Title Prevalence of *Bartonella* Species in Shelter Cats and their Ectoparasites in Southeastern Brazil

Juliana M. Raimundo

UFRRJ: Universidade Federal Rural do Rio de Janeiro

Andresa Guimarães

UFRRJ: Universidade Federal Rural do Rio de Janeiro

Gleice M. Amaro

UFRRJ: Universidade Federal Rural do Rio de Janeiro

Aline T. da Silva

UFRRJ: Universidade Federal Rural do Rio de Janeiro

Caio Junior B. C. Rodrigues

UFRRJ: Universidade Federal Rural do Rio de Janeiro

Huarrisson A. Santos

UFRRJ: Universidade Federal Rural do Rio de Janeiro

Elba R. S. de Lemos

FIOCRUZ: Fundacao Oswaldo Cruz

Alexsandra R. de Mendonça Favacho

FIOCRUZ - Campo Grande/MS

Cristiane Divan Baldani (✉ crisbaldani@gmail.com)

Universidade Federal Rural do Rio de Janeiro <https://orcid.org/0000-0002-4627-1362>

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Abstract

Background: Feline *Bartonella* can be transmitted to humans through cat scratches or bites, and between cats by *Ctenocephalides felis* flea.

Methods: The occurrence of *Bartonella* DNA was assessed in ectoparasites and their cat hosts living in shelters based on the ITS region and *gltA* gene.

Results: *Bartonella* DNA was detected in 47.8% of cat blood samples, in 18.3% of *C. felis* fleas, 13.3% of flea eggs pools and 12.5% of lice pools. *B. henselae* and *B. clarridgeiae* DNA were detected in cat fleas, while *B. henselae*, *B. clarridgeiae*, and *B. koehlerae* in blood samples from bacteremic cats. Cats infested by positive ectoparasites showed approximately twice the chance of being infected.

Conclusions: Our results indicate shelter cats have a high prevalence of *Bartonella* species known to be human pathogens and highlight the importance of controlling their infestation by ectoparasites to avoid cat and human infection.

1. Introduction

Considering One Health concepts, studies approaching bartonellosis are important because this bacterial genus infects a broad variety of animals, are linked to an ever increasing number of human diseases, and are transmitted by arthropod vectors [1]. According to the List of Prokaryotic Names with Standing in Nomenclature (LPSN 2021), the genus *Bartonella* contains 37 species and 3 subspecies. Potential domesticated and wild animal reservoirs includes horse, cats, dogs, rodent, rabbits, ruminants, sea mammals, wild felines, coyotes, deer, elk, and foxes. The list of vectors and potential vectors associated with bacterial transmission includes flies, fleas, ticks, lice, and mites [2]. To date, natural infections in cats have been reported for six *Bartonella* species: *B. henselae*, *B. clarridgeiae*, *B. koehlerae*, *B. bovis*, *B. quintana*, *B. vinsonii* subsp. *Berkhoffii*, and more recently *B. capreoli* [3–5]. Most *Bartonella* species infecting humans are zoonotic, and cats appear to be the primary mammalian reservoir for *B. henselae*, *B. clarridgeiae*, and *B. koehlerae* [6]. Feline *Bartonella* can be transmitted to humans through scratches or bites. Transmission between cats most often occurs via the *Ctenocephalides felis* flea [7–9], which harbors *B. henselae*, *B. clarridgeiae*, *B. quintana*, and *B. koehlerae* DNA [10].

In Brazil, little information is available about *Bartonella* occurrence in animals and humans, and to date no study has verified the occurrence of *Bartonella* spp. in cat shelters in the Southeast region. Therefore, the objective of this study was to investigate the prevalence of *Bartonella* infection in shelter cats and ectoparasites collected from them, and the relationship between bacteremia in cats and their ectoparasites.

2. Materials And Methods

2.1. Cat sample

The study protocol was approved by the animal use ethics committee at Universidade Federal Rural do Rio de Janeiro under process number 027/2014.

