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Low occurrence of *Bartonella* in synanthropic mammals and associated ectoparasites in peri-urban areas from Central-Western and Southern Brazil

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ABSTRACT

Worldwide, *Bartonella* species are known to infect a wide range of mammalian and arthropod hosts, including humans. The current study aimed to investigate the prevalence of *Bartonella* spp. in synanthropic mammals captured in peri-urban areas from Central-Western and Southern Brazil and their ectoparasites. For this aim, 160 mammals belonging to four species, and 218 associated arthropods were sampled. DNA was extracted and subjected to different *Bartonella* screening assays. Additionally, blood samples from 48 small rodents were submitted to liquid BAPGM culture followed by qPCR assay and solid culture. Two out of 55 *Rattus* captured in Santa Catarina state were PCR-positive for *Bartonella* when targeting the *nuoG*, 16S, and ITS loci. Sequences showed high homology with *Bartonella coopersplainsensis*. Conversely, all 48 small rodents, 14 capybaras and 43 opossum DNA samples from animals trapped in Mato Grosso do Sul were *Bartonella* negative in the HRM real time PCR assays targeting the ITS locus and *gltA* gene. Additionally, all mammal-associated ectoparasites showed negativity results based on HRM real time PCR assays. The present study showed, for the first time, the occurrence of *B. coopersplainsensis* in Brazil, shedding some light on the distribution of rats-related *Bartonella* in South America. In addition, the majority of rodents and marsupials were negative for *Bartonella* spp. Since *B. coopersplainsensis* reservoirs - *Rattus* spp. - are widely dispersed around the globe, their zoonotic potential should be further investigated.

1. Introduction

Distinct biotic and abiotic features, such as habitat fragmentation, ectoparasites richness, host density and climate conditions, could affect pathogen transmission dynamics (Greer and Collins, 2007). Furthermore, the absence or the low prevalence of some pathogens in a specific population can be attributed to different factors, including but not limited to a small number of tested animals, refractory hosts, or, in particular cases when a population is established, by invasion/introduction of pathogen-free individuals in a new area (Kosoy and Bai, 2019).

Bartonella species are vector-borne Gram-negative intracellular facultative bacteria that infect a wide range of mammalian and ectoparasite hosts. These bacteria comprise a group showing distinct host specificity, distribution, pathogenesis and genetic diversity features (Arvand et al., 2010; Harms and Dehio, 2012; Mckee et al., 2016;

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Harms et al., 2017; Gutiérrez et al., 2018).

The prevalence of *Bartonella* in rats (*Rattus* sp.), a rodent genus broadly dispersed around the globe, varies widely across different studies, and sampling sites and even among *Rattus* species in a same study (Kosoy et al., 2019). Ecological factors including landscape features, structure population, temperature, ectoparasites richness and sampling zone have been attributed to distinct rates of *Bartonella* infection in rats (Klangthong et al., 2015; Halliday et al., 2015; Peterson et al., 2017; Abreu-Yanes et al., 2018).

Unlike *Bartonella* species and genotypes isolated from *Rattus* genus that are usually highly *Rattus* specific (Buffet et al., 2013; Kosoy et al., 2019), wild rodents species harbor a great diversity of *Bartonella* species (Buffet et al., 2013; Gutiérrez et al., 2015a).

As opposed to rodents, only a few studies have been performed aiming the molecular detection or isolation of *Bartonella* in marsupials. To date, *Bartonella* was only isolated from *M*acropus giganteus from Australia (Fournier et al., 2007). In addition, Bartonella DNA was detected in fleas and ticks collected from marsupials belonging to Bettongia penicillata and Perameles bougainville species from Australia, (Kaewmongkol et al., 2011). Moreover, cat-related Bartonella spp. have been molecularly detected in *Ctenocephalides felis* from an opossum (*Didelphis virginiana*) from the USA (Reeves et al., 2005; Nelder et al., 2009).

