high free-roaming cat and capybara populations that overlapped in an area endemic for BSF. Cats under one year old may be more susceptible to ticks and rickettsial infections, probably due to kittens' less efficient tick self-cleaning habits, thus leading to the potential for disease outbreaks when associated with infestation with infected ticks. Free-roaming and shelter cats may be used as environmental sentinels for human exposure to SFG rickettsiae in such areas.

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References

Aragão H, Fonseca F. Notas de ixodologia: VIII. Lista e chave para os representantes da fauna ixodológica brasileira: notas de ixolodologia. *Mem Inst Oswaldo Cruz* 1961; 59(2): 115-129. http://dx.doi.org/10.1590/S0074-02761961000200001. PMid:13861962.

Barros-Battesti DM, Arzua M, Bechara GH. *Carrapatos de importância médico veterinária da Região Neotropical: Um guia ilustrado para identificação de espécies*. 1a ed. São Paulo: Vox/ICTTD-3/Butantan; 2006.

Bayliss DB, Morris AK, Horta MC, Labruna MB, Radecki SV, Hawley JR, et al. Prevalence of *Rickettsia* species antibodies and *Rickettsia* species DNA in the blood of cats with and without fever. *J Feline Med Surg* 2009; 11(4): 266-270. http://dx.doi.org/10.1016/j.jfms.2008.06.007. PMid:18786845.

Beati L, Kelly PJ, Mason PR, Raoult D. Species-specific BALB/c mouse antibodies to rickettsiae studied by western blotting. *FEMS Microbiol Lett* 1994; 119(3): 339-344. http://dx.doi.org/10.1111/j.1574-6968.1994.tb06910.x. PMid:8050715.

Beninati T, Genchi C, Torina A, Caracappa S, Bandi C, Lo N. Rickettsiae in Ixodid Ticks, Sicily. *Emerg Infect Dis* 2005; 11(3): 509-511. http://dx.doi.org/10.3201/eid1103.040812. PMid:15789496.

Case JB, Chomel B, Nicholson W, Foley JE. Serological survey of vector-borne zoonotic pathogens in pet cats and cats from animal shelters and feral colonies. *J Feline Med Surg* 2006; 8(2): 111-117. http://dx.doi.org/10.1016/j.jfms.2005.10.004. PMid:16434226.

Edwards GP, De Preu N, Shakeshaft BJ, Crealy IV, Paltridge RM. Home range and movements of male feral cats (*Felis catus*) in a semiarid woodland environment in central Australia. *Austral Ecol* 2001; 26(1): 93-101. http://dx.doi.org/10.1111/j.1442-9993.2001.01091.pp.x.

Escola Superior de Agricultura "Luiz de Queiroz" – ESALQ. Universidade de São Paulo – USP. *ESALQ em números* [online]. Piracicaba: USP; 2019 [cited 2019 Oct 22]. Available from: https://www.esalq.usp.br/institucional/esalq-em-numeros

Fortes FS, Santos LC, Cubas ZS, Barros-Filho IR, Biondo AW, Silveira I, et al. Anti-*Rickettsia* spp. antibodies in free-ranging and captive capybaras

from southern Brazil. *Pesq Vet Bras* 2011; 31(11): 1014-1018. http://dx.doi.org/10.1590/S0100-736X2011001100013.

Gheler-Costa C, Verdade LM, Almeida AF. Mamíferos não-voadores do campus "Luiz de Queiroz", Universidade De São Paulo, Piracicaba, Brasil. *Rev Bras Zool* 2002; 19(Suppl. 2): 203-214. http://dx.doi.org/10.1590/S0101-81752002000600021.

Hinkle NC, Koehler PG, Patterson RS. Host grooming efficiency for regulation of Cat Flea (Siphonaptera: Pulicidae) populations. *J Med Entomol* 1998; 35(3): 266-269. http://dx.doi.org/10.1093/jmedent/35.3.266. PMid:9615545.

Horta MC, Labruna MB, Pinter A, Linardi PM, Schumaker TTS. *Rickettsia* infection in five areas of the state of São Paulo, Brazil. *Mem Inst Oswaldo Cruz* 2007; 102(7): 793-801. http://dx.doi.org/10.1590/S0074-02762007000700003. PMid:18094887.

Horta MC, Pinter A, Souza CE, Neto EJR, Souza SSAL, Soares RM, et al. Ocorrência de *Rickettsia bellii* em carrapatos colhidos nos Municípios de Piracicaba, Pedreira, Campinas, Itu e Cordeirópolis, Estado de São Paulo. *Rev Bras Parasitol Vet* 2004; 13(Suppl. 1): 364.