The survey was carried out in six shelters in the Metropolitan Region of Rio de Janeiro State, Brazil, from September 2014 through September 2015. After obtaining shelter owner's permission, approximately 2 mL of blood was aseptically obtained from cats by cephalic phlebotomy, transferred into sterile tubes containing the anticoagulant ethylenediamine tetraacetic acid, and maintained at -80°C until used for molecular analysis.

2.2. Ectoparasite collection and identification

Ectoparasites were retrieved manually from the cats, placed into dry sterile tubes, and stored at -20°C until used. All ectoparasites were morphologically identified to genus or species based on morphological criteria observed with a stereoscopic microscope, according to standard taxonomic keys [11–13].

2.3. Molecular detection

Fleas and ticks were individually packed in separate tubes. And lice were separated in pairs or trio per tube. Some fleas laid eggs inside the tubes, permitting their separation into a single pool per specimen for DNA extraction, as described in literature [14]. Briefly, each flea or tick and pool of flea eggs or lice was ground in 40 μL of TE buffer (10 mM Tris-HCl; 0.5 mM EDTA; pH 9.0) in sterile micro tubes. The final suspension was boiled at 100°C for 30 minutes and maintained at -20°C until tested by PCR. For cats, DNA was extracted from 200 μL of whole blood sample using Kit Relia PrepTM Blood gDNA Miniprep System (Promega®), according to the manufacturer's instructions. For quality assurance, a negative control was processed at the same time as the study samples.

All DNA samples were screened for the presence of *Bartonella* spp. 16S-23S rRNA intergenic spacer region (ITS) using primers 321s and 983as [15], and citrate synthase gene (*gltA*) using primers BhCS.781p and BhCS.1137n [16]. Following amplification, PCR products were subjected to horizontal electrophoresis on 1% agarose gel and stained with GelRed (Biothium, CA, USA). The positive control consisted of *B. henselae* (Houston strain) cultured in HEp-2 cells. All PCR runs were performed with nuclease-free water (Invitrogen, USA) as negative control. In order to prevent PCR contamination, DNA extraction, reaction setup, PCR amplification, and electrophoresis were performed in separated rooms.

Positives flea and cat samples were randomly selected and purified using the Illustra GFX PCR Purification Kit (GE Healthcare, Buckinghamshire England, UK). Purified DNA fragments were submitted to sequence confirmation in an automatic sequencer (ABI3730xl, Applied Biosystem, CA, USA). Sense and antisense sequences were analyzed using DNA Sequence Assembler version 4 software and compared with those deposited in the GenBank DNA database using the Basic Local Alignment Search Tool (BLAST, National Center for Biotechnology Information, available at <https://blast.ncbi.nlm.nih.gov>). The phylogenetic reconstruction was inferred using the Maximum Likelihood method. The nucleotide substitution models were selected based on Bayesian Information Criterion (BIC scores) and Tamura 3-parameter model was used to calculate the evolutionary distances. The combination of phylogenetic

clusters was assessed using a bootstrap test with 1000 replicates to test different phylogenetic reconstructions. The phylogenetic valuation was conducted using the Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0.18.

2.4. Statistical analysis

The relationship between *Bartonella* infection status of cats and their fleas was evaluated by Chi-square test and association between them was expressed as Odds Ratio (OR) at 95% confidence interval. All analysis were implemented using the Bioestat 5.0 statistical software.

3. Results

A total of 115 fleas, 21 lice and one tick were collected from 46 cats. All flea and lice specimens were identified as *Ctenocephalides felis* and *Felicola subrostratus* adults, respectively. The tick specimen was identified as a *Rhipicephalus sanguineus* nymph. All sampled cats presented ectoparasites on their bodies. On average, approximately three fleas or lice were collected per cat (range 1–9 fleas and 2–4 lice).

Overall, 47.8% (22/46) of cats tested positive for *Bartonella* DNA according to both ITS and *gltA* gene (Table 1). Sequencing confirmed *Bartonella henselae*, *Bartonella clarridgeiae*, and *Bartonella koehlerae* infection among the blood samples. Cases in which ITS and *gltA* sequences from a single same cat corresponded to different feline *Bartonella* species were considered coinfections.