In Brazil, limited studies have been carried out addressing the detection and isolation of Bartonella in synanthropic small mammals. For instance, rat-related Bartonella spp. were isolated from five out of 26 (19%) R. norvegicus sampled in Salvador, Northeast Brazil (Costa et al., 2014). Additionally, Bartonella DNA was detected in two out of 29 R. rattus (6.8%) sampled from four Brazilian states (Gonçalves et al., 2016). Concerning wild rodents, a Bartonella species phylogenetic related to the Bartonella vinsonii complex (Kosoy et al., 2012) has been detected in Cricetidae rodents from 12 Brazilian states (Favacho et al., 2015; Gonçalves et al., 2016; Rozental et al., 2017; de Sousa et al., 2018).

Only two studies have been performed aiming to detect *Bartonella* DNA in marsupials from Brazil. In both studies, none of the 68 marsupials' specimens from four different species were positive for *Bartonella* DNA (Fontalvo et al., 2017; de Sousa et al., 2018).

Since rodent-related Bartonella comprise some zoonotic species coupled with few studies targeting Bartonella in marsupials, we investigate here the presence of this bacterial group in synanthropic mammals and associated ectoparasites sampled in localities in Central-Western and Southern Brazil.

2. Materials and methods

2.1. Sampling sites, mammals capture and biological samples collection

Between September 2016 and August 2018, 160 mammal specimens belonging to four different species were sampled in distinct sites of Campo Grande municipality (-20^0 42' 30" S, -54^0 61' 60" W), Mato Grosso do Sul state (MS), Central-Western Brazil, and Três Barras city (26° 8' 42" S, 50° 22' 53" W), Santa Catarina state (SC), Southern Brazil (**Table 1**). In Campo Grande municipality, 48 small rodents (*Rattus rattus* [n = 39] and *Mus musculus* [n =9]) were trapped in urban area (4 sites) and urban forest fragments (4 sites). Additionally, 14 capybaras (*Hydrochoerus hydrochaeris*), and 43 marsupials (*Didelphis albiventris*) were trapped in three and six urban forest fragments, respectively, in Campo Grande city. Additionally, 55 black rats (*R. rattus*) were captured in thirteen urban sites in Três Barras city, SC (**Fig. 1**).

All capture procedures were performed as previously described (Nantes et al., 2019; Gonçalves et al., 2020a). Briefly, the small mammals were captured using Tomahawk (45 × 17,5 × 15 cm) and Sherman (42 × 11,5 × 14 cm) live traps baited with a mix of bananas, paçoca, oat flakes and tinned sardines. The small rodents were chemically immobilized using a combination of ketamine hydrochloride (100 mg/mL) and acepromazine (10 mg/mL) (1:9) intramuscularly. When the death of anesthetized small rodents did not occur after exsanguination, the euthanasia was performed through intracardiac injection of 19.1% potassium chloride (2 ml/kg). Thereafter blood and/or spleen fragments were collected under sterile conditions. On the other hand, marsupials were anesthetized with a chemical association of Ketamine (20 mg/kg) and Xylazine (2 mg/kg) intramuscularly. Blood samples were collected from the marsupials' lateral caudal veins and placed to DNase and RNase-free anticoagulant ethylenediaminetetraacetic acid (EDTA)-containing microtubes. Finally, after chemical immobilization using an anesthetic dart containing TELAZOL® 100 (4mg/kg), blood samples were collected from the capybaras' femoral vein into EDTA-buffered vacutainer tubes (Gonçalves et al., 2020a). Except for the small rodents trapped in Campo Grande, for which blood samples were kept at -80°C for *Bartonella* culture, all other samples (blood or spleen) were kept on ice until arrival to the laboratory and stored at -20°C until DNA extraction. The following data were recorded from each animal: the presence of ectoparasites, gender, weight, and sampling point.

All sampled animals were checked for the presence of ectoparasites. Once collected, the ectoparasites were placed in microtubes containing absolute ethanol (Merck®) and maintained at -20°C until morphological identification and DNA extraction. The morphological identification was performed using previously described taxonomic keys (Onofrio et al., 2005; Martins et al., 2010; Linardi et al., 2014; Anholt et al., 2014; Pereira et al., 2017).

