Rickettsia felis in *Ctenocephalides felis felis* from Five Geographic Regions of Brazil

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Abstract. This study evaluated rickettsial infection in 701 *Ctenocephalides felis felis* fleas that were collected from dogs and cats in 31 municipalities, encompassing all regions and major biomes of Brazil. A total of 268 (38.2%) fleas from 30 municipalities were polymerase chain reaction (PCR) positive for the rickettsial *gltA* gene. The PCR products from 44 fleas, consisting of at least 1 PCR-positive flea from each of 30 municipalities, generated DNA sequences identical to *Rickettsia felis*. Rickettsial prevalence was highly variable among 30 municipalities, with values ranging from 2.9% to 100%. Significantly higher infection rates by *R. felis* were associated with the Pampa biome (southern Brazil), and the temperate climate that prevails in southern Brazil. In contrast, lowest *R. felis*-infection rates were significantly associated with the Caatinga biome, and its semiarid climate. Further studies should evaluate the effect of temperature and moisture on the *R. felis* infection in *Ctenocephalides felis* fleas world widely.

INTRODUCTION

Rickettsia felis, an obligate intracellular Gram-negative bacterium, was first described infecting internal tissues of the flea *Ctenocephalides felis felis* in the United States.^{1,2} Since then, the bacterium has been reported infecting a number of invertebrate species, including fleas, mosquitoes, ticks, mites, and booklice.^{3–8} Despite an apparent broad host range, the majority of the literature reports of *R. felis* have been on fleas of the genus *Ctenocephalides*; namely, *C. felis felis* and *Ctenocephalides canis* collected from domestic dogs and cats.^{9–12} In Brazil, *R. felis* was reported infecting *Ctenocephalides felis* fleas in the states of São Paulo, Minas Gerais, and Rio de Janeiro, all in the Southeastern region of the country.^{13–15}

Rickettsia felis has been reported globally as an emerging disease agent. Human disease attributed to *R. felis* infection, have been reported in all continents, mostly during the last 5 years.^{9,16–18} In Brazil, *R. felis* was associated with a febrile rash in two patients from the state of Minas Gerais.¹⁶

Brazil is a continental country, divided into five geopolitical regions (North, Northeast, Middle-West, Southeast, and South) and six major biomes: Amazon (rainforest), Caatinga (semiarid), Cerrado (savannah), Pantanal (wetlands), Pampa (open fields), and the Atlantic rainforest. Because only a few studies have investigated *R. felis* infection in Brazil (all in the Southeast region), this study evaluated rickettsial infection in fleas collected from different areas of all five geopolitical regions of the country, encompassing the six major biomes.

MATERIAL AND METHODS

From 2006 to 2009, fleas were collected on domestic dogs and cats of 31 municipalities (mean: 23 fleas per municipality) among the five geopolitical regions of Brazil: North (5 municipalities); Northeast (7); Middle-West (4); Southeast (8); and South (7) (Table 1). Sampling localities were chosen by convenience, depending on the availability of collaborators in each locality, as stated in the Acknowledgment section. Care was taken to include only fleas that were collected from animals living in outdoor conditions. Collected fleas were

preserved in absolute ethanol and brought to the laboratory, where they were identified to species following Linardi and Guimarães,¹⁹ and individually submitted to DNA extraction by boiling at 100°C for 20 min following Horta and others.¹⁴ Fleas were individually tested for the presence of *Rickettsia* by polymerase chain reaction (PCR) using primers CS-78 and CS-323, targeting a 401 fragment of the rickettsial *gltA* gene.²⁰ In each PCR run, *Rickettsia parkeri* DNA was used as a positive control, and at least two negative controls (water). The PCR products were DNA sequenced and submitted to basic local alignment search tool (BLAST) analysis to determine similarities to other *Rickettsia* species.²¹