Table 1
Prevalence of *Bartonella* DNA in cats and their ectoparasites in shelters, Rio de Janeiro, Brazil

Cats					
Ectoparasites					
Flea					
DNA samples	Blood	Adults	Eggs*	Lice*	Ticks
Total of samples	46	115	15	8	1
Total PCR positive no.(%)	22 (47.8)	21 (18.3)	2 (13.3)	1(12.5)	0 (0.0)
<i>gltA</i> positive no.(%)	21 (45.7)	18 (15.7)	2 (13.3)	1(12.5)	0 (0.0)
ITS positive no.(%)	12 (26.1)	5 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)
*Number of pools					
<p><i>Bartonella</i> DNA was detected in 18.3% (21/115) of <i>C. felis</i> fleas, of which 15.7% (18/115) were by means of the <i>gltA</i> gene and 4.3% (5/115) by the ITS region. Bacterial DNA was amplified for both ITS and <i>gltA</i> fragments in three samples. Among 15 pools of eggs laid by fleas and 8 pools of lice, 13.3% and 12.5% showed amplification of the expected <i>Bartonella</i> spp. <i>gltA</i> gene, respectively. <i>Bartonella henselae</i> DNA was detected in cat fleas and their respective eggs, while <i>Bartonella clarridgeiae</i> DNA was only identified in fleas. No eggs or lice tested positive for the ITS region. No amplification of <i>Bartonella</i> DNA was obtained in the <i>Rhipicephalus sanguineus</i> nymph. <i>Bartonella henselae</i> was the predominant species in both fleas and cats (Table 2).</p>					

Table 2
Bartonella species in cats and their fleas (99 to 100% identities), Rio de Janeiro, Brazil

		Ectoparasites	
		Flea	
Shelter 1	Host cat	Adult	Eggs
211	<i>B. henselae</i>	<i>B. henselae</i>	<i>B. henselae</i>
208	Negative	*	Negative
76	*	*	Negative
Shelter 2			
175	<i>B. clarridgeiae</i>	<i>B. henselae</i>	<i>B. henselae</i>
171	<i>B. koehlerae</i>	*	Negative
168	<i>B. henselae</i>	Negative	Negative
172	<i>B. henselae</i>	Negative	Negative
178	*	Negative	Negative
180	Negative	*	Negative
173	Negative	*	Negative
Shelter 3			
205	<i>B. clarridgeiae</i>	*	Negative
207	<i>B. clarridgeiae</i>	*	Negative
201	<i>B. clarridgeiae</i> + <i>B. henselae</i>	Negative	Negative
206	Negative	<i>B. clarridgeiae</i>	Negative
202	<i>B. clarridgeiae</i>	Negative	Negative
Shelter 4			
117	<i>B. henselae</i>	<i>B. henselae</i>	Negative
116	<i>B. henselae</i>	*	Negative
Shelter 5			
132	Negative	<i>B. clarridgeiae</i>	Negative
131	<i>B. clarridgeiae</i>	Negative	Negative

*Positive sample, but showing weak bands whose DNA concentration was too low to be sequenced.

Ectoparasites			
129	<i>B. henselae</i>	Negative	Negative
Shelter 6			
193	<i>B. henselae</i>	Negative	Negative
191	*	<i>B. henselae</i>	Negative
195	<i>B. henselae</i>	Negative	Negative
200	<i>B. henselae</i>	Negative	Negative
198	Negative	*	Negative
*Positive sample, but showing weak bands whose DNA concentration was too low to be sequenced.			

At least one *Bartonella* species was detected in fleas in each shelter. Additionally, all bacteria species detected in each shelter's fleas were also identified in at least one of its cats. *Bartonella* spp. was also amplified from fleas belonging to apparently uninfected cats and from bacteremic cats infested by negative fleas. Not all fleas had the same *Bartonella* species as their hosts. Two of three fleas collected from infected cats carried the same *Bartonella* species as their cat hosts (Table 2). Whereas 60% (9/15) of cats infested by positive ectoparasites was bacteremic, the prevalence was only 40% (10/25) in those infested by negative ectoparasites. Although cats infested by positive ectoparasites, especially fleas, had more than twice the chance of being infected, there was no statistical correlation between cats' bacteremia status and parasitism by positive fleas (Table 3).