All animal captures were in accordance with the licenses obtained from the Instituto Chico Mendes de Conservação da Biodiversidade (license number 56912-2), Imasul (license number 001/2017) and endorsed by the Ethics Committee of FCAV/UNESP and Contestado Universities under the numbers: 01952/18 and 15/16.

2.2. Bartonella isolation

The 48 blood samples obtained from the small rodents trapped in Campo Grande municipality were taken to the laboratory for bacterial culturing. Initially, the blood samples were subjected to a pre-enrichment culture as previously described (Maggi et al., 2005; Duncan et al., 2007). Briefly, the EDTA-anticoagulated blood samples were thawed at room temperature and an aliquot of 200 µL was inoculated into filter cap cell culture flasks (Corning®) containing 2 mL of liquid *Bartonella* alphaproteobacterium growth medium (BAPGM – pH 6.2) supplemented with 10% of defibrinated sheep blood. Sheep blood samples were confirmed to be *Bartonella*-negative using a previously described qPCR assay (André et al., 2016). The flasks were incubated for 7 days at 37°C in 5% CO_2 in a water-saturated atmosphere and maintained under a constant shaking motion. A negative control flask (only liquid culture) was prepared and incubated simultaneously with all blood samples. After 7 days of incubation an aliquot of 500 µL was submitted to DNA extraction using a commercial kit (InstaGene Matrix - BIORAD®) followed by a *Bartonella* screening using the qPCR assay targeting the *nuoG* gene describe elsewhere (André et al., 2016). In addition, 300 µL aliquot was sub-inoculated onto an agar chocolate plate, which was maintained in incubation as described above during four weeks. The plates were examined twice a week for the presence of *Bartonella*-like colonies. If *Bartonella*-like colonies were observed, the colonies were submitted to DNA extraction, qPCR confirmation (André et al., 2016), conventional PCR targeting the *gltA* gene (Birtles and Raoult, 1996) and sequencing.

2.3. DNA extraction and quality

DNA was extracted from ten mg of each small rodent spleen tissue, and from 200 µL of each blood sample from capybaras and marsupials, using the DNeasy® Blood & Tissue Kit (Qiagen®, Valencia, California, USA), according to manufacturer's instructions. Furthermore, the

Table 1
Number and animal species positive to *Bartonella*.

Site	Animal species	Sample type	N^0 of sampled animals	Occurrence of *Bartonella* % (N^0)	Ectoparasite species	N^0 of sampled ectoparasites	Occurrence of *Bartonella* % (N^0)
	Mammals				Arthropods		
Santa Catarina	Rodentia						
	R. rattus	DNA from spleen tissues	55	3.6% (2/55)	*Tunga caecata*	2	Not tested
					Notoedres muris	1	Not tested
					Myocoptes sp.	1	Not tested
Campo Grande	Rodentia						
	R. rattus	DNA from spleen tissues and liquid culture	39	0% (0/39)	*Amblyomma* sp.[a]	62	0% (0/9)[b]
					Polyplax spinulosa	6	0% (0/2)[b]
	M. musculus	DNA from spleen tissues and liquid culture	9	0% (0/9)	-	-	-
	H. hydrochaeris	DNA from whole blood	14	0% (0/14)	*A. dubitatum*	42	0% (0/42)
					A. sculptum	36	0% (0/33)[c]
					Amblyomma sp.[a]	2	0% (0/2)
	Didelphimorphia						
	D. albiventris	DNA from whole blood	43	0% (0/43)	*A. dubitatum*	70	0% (0/28)[b]

[a] *Amblyomma* sp. refers to larvae sampled – In these specimens only the genus was reported.
[b] Ectoparasites-DNA pool samples.
[c] Three out of 36 *Amblyomma*-DNA samples were negative to the endogenous control (16S rRNA).

collected ectoparasites were submitted to DNA extraction individually and/or in pools (tick nymphs were pooled up to three individuals, larvae up to seven individuals) from the same host. The lice were pooled up to two specimens from the same host, using the commercial kit mentioned above.