The proportions of *Rickettsia*-infected fleas were compared between geopolitical regions (North, Northeast, Middle-West, Southeast, South), between biome types (Amazon, Caatinga, Cerrado, Atlantic rainforest, Pampa, Pantanal), and between major Climate types (Equatorial, Temperate, Tropical Central Brazil, Tropical Oriental Northeast, Tropical Equatorial Zone) by the χ^2 distribution, using the program Minitab 16.1.0. Values were considered significantly different when $P < 0.05$. The geographical distributions of the sampled municipalities according to biome and climate types were retrieved from the official database of the Brazilian Institute of Geography and Statistics (<http://www.ibge.gov.br/>). For comparison purposes, the Pantanal biome was considered as belonging to the Cerrado biome, following Junk and others.²²

RESULTS

A total of 701 fleas were collected, 665 from dogs and only 36 from cats. All fleas were identified as *C. felis felis*. A total of 268 (38.2%) individual fleas, 255 from dogs and 13 from cats, yielded PCR products of the expected size for the rickettsial *gltA* gene. Only one of the 31 municipalities did not yield at least one PCR-positive flea (Table 1). Rickettsial prevalence was highly variable among 30 municipalities, with values ranging from 2.9% to 100%. The *gltA* product from 44 fleas, consisting of at least one or two PCR-positive fleas from each of 30 municipalities, yielded DNA sequences identical to each other, and 100% identical to the corresponding sequence of the *R. felis* type strain (GenBank accession no. CP000053).

The geographic distributions of the 31 municipalities sampled in this study are shown in Figure 1, according to the geopolitical region, biome type, and climate type. The proportions of infected fleas were significantly different ($P < 0.05$) between

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TABLE 1
Rickettsial infection in *Ctenocephalides felis felis* fleas collected from dogs and cats in the five geopolitical regions of Brazil

Region	Municipality	State	No. in Figure 1	No. infected fleas/no. tested fleas (%)	
				Municipality	Region
North	Belém	Pará	1	1/25 (4)	38/111 (34.2)
	Macapá	Amapá	2	0/20 (0)	
	Manaus	Amazonas	3	18/20 (90)	
	Marabá	Pará	4	17/21 (81)	
	Monte Negro	Roraima	5	2/25 (8)	
Northeast	Fortaleza	Ceará	6	2/25 (8)	33/168 (19.6)
	Jaboatão dos Guararapes	Pernambuco	7	10/24 (41.7)	
	Mossoró	Rio G. do Norte	8	2/25 (8)	
	Natal	Rio G. do Norte	9	2/20 (10)	
	Patos	Paraíba	10	7/20 (35)	
	Salvador	Bahia	11	9/20 (45)	
	Teresina	Piauí	12	1/34 (2.9)	
Middle-West	Brasília	Distrito Federal	13	7/22 (31.8)	25/88 (28.4)
	Cuiabá	Mato Grosso	14	1/25 (4)	
	Dourados	Mato G. do Sul	15	14/20 (70)	
	Poconé	Mato Grosso	16	3/21 (14.3)	
Southeast	Juiz de Fora	Minas Gerais	17	8/20 (40)	68/188 (36.3)
	Montes Claros	Minas Gerais	18	17/20 (85)	
	Presidente Prudente	São Paulo	19	11/21 (52.4)	
	Rio de Janeiro	Rio de Janeiro	20	3/26 (11.5)	
	São José do Rio Preto	São Paulo	21	10/38 (26.3)	
	São Paulo	São Paulo	22	7/20 (35)	
	Uberlândia	Minas Gerais	23	6/22 (27.3)	
	Vila Velha	Espírito Santo	24	6/21 (28.6)	
South	Araranguá	Santa Catarina	25	20/20 (100)	104/146 (71.2)
	Bandeirantes	Paraná	26	10/20 (50)	
	Blumenau	Santa Catarina	27	19/20 (95)	
	Cândido de Abreu	Paraná	28	15/25 (60)	
	Pelotas	Rio G. do Sul	29	19/20 (95)	
	Rolândia	Paraná	30	7/20 (35)	
	Santa Maria	Rio G. do Sul	31	14/21 (66.7)	
Total					268/701 (38.2)