Table 3
Statistical analysis of *Bartonella* infection status of cat hosts and fleas, Rio de Janeiro, Brazil

		Cat blood		p-value	Odds ratio	CI (95%)
		Positive	Negative			
Fleas	Positive	9 (60.0) ^a	6 (40.0)	0.3685	2.25	0.61–8.31
	Negative	10 (40.0) ^a	15 (60.0)			
^a Same letters in the same column did not differ by Chi-square test at 5% significance.						

All sequences showed 99 to 100% homology with *B. henselae*, *B. clarridgeiae* and *B. koehlerae* reference sequences. Sequences were deposited into Genbank under accession no.: MT112180 - MT112197 and MT095045 - MT095055 for *gltA* gene and ITS region, respectively (Fig. 1; 2).

4. Discussion

One Health is an initiative aiming to bring together human, animal, and environmental health and plays a significant role in zoonosis prevention and control [17]. The increasingly close health relationship between humans and their domestic animals, especially cats, is conspicuous. According to the Brazilian Association of Pet Products Industry, the cat population has shown an accelerated annual growth in Brazil. Based on these facts, zoonosis studies have become ever more important. From a public health perspective, cats are a major reservoir host for at least three zoonotic *Bartonella* species (*B. henselae*, *B. clarridgeiae*, and *B. koehlerae*) and they are commonly infested by *C. felis* fleas, which also represent the great majority of fleas observed in peoples' homes [18]. To the best of our knowledge, this is the first study in Brazil to investigate *Bartonella* DNA in shelter cats and their ectoparasites.

The overall prevalence of *Bartonella* DNA was 47.5% in cat blood, 18.3% in fleas, 13.3% in flea egg pools, and 12.5% in lice pools. The occurrence of *Bartonella* DNA was higher than previously reported, especially in shelter cats [19–25]. However, a previous study reported 97.3% positivity in one shelter in Rio de Janeiro, Brazil [26]. Risk factors which appear to influence the occurrence of bacteremia in cats includes age, flea infestation status, neutering status, historic of fights, outdoor access and multiple cat households [25, 27–29]. Flea infestation status is particularly important as all cats in this study had ectoparasites on their body surface. *Bartonella* DNA detected in *C. felis* fleas varies worldwide, with prevalence ranging from 7.3–75.6% [10, 20, 30–32]. *Bartonella* DNA was not detected in *R. sanguineus* ticks or *F. subrostratus* lice collected from shelter cats in Taiwan [30].

This study confirmed *Bartonella henselae*, *Bartonella clarridgeiae*, and *Bartonella koehlerae* single infection as well as coinfection by *B. henselae* and *B. clarridgeiae* in feline blood samples. Occurrence of concurrent infection by two or more *Bartonella* species are uncommon in the literature, being documented in low percentages of cats or being absent [5, 31, 33]. The *Bartonella* species encountered in the present study in cat fleas (*B. henselae* and *B. clarridgeiae*) have also been detected in cat fleas in previous studies [20, 34–36].

In all shelters, *Bartonella* spp. were detected in fleas and their hosts. In one shelter, the *Bartonella* species detected in fleas and their eggs were different from those in their respective hosts. However, the discrepant bacteria species was detected in other cats sharing the same environment. For such non coincident cases, it is possible that fleas previously fed on different bacteremic cats from the one on which they were collected.

Interestingly, bacteria DNA was detected in fleas collected from non-bacteremic hosts and detected in cats harboring negative fleas. It is noteworthy that in this study, fleas collected from cats represent a sample of the real flea population present on cats and in the local environment. Thus, newly emerged or not yet infected fleas may have been collected. Examples of different bacterial species in fleas and cat hosts has been documented previously [31, 36–39].