In order to discard the presence of PCR inhibitors, all extracted mammal DNA samples were used as a template in an internal control PCR targeting the mammal *gapdh* gene as previously described (Birkenheuer et al., 2003). Likewise, all arthropod DNA samples were submitted to internal control targeting the 16S rRNA as previously described (Black and Piesman, 1994). Internal control-PCR positive samples were subsequently submitted to *Bartonella* screening High Resolution Melt (HRM) real-time PCR assays targeting the ITS locus and gltA gene. Additionally, DNA samples from the rodents trapped in SC were screened using a qPCR assay targeting the nuoG gene.

2.4. Molecular detection of Bartonella DNA in mammals and associated ectoparasites from Campo Grande city, MS

Initially, DNA samples were screened for *Bartonella* DNA using an HRM real-time PCR assay targeting a fragment of approximately 200 bp of the 16S–23S internal transcribed spacer (ITS) locus, as previously described (Maggi and Breitschwerdt, 2005; Gutiérrez et al., 2013). To confirm the results, all DNA samples were submitted to an additional

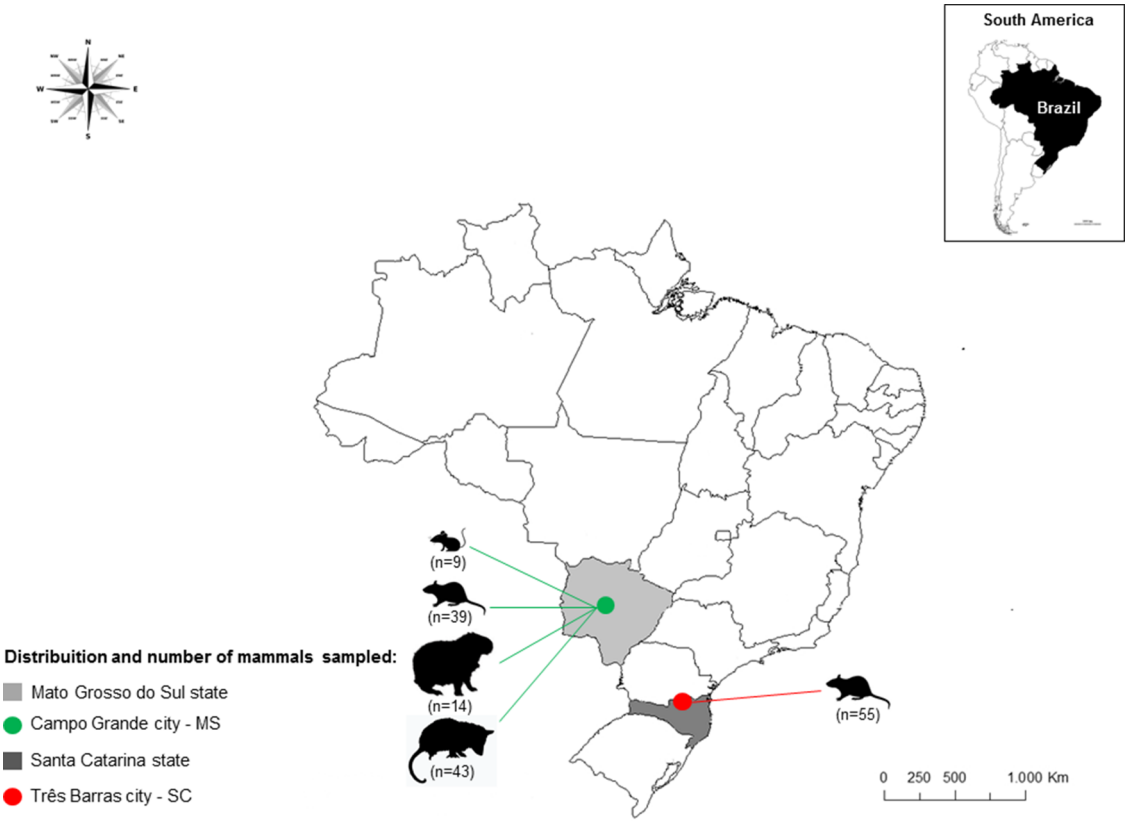

Fig. 1. Sampling sites, number and distribution of mammals sampled in Campo Grande, MS and Três Barras, SC, Brazil.