the five geopolitical regions, with the South region having the highest prevalence (71.2%), and the Northeast region the lowest prevalence (19.6%) (Table 1). The proportions of infected fleas were significantly different ($P < 0.05$) between biome, with the highest rickettsial prevalence in Pampa (33 of 41; 80.5%), followed by Atlantic rainforest (141 of 297; 47.5), Amazon (38 of 111; 34.2%), Atlantic rainforest (44 of 148; 29.7), and Caatinga (12 of 104; 11.5%). The proportions of infected fleas were significantly different ($P < 0.05$) between climate types, with the highest rickettsial prevalence in Temperate (87 of 106; 82.1%), followed by Tropical Central Brazil (110 of 206; 53.4%), Equatorial (38 of 111; 34.2%), Tropical Oriental Northeast (21 of 64; 32.8%), and Tropical Equatorial Zone (12 of 104; 11.5%).

DISCUSSION

This study shows that the infection by *R. felis* is widespread along flea populations in continental Brazil, encompassing all geopolitical regions or all biome types. Although we generated DNA sequences for only 1–2 fleas per locality, we assume that the remaining PCR-positive fleas referred to *R. felis* because it has been the major agent (most of time the sole agent) infecting *C. felis* worldwide.^{9,10} Rickettsial infection

rates were highly variable among flea populations from the 31 sampled localities, in accordance with literature reports that have shown that *R. felis* is found infecting 0% to 100% of fleas from populations sampled worldwide.¹⁰ Interestingly, significantly higher infection rates by *R. felis* were associated with the Pampa biome (southern Brazil), and the temperate climate that prevails in southern Brazil. This part of Brazil is characterized by having the lowest winter temperatures of the country, typically below 10°C, with occasional snow.²³ In contrast, lowest *R. felis*-infection rates were significantly associated with the Caatinga biome, and its Tropical Equatorial zone climate. This climate is characterized mainly by having the lowest precipitation rates of Brazil, in strong association with the Caatinga biome, forming the only semiarid region of Brazil.²³ *Rickettsia felis*-infection rates were of intermediate values among the remaining biomes or climate types of Brazil, where temperature is not as cold as in Pampas, or where precipitation rates are not as low as in the Caatinga. Because all flea samples were collected from animals living at outdoors conditions, we can assume that free-living stages of these fleas were, at some extent, affected by the different temperature and precipitation rate conditions inherent to each biome.

This is the first study that associates flea-*R. felis* infection with environmental conditions. Because it has been shown

FIGURE 1. Municipalities sampled in this study, according to geopolitical regions (A), biomes (B), and climate zones (C) of continental Brazil. Numbers refer to municipality names in Table 1.

that infection rates by *R. felis* is highly variable among flea populations in all continents of the world,^{9–12} further studies should evaluate the effect of temperature and moisture on the *R. felis* infection in *Ctenocephalides* fleas.

Recent studies have considered *R. felis* as a global emerging pathogen.²⁴ This scenario, in conjunction with our present study, indicates that humans are at risk of acquiring *R. felis*-caused spotted fever in all regions of Brazil, because a large number of recent studies have reported that *Ctenocephalides* fleas are major vectors of *R. felis*.^{10,24,25} Contrastingly, only two cases of human infection by *R. felis* have been reported in Brazil,¹⁶ where modern diagnostic tools are available for surveillance of spotted fever in some areas (Southeastern region) that have reported hundreds of cases of tick-borne spotted fever, caused mainly by *Rickettsia rickettsii*.^{26,27} Interestingly, one study in Rio de Janeiro (southeastern Brazil) reported no history of spotted fever among 15 persons that were frequently bitten by *R. felis*-infected fleas.²⁸ Indeed, further studies are needed to evaluate the real risk of humans to acquire spotted fever caused by *R. felis* in Brazil, because there is no doubt that humans live in close association with *R. felis*-infected fleas throughout the country.

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