The presence of *Bartonella* DNA in *C. felis* fleas collected in bacteremic cats also suggests that these ectoparasites play an essential role in the transmission of *Bartonella* species to cats. A study has shown *C. felis* to be a potential vector for *Bartonella* species including those for which cats serve as natural reservoir, such as *B. henselae* and *B. clarridgeiae* [40]. In fact, there is a positive correlation between previous or current flea infestation and *Bartonella* bacteremia in shelter cats [25]. Although there was no statistical association, cats infested by fleas have at least twice the chance of becoming infected by *Bartonella* species. Similarly, previous studies found no apparent correlation [37, 39].

To the best of the authors' knowledge, *Bartonella* DNA was detected for the first time in lice collected from bacteremic cat. This cat was infested with lice at the time of collection, had historic of flea infestation and lived at shelter 6, where *Bartonella* species DNA was also detected in fleas and contactant cats. Considering the flea importance on *Bartonella* transmission between cats [9, 25] and the absent link of lice infestation to *Bartonella* infection in cats in this and previous studies [25, 30], it is necessary to be cautious to infer that the louse was responsible for the transmission of bacteria to the cat in the present study. In order to analyse the importance of the lice as a vector of *Bartonella* spp. to cats, one must invest on further studies.

The possibility of vertical *Bartonella* spp. transmission among fleas remains as a hypothesis. In our study, *B. henselae* DNA was detected in naturally infected fleas and their respective eggs. Consistent with our findings, *Bartonella* DNA was detected in reproductive tissues (ovary) of flea species collected from several mammals, suggesting that transovarian transmission of this organism among fleas may be possible [41]. However, no *Bartonella* DNA was amplified in eggs laid by infected fleas, but the authors concluded that their results cannot be extended to natural conditions [40]. Knowledge of *Bartonella* behavior and dispersal in fleas is limited and the question of whether fleas can acquire *Bartonella* by mechanisms other than ingestion of infected blood remains. Additional studies are needed to validate the vertical transmission hypothesis. According to a study evaluating ticks as a possible bacterial vector, transovarian transmission was not supported because no bacterial amplification was obtained in larvae even though *B. henselae* DNA was detected in the respective egg pools laid by female ticks that had fed on infected blood [42].

This study shows that three distinct *Bartonella* species occur in shelter cats in metropolitan region of Rio de Janeiro and that *B. henselae* and *B. clarridgeiae* circulate among fleas collected from them, reinforcing the importance of this ectoparasite in bacterial transmission between cats. For public health purposes, it is important to note that *Bartonella* species identified in ectoparasites and their host cats are agents associated with human disease. Thus, ectoparasites control measures should be implemented to prevent flea infestation and, consequently, *Bartonella* infection in cats and the humans with whom they have close contact.

Declarations

5. Consent for publication

Not applicable.

6. Competing interests

The authors declare that they have no competing interests.

7. Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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9. Author's contributions

JMR is the first author, conducted the experiments, analyzed all data and wrote the manuscript. AG performed data collection and helped in phylogenetical analysis. GMA, ATS and CJCR contributed in blood sample collection. HAS and ERS� provided access to biology molecular equipment. ARMF is the co-advisor, provided access to biology molecular equipment and helped to draft the paper. CBD is the advisor, conceived of the study, participated in its design and coordination and helped to draft the paper. All authors read, revised and approved the final manuscript.

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References

1. Regier Y, O'Rourke F, Kempf VAJ. *Bartonella* spp. - a chance to establish One Health concepts in veterinary and human medicine. *Parasit Vectors*. 2016;9:261. doi: 10.1186/s13071-016-1546-x.
2. Baldani CD, Santos HA, Massard CL. The Family Bartonellaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*. 4th ed. London: Springer-Verlag Berlin Heidelberg; 2014. pp. 81–114.
3. Varanat M, Travis A, Lee W, Maggi RG, Bissett SA, Linder KE, et al. Recurrent osteomyelitis in a cat due to infection with *Bartonella vinsonii* subsp. *berkhoffii* genotype II. *J Vet Intern Med*. 2009;23:1273–77. doi:10.1111/j.1939-1676.2009.0372.x.
4. Chomel BB, Kasten RW. Bartonellosis, an increasingly recognized zoonosis. *J Appl Microbiol*. 2010;109:743–50. doi:10.1111/j.1365-2672.2010.04679.x.