HRM real-time PCR assay targeting the *gltA* gene (approx. 350 bp), as previously described (Sofer et al., 2015). Briefly, the amplification reaction was performed using the StepOnePlus (Applied Biosystems) real-time system. The amplification protocol used was as follows: 3 min at 95°C, followed by 40 cycles of 30 s at 95°C, 20 s at 65°C for both targets (data collection on HRM reporter), and 5 s at 72°C. The HRM stage was performed at the end of the cycling as follows: 15 s at 95°C, followed by a temperature increase from 70 to 95°C (data collection set in 0.3%, HRM reporter). PCR was carried out in 20 μL reaction volumes containing 0.5 μL of 10 mM of each primer, 0.6 μL of 50 μM solution of Syto9 (Invitrogen®, CA, US), 10 μL of Dream Taq Hot Start PCR Master Mix (Thermo Fisher Scientific®, San Jose, CA, USA), 6.4 μL ultrapure PCR water (Thermo Fisher Scientific®, San Jose, CA, USA), and 2 μL of DNA. DNA of *Bartonella krasnovii* (Gutiérrez et al., 2020) and ultra-pure water were used as positive and non-template controls, respectively, in all real-time PCR assays,

2.5. Molecular detection and characterization of Bartonella DNA in rodents from Três Barras, SC

The quantification and screening for the *Bartonella* DNA was performed using a qPCR assay targeting a fragment of 83 bp of the *nuoG* gene as described elsewhere (André et al., 2016). Briefly, the qPCR assay was performed using the 10 μL PCR mixtures contained 5 μL of Go Taq® Probe qPCR Master Mix, dTTP (Promega) with a final concentration of 1,2 μM of each primer (F-Bart [5'-CAATCTTCTTTTGCTT CACC-3'] and R-Bart [5'- TCAGGGCTTTATGTGAATAC-3'] and hydrolysis probe (TexasRed-5'-TTYGTCATTTGAACACG-3'[BHQ2a-Q]-3') and 1 μL of DNA sample. The amplification conditions were used as follows: 95°C for 3 minutes followed by 40 cycles of 95°C for 10 minutes and 52.8°C for 30 seconds) (André et al., 2016). Serial dilutions were performed with the aim of constructing standard curves with different concentrations of plasmid DNA (pIDT Smart; Integrated DNA Technologies) (2.0×10^7 to $2.0 \times 10^{\circ}$ copies/μl).The number of plasmid copies was determined in accordance with the formula (x grams per microliter of DNA/[plasmid size (base pairs) x 660]) x 6.022×10^{23} x plasmid copies per microliter. Amplification efficiency (E) was calculated from the slope of the standard curve in each run using the formula $E = 10^{-1/\text{slope}}$.

The positive DNA samples in the molecular screening for *Bartonella* were submitted to additional PCR assays targeting the *gltA* (Birtles and Raoult, 1996), *rpoB* (Renesto et al., 2001), *nuoG* (Colborn et al., 2010), *groEL* (Zeaiter et al., 2002 and Paziewska et al., 2011), 16S rRNA (Dauga et al., 1996), and ITS (Diniz et al., 2007). *Bartonella bovis* DNA (Gonçalves et al., 2020b) and ultra-pure water were used as positive and non-template controls, respectively, in all PCR assays. Thereafter, the amplicons obtained were purified using the EXOSAP-IT® (Applied Biosystems). Purified amplified DNA fragments were submitted to sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyser – Applied Biosystem/ Perkin Elmer) (Sanger et al., 1997). Finally, consensus sequences were obtained through the analysis of electropherograms using the Phred-Phrap program with a Phred quality score (peaks around each base call) established at ≥20 (99% in the accuracy of the base call) (Ewing et al 1998).