Horta MC, Scott FB, Correia TR, Fernandes JI, Richtzenhain LJ, Labruna MB. *Rickettsia felis* infection in cat fleas *Ctenocephalides felis felis. Braz J Microbiol* 2010; 41(3): 813-818. http://dx.doi.org/10.1590/S1517-83822010000300035. PMid:24031560.

Horta MC. *Estudo epidemiológico de Rickettsia felis em áreas endêmicas e não-endêmicas para febre maculosa no Estado de São Paulo* [tese]. São Paulo: Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia; 2006. http://dx.doi.org/10.11606/T.10.2006.tde-12092007-112431.

Instituto Brasileiro de Geografia e Estatística – IBGE. *Piracicaba Panorama* [online]. Rio de Janeiro: IBGE; 2017 [cited 2019 Oct 22] Available from: https://cidades.ibge.gov.br/brasil/sp/piracicaba/panorama

Kelly PJ, Köster L, Li J, Zhang J, Huang K, Branford GC, et al. Survey of vector-borne agents in feral cats and first report of *Babesia gibsoni* in cats on St Kitts, West Indies. *BMC Vet Res* 2017; 13(1): 331. http://dx.doi.org/10.1186/s12917-017-1230-1. PMid:29132371.

La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. *J Clin Microbiol* 1997; 35(11): 2715-2727. PMid:9350721.

Labruna MB, Krawczak FS, Gerardi M, Binder LC, Barbieri ARM, Paz GF, et al. Isolation of *Rickettsia rickettsii* from the tick *Amblyomma sculptum* from a Brazilian Spotted Fever-endemic area in the Pampulha Lake region, southeastern Brazil. *Vet Parasitol Reg Stud Rep* 2017; 8: 82-85. http://dx.doi.org/10.1016/j.vprsr.2017.02.007. PMid:31014644.

Labruna MB, Ogrzewalska M, Soares JF, Martins TF, Soares HS, Moraes-Filho J, et al. Experimental infection of *Amblyomma aureolatum* ticks with *Rickettsia rickettsii. Emerg Infect Dis* 2011; 17(5): 829-834. http://dx.doi.org/10.3201/eid1705.101524. PMid:21529391.

Labruna MB, Santos FC, Ogrzewalska M, Nascimento EM, Colombo S, Marcili A, et al. Genetic identification of rickettsial isolates from fatal cases of Brazilian spotted fever and comparison with *Rickettsia rickettsii* isolates from the American continents. *J Clin Microbiol* 2014; 52(10): 3788-3791. http://dx.doi.org/10.1128/JCM.01914-14. PMid:25078908.

Labruna MB, Walker DH. *Rickettsia felis* and changing paradigms about pathogenic Rickettsiae. *Emerg Infect Dis* 2014; 20(10): 1768-1769. http://dx.doi.org/10.3201/eid2010.131797. PMid:25271441.

Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A, et al. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian Spotted Fever is endemic. *J Clin Microbiol* 2004; 42(1): 90-98. http://dx.doi.org/10.1128/JCM.42.1.90-98.2004. PMid:14715737.

Labruna MB. Ecology of Rickettsia in South America. *Ann N Y Acad Sci* 2009; 1166(1): 156-166. http://dx.doi.org/10.1111/j.1749-6632.2009.04516.x. PMid:19538276.

Mangold AJ, Bargues MD, Mas-Coma S. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). *Parasitol Res* 1998; 84(6): 478-484. http://dx.doi.org/10.1007/s004360050433. PMid:9660138.

Martins TF, Onofrio VC, Barros-Battesti DM, Labruna MB. Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brasil: descriptions, redescriptions, and identification key. *Ticks Tick Borne Dis* 2010; 1(2): 75-99. http://dx.doi.org/10.1016/j.ttbdis.2010.03.002. PMid:21771514.

Matthewman L, Kelly P, Hayter D, Downie S, Wray K, Bryson N, et al. Domestic cats as indicators of the presence of spotted fever and typhus group Rickettsiae. *Eur J Epidemiol* 1997; 13(1): 109-111. http://dx.doi.org/10.1023/A:1007375718204. PMid:9062789.

Nieri-Bastos FA, Marcili A, De Sousa R, Paddock CD, Labruna MB. Phylogenetic evidence for the existence of multiple strains of *Rickettsia parkeri* in the new world. *Appl Environ Microbiol* 2018; 84(8): e02872-e17. http://dx.doi.org/10.1128/AEM.02872-17. PMid:29439989.

Novakova M, Costa FB, Krause F, Literak I, Labruna MB. *Rickettsia vini* n. sp. (Rickettsiaceae) infecting the tick *Ixodes arboricola* (Acari: ixodidae). *Parasit Vectors* 2016; 9(1): 469. http://dx.doi.org/10.1186/s13071-016-1742-8. PMid:27565956.