5. Gil H, Escudero R, Pons I, Rodríguez-Vargas M, García-Esteban C, Rodríguez-Moreno I, et al. Distribution of *Bartonella henselae* variants in patients, reservoir hosts and vectors in Spain. PLoS One. 2013;8(7):e68248. doi:10.1371/journal.pone.0068248.
6. Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. Vet Res. 2005;36:383–410. doi:10.1051/vetres:2005009.
7. Chomel BB, Kasten RW, Floyd-Hawkins K, Chi B, Yamamoto K, Roberts-Wilson J, et al. Experimental transmission of *Bartonella henselae* by the cat flea. J Clin Microbiol. 1996;34(8):1952–6. PMID: 8818889.
8. Kordick DL, Brown TT, Shin K, Breitschwerdt EB. Clinical and pathologic evaluation of chronic *Bartonella henselae* or *Bartonella clarridgeiae* infection in cats. J Clin Microbiol. 1999;37(5):1536–47. PMID: 10203518.
9. Guptill L. *Bartonella* infections in cats: what is the significance? Pract. 2012;34:434–45. doi:10.1136/inp.e5704.
10. Rolain JM, Franc M, Davoust B, Raoult D. Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas. France Emerg Infect Dis. 2003;9(3):338–42. doi:10.3201/eid0903.020278.
11. Linardi PM, Santos JLC. *Ctenocephalides felis felis* vs. *Ctenocephalides canis* (Siphonaptera: Pulicidae): some issues in correctly identify these species. Rev Bras Parasitol Vet. 2012;21(4):345–54. doi:10.1590/s1984-29612012000400002.
12. Barros-Battesti DM, Arzua M, Bechara GH. Carrapatos de importância médico-veterinária da Região Neotropical: Um guia ilustrado para identificação de espécies. São Paulo: International Consortium on Ticks and Tick-borne Diseases, Butantan; 2006.223pp.
13. Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. Parasitologia veterinária. 2 ed. Rio de Janeiro, Guanabara Koogan, 1998. 304 pp.
14. Horta MC, Labruna MB, Pinter A, Linardi PM. *Rickettsia* infection in five areas of the state of São Paulo, Brazil. Mem Inst Oswaldo Cruz. 2007;102:793–801. doi:10.1590/s0074-02762007000700003.
15. Maggie RG, Breitschwerdt EB. Potential limitations of the 16S-23S rRNA intergenic region for molecular detection of *Bartonella* species. J Clin Microbiol. 2005;43:1171–76. doi:10.1128/JCM.43.3.1171-1176.2005.
16. Norman AF, Regnery R, Jameson P, Greene C, Krause DC. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol. 1995;33(7):1797–803. PMID: 7545181.
17. Bidaisee S, Macpherson C. Zoonoses and One Health: A Review of the Literature. J Parasitol Res. 2014;2014:874345. doi:10.1155/2014/874345.
18. Bitam I, Dittmar K, Parola P, Whiting MF, Raoult D. Fleas and flea-borne diseases. Int J Infect Dis. 2010;14:e667–76. doi:10.1016/j.ijid.2009.11.011.