3. Results

3.1. Ectoparasites and DNA extraction quality

Ectoparasites were found in 7.7% (8/103) of small rodents sampled in Campo Grande, MS and Três Barras, SC. Among them, 62 ticks (*Amblyomma* spp.), six lice (*Polyplax spinulosa*), two mites (one *Notoedres muris* and one *Myocoptes* sp.) and two fleas (*Tunga caecata* – including one specimen found in a *Bartonella*-positive rat - #27) were sampled. Also, 71.4% (10/14) of trapped capybaras in Campo Grande were infested with ticks (*Amblyomma* spp.). Lastly, ticks (*Amblyomma* spp.) were observed in 32.5% (14/43) of the sampled opossums. All ectoparasite species sampled are shown in **Table 1**.

Except for three tick-DNA samples obtained from capybaras, all arthropod and mammal DNA samples were positive for 16S rRNA and *gapdh* internal control PCR assays, respectively. The tick samples that were found negative for arthropod-16S rRNA by PCR were excluded from subsequent analyses.

3.2. Bartonella isolation

None of 48 rodent blood samples submitted to the liquid pre-enrichment culture were positive by the *Bartonella* qPCR assay. Likewise, no *Bartonella*-like colonies were observed on the agar chocolate plates during a period of four weeks of incubation.

3.3. Bartonella prevalence and BLASTn results

Two (#22 and #27) out of 55 rats (3.6%) sampled in SC were positive by the qPCR assay for *Bartonella* based on the *nuoG gene*. The #22 and #27 DNA samples showed absolute quantification of 4.7×10^1 and 3.1×10^1 copies/μL, respectively. On the other hand, all mammal and arthropod specimens trapped in MS were negative by the qPCR assays for *Bartonella* targeting the ITS locus and *gltA* gene. The efficiency mean of the qPCR assays was $E = 98.1\%$ ([ranging from 91.4% to 103.9%]; slope = -3.371; $r^2 = 0.997$).

The two *nuoG*-qPCR positive samples were confirmed positive by conventional PCR assays targeting the *nuoG* and 16S rRNA genes and ITS locus. While the #22 and #27 DNA samples were positive in all above referred PCR assays, the #27 DNA sample showed weak band intensity in *nuoG* and 16S assays, precluding its sequencing. Both ITS sequences shared 99.6% identity to *Bartonella coopersplainsensis* (accession number EU111770) isolated from an Australian *Rattus leucopus*. The only 16S sequence obtained (#22) in the present study shared 100% identity to both *Bartonella* sp. (accession number AY993935) detected in *Rattus tanezumi* from China and *B. coopersplainsensis* (accession number NR_1116177) isolated from *R. leucopus* from Australia. Also, the obtained *nuoG* sequence (#22) was identical (100% identity) to *Bartonella* sp. (JX131666) detected from *R. tanezumi* from South Africa. The *nuoG*, ITS and 16S sequences showed query coverages ranging from 81% to 100%. The sequences were deposited in GenBank database under accession numbers: *nuoG*: MT302378; ITS: MT271770-MT271771 and 16S: MT267730.

4. Discussion

In the current study, only two out of 55 rats trapped in SC were positive for *Bartonella* DNA. However, all DNA samples obtained from mammals' blood samples or ectoparasites sampled in MS were negative on the screening real-time PCR for *Bartonella* DNA targeting two different genomic regions. Considering that all DNA samples from MS were positive for the internal control (*gapdh*), confirming the presence of amplifiable DNA, the absence of *Bartonella* DNA suggests that all animals screened were not infected with *Bartonella* or that these animals presented an extremely low number of circulating *Bartonella* organisms, at least below the limit of detection of the HRM real-time PCR assays performed, which was previously estimated to be < 1 CFU per μl for the ITS locus (Gutiérrez et al., 2015b). In addition, we carried out the liquid BAPGM culture followed by qPCR approach aiming to improve the sensitivity of *Bartonella* diagnosis in the small rodent blood samples from MS. This approach has greatly facilitated the *Bartonella* detection from the blood samples of several animal species (Breitschwerdt, 2014). Nonetheless, none rodent blood sample subjected to BAPGM approach was positive, supporting the hypothesis that these animals were *Bartonella*-free.