Perez CA, Almeida AF, Almeida A, Carvalho VHB, Balestrin DC, Guimarães MS, et al. Carrapatos do gênero *Amblyomma* (Acari: Ixodidae) e suas relações com os hospedeiros em área endêmica para febre maculosa no estado de São Paulo. *Rev Bras Parasitol Vet* 2008; 17(4): 210-217. http://dx.doi.org/10.1590/S1984-29612008000400008. PMid:19265580.

Piranda EM, Faccini JL, Pinter A, Saito TB, Pacheco RC, Hagiwara MK, et al. Experimental infection of dogs with a Brazilian strain of *Rickettsia rickettsii*: clinical and laboratory findings. *Mem Inst Oswaldo Cruz* 2008; 103(7): 696-701. http://dx.doi.org/10.1590/S0074-02762008000700012. PMid:19057821.

Polo G, Labruna MB, Ferreira F. Satellite hyperspectral imagery to support tick-borne infectious diseases surveillance. *PLoS One* 2015; 10(11): e0143736. http://dx.doi.org/10.1371/journal.pone.0143736. PMid:26599337.

Sangioni LA, Horta MC, Vianna MCB, Gennari SM, Soares RM, Galvão MAM, et al. Ricketsial infection in animals and Brazilian Spotted Fever endemicity. *Emerg Infect Dis* 2005; 11(2): 265-269. http://dx.doi.org/10.3201/eid1102.040656. PMid:15752445.

São Paulo. Secretaria de Estado da Saúde. *Casos autóctones confirmados de febre maculosa brasileira no estado de São Paulo de 2007 a 2019* por município de LPI e evolução*. [online]. 2019. [cited june 2019] Available from: http://www.saude.sp.gov.br/resources/sucen/homepage/destaques/nova-pasta-de-midia/casos__fmb_2007-2018_2.pdf

Segura F, Pons I, Miret J, Pla J, Ortuño A, Nogueras MM. The role of cats in the eco-epidemiology of spotted fever group diseases. *Parasit Vectors* 2014; 7(353): 1-13. http://dx.doi.org/10.1186/1756-3305-7-353. PMid:25084969.

Solano-Gallego L, Hegarty B, Espada Y, Llull J, Breitschwerdt E. Serological and molecular evidence of exposure to arthropod-borne organisms in cats

from northeastern Spain. *Vet Microbiol* 2006; 118(3-4): 274-277. http://dx.doi.org/10.1016/j.vetmic.2006.07.010. PMid:16919405.

Silveira I, Pacheco RC, Szabó MPJ, Ramos HGC, Labruna MB. *Rickettsia parkeri* in Brazil. *Emerg Infect Dis* 2007; 13(7): 1111-1113. http://dx.doi.org/10.3201/eid1307.061397. PMid:18214195.

Souza CE, Moraes-Filho J, Ogrzewalska M, Uchoa FC, Horta MC, Souza SS, et al. Experimental infection of capybaras *Hydrochoerus hydrochaeris* by *Rickettsia rickettsii* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense. Vet Parasitol* 2009; 161(1-2): 116-121. http://dx.doi.org/10.1016/j.vetpar.2008.12.010. PMid:19147293.

Stephenson N, Blaney A, Clifford D, Gabriel M, Wengert G, Foley P, et al. Diversity of rickettsiae in a rural community in northern California. *Ticks Tick Borne Dis* 2017; 8(4): 526-531. http://dx.doi.org/10.1016/j.ttbdis.2017.02.014. PMid:28274808.

Szabó MP, Nieri-Bastos FA, Spolidorio MG, Martins TF, Barbieri AM, Labruna MB. *In vitro* isolation from *Amblyomma ovale* (Acari: Ixodidae) and ecological aspects of the Atlantic rainforest *Rickettsia*, the causative agent of a novel spotted fever rickettsiosis in Brazil. *Parasitology* 2013; 140(6): 719-728. http://dx.doi.org/10.1017/S0031182012002065. PMid:23363571.

Wedincamp JJ Jr, Foil LD. Infection and seroconversion of cats exposed to cat fleas (*Ctenocephalides felis* Bouché) infected with *Rickettsia feli*s. *J Vector Ecol* 2000; 25(1): 123-126. PMid:10925806.

Zavala-Velazquez JE, Yu XJ, Walker DH. Unrecognized spotted fever group rickettsiosis masquerading as dengue fever in Mexico. *Am J Trop Med Hyg* 1996; 55(2): 157-159. http://dx.doi.org/10.4269/ajtmh.1996.55.157. PMid:8780453.