19. Bergmans AMC, Jong CMA, Amerongen GV, Schot ACS, Schouls LM. Prevalence of *Bartonella* species in domestic cats in the Netherlands. J Clin Microbiol. 1997;35(9):2256–61. PMID: 9276397.
20. Alves AS, Milhano N, Santos-Silva M, Santos AS, Vilhena M, de Sousa R. Evidence of *Bartonella* spp., *Rickettsia* spp. and *Anaplasma phagocytophilum* in domestic, shelter and stray cat blood and fleas, Portugal. Clin Microbiol Infect. 2009;15:1–3. doi: 10.1111/j.1469-0691.2008.02636.x
21. Namekata DY, Kasten RW, Boman DA, Straub MH, Siperstein-Cook L, Couvalaire K, et al. Oral shedding of *Bartonella* in cats: Correlation with bacteremia and seropositivity. Vet Microbiol. 2010;146:371–75. doi:10.1016/j.vetmic.2010.05.034.
22. Staggemeier R, Venker CA, Klein DH, Petry M, Spilki FR, Cantarelli VV. Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in cats in the south of Brazil: a molecular study. Mem Inst Oswaldo Cruz. 2010;105(7):873–78. doi:10.1590/s0074-02762010000700006.
23. Miceli NG, Gavioli FA, Gonçalves LR, André MR, Sousa VRF, Sousa KCM, et al. Molecular detection of feline arthropod-borne pathogens in cats in Cuiabá, state of Mato Grosso, central-western region of Brazil. Rev Bras Parasitol Vet. 2013;22(3):385–90. doi:10.1590/S1984-29612013000300011.
24. Braga IA, Dias ISO, Chitarra CS, Amude AM, Aguiar DM. Molecular detection of *Bartonella clarridgeiae* in domestic cats from Midwest Brazil. Braz J. Infect Dis. 2015;19(4):451–52. doi: 10.1016/j.bjid.2015.05.002.
25. Raimundo JM, Guimarães A, Amaro GM, Silva AT, Botelho CFM, Massard CL, et al. Molecular survey of *Bartonella* species in shelter cats in Rio de Janeiro: clinical, hematological, and risk factors. Am J Trop Med Hyg. 2019;100(6):1321–27. doi:10.4269/ajtmh.18-0585.
26. Souza AM, Almeida DNP, Guterres A, Gomes R, Favacho ARM, Moreira NS, et al. Bartonelose: análise molecular e sorológica em gatos do Rio de Janeiro – Brasil. Rev Bras Ciências Vet. 2010;17(1):7–11. <http://dx.doi.org/10.4322/rbcv.2014.135>.
27. Chomel BB, Abbott RC, Kasten RW, Floyd-Hawkins KA, Kass PH, Glaser CA, et al. *Bartonella henselae* prevalence in domestic cats in California: risk factors and association between bacteremia and antibody titers. J Clin Microbiol. 1995;3(9):2445–50. PMID: 7494043.
28. Gurfield AN, Boulouis HJ, Chomel BB, Kasten RW, Heller R, Bouillin C, et al. Epidemiology of *Bartonella* infection in domestic cats in France. Vet Microbiol. 2001;80:185–98. doi:10.1016/s0378-1135(01)00304-2.
29. Mazurek L, Carbonero A, Skrzypczak M, Winiarczyk S, Adaszek L. Epizootic situation of feline *Bartonella* infection in eastern Poland. J Vet Res. 2020; 64. doi:10.2478/jvetres-2020-0019.
30. Tsai Y, Lin CC, Chomel BB, Chuang ST, Tsai KH, Wu WJ, et al. *Bartonella* infection in shelter cats and dogs and their ectoparasites. Vector Borne Zoonotic Dis. 2011;11(8):1023–30. doi:10.1089/vbz.2010.0085.
31. Gutiérrez R, Nachum-Biala Y, Harrus S. Relationship between the presence of *Bartonella* species and bacterial loads in cats and cat fleas (*Ctenocephalides felis*) under natural conditions. Appl Environ Microbiol. 2015;81:5613–21. doi:10.1128/AEM.01370-15.