The rat-associated *Bartonella* prevalence rates vary widely among the different studies and sampling zones within the same study

(Kosoy and Bai, 2019). Several biotic and abiotic features have been attributed to prevalence variations. Peterson et al. (2017) reported that the *Bartonella* prevalence in rats ranged from 0 to 97% among the different sampling sites. In addition to environmental features, the authors reported that co-occurrence of rat species (*R. rattus* and *R. norvegicus*), flea infestation, and age class were significant predictors for *Bartonella* prevalence (Peterson et al., 2017). Likewise, during a study performed in Asembo and Kibera, Kenya, the *Bartonella* prevalence in *Rattus* varied from 0 to 60% according to the trapping sites (Halliday et al.,2015). Different from Asembo sampling site that is more rural, the Kibera study site is a neighborhood within Nairobi - the Kenyan capital - few miles from the Nairobi center, thus is likely to have a higher international connectivity with a higher rodent movement through sea trade. Thereby, the higher prevalence observed at the Kibera might be explained by the repeated introduction of *Rattus*-associated *Bartonella* species to this site.

In the above scenario, Kosoy and Bay (2019) analyzing the prevalence of *Bartonella* in rats around the world, highlighted that *Bartonella* infection rates in rat populations are very high. However, the authors noticed that rats in some cities were not infected with *Bartonella*. One of the explanations raised by the authors is that the absence of *Bartonella* in some rat populations could be attributed to a kind of "island syndrome", in which at some point, certain parasites are absent mainly when a new rat population is formed by the introduction of a small number of individuals. Therefore, this could explain the absence or low prevalence of *Bartonella* in rats in some studies outside of Asia (Kosoy and Bay, 2019), as well as in our study.

Curiously, Ellis et al. (1999) reported that rats from US coastal cities (e.g. Baltimore, Miami and Los Angeles) were *Bartonella* positive, while rats sampled in non-coastal cities (Reno and Spencer) were *Bartonella*-free. Distinct from the first report of rat-associated *Bartonella* in Brazil, in which 19% *R. norvegicus* (n = 26) trapped in Salvador, a coastal city, were positive (Costa et al., 2014), the rats sampled in the present study in non-coastal cities of Campo Grande, MS, and Três Barras, SC, far approximately 1.000 Km and 170 Km, respectively, from the Brazilian coast, showed to be *Bartonella*-free and/or a low prevalence (3.6%) for this group of bacteria. Although a low number of rats have been analyzed (n = 39 in MS and n = 55 in SC), the molecular absence and the low *Bartonella*-DNA prevalence in rats could be attributed to possible isolation of the rat populations explored in this study.

Considering that rats were introduced to Brazilian territories during the European colonization (only about 500 years ago) (Hingston et al., 2016), we can speculate that these rat populations were established by *Bartonella*-free individuals or by only a few individuals harboring *Bartonella*. Likewise, the continuous arrival of new rats through seaports, as probably has happened in Salvador city, may have played a crucial role in the prevalence of rat-related *Bartonella* in coastal cities. Finally, the low prevalence of rat fleas, that are considered key players in the *Bartonella* cycle (Gutiérrez et al., 2015a) during the ectoparasites survey, could have influenced the *Bartonella* prevalence found. However, new studies sampling a higher number of rats in different sites, coastal and non-coastal regions, are much necessary to solve this enigma.

In Brazil, the overall *Bartonella* prevalence in wild rodents varies from 0% to 42.9% (Favacho et al., 2015; Gonçalves et al., 2016; Rozental et al., 2017; Fontalvo et al., 2017; de Sousa et al., 2018). Similar to Fontalvo et al. (2017) that reported negative results for *Bartonella* DNA in all small wild rodents (n = 38) trapped in Pernambuco state, Northeastern Brazil, all capybaras (n =14) analyzed in the current study showed negative results in the HRM real-time PCR assays. Despite the fact that several flea species have been reported in small rodents from Brazil (Carvalho et al., 2001), fleas were not observed on rodents at the time of sampling nor in the present study neither in the study performed by Fontalvo et al. (2017). Conversely, de Sousa et al. (2018) reported a *Bartonella* prevalence of 31.8% (35/110) among wild rodents trapped in the central region of Pantanal, municipality of Corumbá, MS – far approximately 350 km from our sampling sites in Campo Grande municipality, MS. Also, 75 fleas (*Polygenis bohlsi bohlsi*) were collected from 16 (14.5%) rodents. Finally, the authors reported *Bartonella* DNA in three (7.8%) flea pools, emphasizing the role of fleas in the rodent-associated *Bartonella* prevalence.

Capybaras have a broad distribution in South America. Usually, this rodent species is infested by ticks (Szabó et al., 2013), and only a few records of fleas in this rodent species were reported (Linardi and de Avelar, 2014). Although the rodent-associated *Bartonella birtlesii* transmission under experimental condition by *Ixodes ricinus* tick has been demonstrated (Reis et al., 2011), the role of ticks in rodent-related *Bartonella* transmission remains unclear (Harrison et al., 2012). Therefore, the absence of fleas may have influenced the *Bartonella* prevalence found in capybaras. Considering that a limited number of capybaras were screened, these results should be interpreted with caution. Thereby, future studies are needed in order to shed light on the role of capybaras as hosts for *Bartonella* in South America.

Except for the findings from Australia (Fournier et al., 2007; Kaewmongkol et al., 2011), no study has reported the detection of Bartonella DNA in marsupials from America. Marsupials have a wide distribution in the American continents, but, only two studies reported the occurrence of Bartonella in ectoparasites from marsupials (Reeves et al., 2005; Nelder et al., 2009). Nonetheless, it is necessary to emphasize that the *Bartonella*-positive ectoparasites found in *D. virginiana* were the cat flea, *C. felis* - a common finding in the USA (Abramowicz et al., 2012; Blanton et al., 2016) - in which only cat-associated *Bartonella* DNA (*B. clarridgeiae* and *B. henselae*) were detected. The fact that the marsupials were not screened in both above mentioned studies, the role of these mammals in the *Bartonella* epidemiological cycles remains vague.

Only two studies have previously investigated the occurrence of *Bartonella* in marsupials from Brazil. None of the 68 marsupials sampled in central-western and northeastern Brazil were positive for *Bartonella* (Fontalvo et al., 2017; de Sousa et al, 2018), as well as reported in the present study. Additionally, all ectoparasites including *Polygenis bohlsi bohlsi* fleas (n = 5) collected from *Monodelphys domestica* and *Thylamus macrurus* (de Sousa et al., 2018) and *C. felis* fleas (n = 3) collected from *D. albiventris* (Fontalvo et al. 2017) showed to be negative for *Bartonella* DNA. These findings raise interesting questions about the role of the American marsupials in the *Bartonella* life cycle. Are marsupials refractory hosts for *Bartonella* infection? Could the marsupial's immune system promote the clearance of *Bartonella* infection?

In the current study, although with a low prevalence, we showed, for the first time, the occurrence of *B. coopersplainsensis* in rats trapped in Brazil. This *Bartonella* species has not been reported in humans yet. However, since its description (Gundi et al., 2009), *B. coopersplainsensis* has been detected from *Rattus* collected in Southeastern Asia (Saisongkorh et al., 2009; Jiyipong et al., 2012; Tay et al., 2014), New Zealand (Helan et al., 2018), Spain and Italy (Obiegala et al., 2019), and from wild rodents (*Apodemus agrarius*) from China (Li et al., 2015) and Lithuania (Mardosaitè-Busaitienè et al., 2019). The absence of positive results in conventional PCR assays based on *gltA* and *rpoB* genes might have been associated to the low number of *Bartonella* DNA copies present in the sampled rodent samples. Although the three amplified regions (16S rRNA, *nuoG* and ITS) shows a lower discriminatory power when compared with *gltA* and *rpoB* genes (La Scola et al., 2003), they provided sufficient discriminatory information for the identification of *B. coopersplainsensis* infecting *R. rattus* in the current study. Additionally, we emphasized that most screened DNA samples were *Bartonella*-free. Therefore, ecological and biological factors (e.g. rodents' geographic isolation and absence of fleas) may also have influenced the prevalence observed. Finally, since *B. coopersplainsensis* reservoirs - *Rattus* spp. - are widely dispersed around the world, their zoonotic potential should be further investigated.

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