32. Rizzo MF, Billeter SA, Osikowicz L, Luna-Caipo DV, Cáceres AG. Fleas and flea-associated *Bartonella* species in dogs and cats from Peru. *J Med Entomol Soc Am*. 2015;52(6):1374–77. doi:10.1093/jme/tjv137.
33. André MR, Dumler JS, Herrera HM, Gonçalves LR, de Souza KCM, Scorpio DG, et al. Assessment of a quantitative 5' nuclease real-time polymerase chain reaction using the nicotinamide adenine dinucleotide dehydrogenase gamma subunit (nuoG) for *Bartonella* species in domiciled and stray cats in Brazil. *J Feline Med Surg*. 2015;18(10):783–90. doi:10.1177/1098612X15593787.
34. Assarasakorn S, Veir JK, Hawley JR, Brewer MM, Morris AK, Hill AE, et al. Prevalence of *Bartonella* species, hemoplasmas, and *Rickettsia felis* DNA in blood and fleas of cats in Bangkok, Thailand. *Res Vet Sci*. 2012;93:1213–16. doi:10.1016/j.rvsc.2012.03.015.
35. Rojas N, Troyo A, Castillo D, Gutierrez R, Harrus S. Molecular detection of *Bartonella* species in fleas collected from dogs and cats from Costa Rica. *Vector Borne Zoonotic Dis*. 2015;15(10):630–32. doi:10.1089/vbz.2015.1799.
36. Fontalvo MC, Favacho ARM, Araújo AC, Santos MN, Oliveira GMB, Aguiar DM, et al. *Bartonella* species pathogenic for humans infect pets, free-ranging wild mammals and their ectoparasites in the Caatinga biome, Northeastern Brazil: a serological and molecular study. *Braz J Infect Dis*. 2017;21(3):290–96. doi:10.1016/j.bjid.2017.02.002.
37. La Scola B, Davoust B, Boni M, Raoult D. Lack of correlation between *Bartonella* DNA detection within fleas, serological results, and results of blood culture in a *Bartonella*-infected stray cat population. *Clin Microbiol Infect*. 2002;8(6):345–51. doi:10.1046/j.1469-0691.2002.00434.x.
38. Gabriel MW, Henn J, Foley JE, Brown RN, Kasten RW, Brown RN, et al. Zoonotic *Bartonella* in fleas collected on gray foxes (*Urocyon cinereoargenteus*). *Vector Borne Zoonotic Dis*. 2009;9(6):597–602. doi:10.1089/vbz.2008.0134.
39. Bai Y, Rizzo MF, Alvarez D, Moran D, Peruski LF, Kosoy M. Coexistence of *Bartonella henselae* and *Bartonella clarridgeiae* in population of cats and their fleas in Guatemala. *J Vector Ecol*. 2015;40(2):327–32. doi:10.1111/jvec.12171.
40. Bouhsira E, Ferrandez Y, Liu M, Franc M, Boulouis HJ, Biville F. *Ctenocephalides felis* an in vitro potential vector for five *Bartonella* species. *Comp Immunol Microbiol Infect Dis*. 2013;36:105–11. doi:10.1016/j.cimid.2012.10.004.
41. Brinkerhoff RJ, Kabeya H, Inoue K, Bai Y, Maruyama S. Detection of multiple *Bartonella* species in digestive and reproductive tissues of fleas collected from sympatric mammals. *ISME J*. 2010;4:955–58. doi:10.1038/ismej.2010.22.
42. Cotté V, Bonnet S, Le Rhun D, Le Naour E, Chauvin A, Boulouis H-J, et al. Transmission of *Bartonella henselae* by *Ixodes ricinus*. *Emerg Infect Dis*. 2008;14(7):1074–80. doi:10.3201/eid1407.071110.

Figures

Figure 1

Phylogenetic relationship of *Bartonella* species detected in shelter cats and ectoparasites based on *gltA* gene. Phylogenetic position of *Bartonella* species isolates from shelter cats (●), cat fleas (▲) and cat flea eggs (Δ), Rio de Janeiro, Brazil. The phylogenetic tree was constructed using the Maximum (T92+G) and the numbers on the tree nodes indicate bootstrap values with 1000 replicates. Accession numbers are indicated. *Bartonella bacilliformis* was used as outgroup. Scale bar indicates nucleotide substitutions per site.

Figure 2

Phylogenetic relationship of *Bartonella* species detected in shelter cats and fleas based on ITS region. Phylogenetic position of *Bartonella* sp. isolates from shelter cats (●) and their fleas (▲), Rio de Janeiro, Brazil. The phylogenetic tree was constructed using the Maximum Likelihood (T92+I) and the numbers on the tree nodes indicate bootstrap values with 1000 replicates. Accession numbers are indicated. *Bartonella bacilliformis* was used as outgroup. Scale bar indicates nucleotide substitutions per site.

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