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HEALTH SCIENCES

Bartonella in Norway rats (*Rattus norvegicus*) from the urban slum environment in Brazil

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Abstract: *Bartonella* are rodent-borne bacteria that cause varied human etiologies. Studies on synanthropic rodents are rare, causing gaps in epidemiological knowledge. We tested bloodclot samples from 79 rats from an urban slum in Salvador, Brazil through PCR targeting *gltA* gene. Nine samples (11.4%) were positive: six had 100% identity with *Bartonella* sp. isolate JF429580 and 99.5% with *B. queenslandensis strain AUST/NH8*; three were 100% identical to isolate JF429532 and 99.7% to *B. tribocorum*. This is the second report on urban rat *Bartonella* indicating bacterial circulation at detectable rates. Its presence in rats from vulnerable human settlements demands public health attention.

Key words: zoonosis, synanthropic fauna, one health, vulnerable human settlements, urban rodents, neglected tropical diseases.

INTRODUCTION

Infections by bacteria from the genus *Bartonella* represent an array of neglected health issues that can range from asymptomatic chronic infections, to serious, and potentially fatal manifestations (Lins et al. 2019, Chomel et al. 2003, Rolain et al. 2004). Mammals are the primary hosts, with emphasis on rodents, both wild and synanthropic (Gonçalves et al. 2016, Gonçalves-Oliveira et al. 2020).Human infections are associated to close proximity to the hosts and their fleas, and unsanitary conditions. At least 18 genotypes of *Bartonella* are associated to human infections (Lins et al. 2019, Frank et al. 2018).

Synanthropic rats (genus *Rattus*) are key hosts of zoonotic pathogens in poor urban settlements, where environmental conditions are prone to rodent infestation, and human populations are forced to coexistence closely, contributing to rat-borne spillover transmissions (Costa et al. 2014b). Rodents have recently been identified as carriers of *Bartonella* spp. in sylvatic and urban contexts (Costa et al. 2014a, Gonçalves et al. 2016). This context poses rats as key subjects for One Health research, allowing to understand pathogen circulation, predict disease incidence, and inform public health policies (Costa et al. 2014b, 2021).

However, in spite of its public health relevance and evidence of circulation in wild and urban fauna, the ecoepidemiology of *Bartonella* is still understudied and underestimated (Lins et al. 2019). Brazil suffers with particular lack of studies (Silva et al. 2019), especially evaluating non-human hosts and their potential as sources of infection (Braga et al. 2012). The present study reports the presence and molecular characterization of *Bartonella* in rats captured in poor, urban communities in Salvador, Bahia, Brazil.

MATERIALS AND METHODS

The present study was conducted in 2012 as a pilot to test a systematic rodent sampling methodology (see Panti-May et al. (2016)) at Pau da Lima, a poor urban community in the city of Salvador, Bahia, Brazil. This area is characterized by inadequate and/or absent coverage of urban sanitation services (trash collection, sewage and drainage systems), and is known for high rodent infestation and high incidence of rodent-associated zoonosis; the area is subdivided in three valleys, and this study was conducted in Valley 1 (for a full characterization, see Panti-May et al. (2016)). One hundred and eight sampling points were semi-randomized within the study area in the peridomicile (both around households and within yards of the houses), with two live traps per point for four nights. Captured animals were taxonomically identified by trained biologists during necropsy. Procedures for tissue sampling and *Bartonella* spp. detection was performed by PCR, with material extracted from blood clot samples following the methodology of Kosoy et al. (1997), briefly the samples were extracted using the QIAamp Tissue Kit (Qiagen Inc., California, USA). Amplification was performed using primers targeting the *gltA* gene, 0.5 µM of each primer, 10 µL of the sample in a mix of 50 mM KC1, 10 mM Tris-HC1, 1.5 mM MgC12, 0.001% gelatin, 0.1% Brij-35, 200 µM of each deoxynucleotide triphosphate, and 0.2 U of thermostable AmpliTaq DNA polymerase (Perkin-Elmer-Cetus, Connecticut, USA). Incubation happened at 95°C for two minutes, amplification occurred for 40 cycles at: 95°C (1 minute), 50°C (1 minute), and 72°C (1 minute). All amplifications were verified by an agarose (2%) in Tris-borate-EDTA buffer (0.1 M Tris, 0.09 M of boric acid, 0.001 M of EDTA) electrophoresis. Purification was performed using Wizard PCR Preps (Promega, Wisconsin, USA) for sequencing. Sequencing was performed using a Cetus 9600 thermocycler (Perkin-Elemer-Cetus), where the PCR results were sequenced in both directions with PRISM dye-terminator cycle sequencing kit (Applied Biosystems Inc.), following all manufacturers' specifications. Resolution was obtained using an ABI-Prism autosequencer (Applied Biosystems, Inc.) by polyacrylamide gel (4%) electrophoresis, with the reaction performed at 51°C and constant 1500V. All activities were conducted with approval from the Ethics Committee on Use of Animals of the Oswaldo Cruz Foundation, Salvador, Bahia, Brazil (protocol 003/2012), and the Yale University's Institutional Animal Care and Use Committee (IACUC), New Haven, Connecticut (protocol number 2012–11498).

RESULTS

A total of 117 Brown rats (*Rattus norvegicus*) were captured, but only 79 had blood clots were collected and used for *Bartonella* detection by PCR. Nine samples were positive (Table I): six had 100% identity with *Bartonella* sp. isolate JF429580 (Gundi et al. 2012) and 99.5% identity with *B. queenslandensis strain AUST/NH8* (3 mature females, one immature female, one mature male, and one male of unknown age); while the remaining three samples were 100% identical to *Bartonella* sp. isolate JF429532 (Gundi et al. 2012) and 99.7% to *B. tribocorum* (all females, 2 immatures and 1 mature). Overall positivity rate for the samples tested was 11.4%.

Table I. Results for the PCR tests performed on rat blood samples to detect Bartonella sp., with results indicating whether the gltA gene was positively detected and posterior sequence (Seq) identity.

Tracking ID	Tissue sample	gltA	Seq
127268	Blood clot		
127267	Blood clot		
127266	Blood clot		
127271	Blood clot		
127276	Blood clot		
127275	Blood clot		
127280	Blood clot		
127279	Blood clot		
127278	Blood clot		
127283	Blood clot		
127282	Blood clot		
127281	Blood clot		
127286	Blood clot		
127285	Blood clot		
127284	Blood clot		
127287	Blood clot		
127292	Blood clot		
127291	Blood clot		
127290	Blood clot	pos	100% JF429532, 99.7% similarity to B. tribocorum
127295	Blood clot		
127294	Blood clot		
127293	Blood clot	pos	100% JF429580, 96.5% similarity to B. queenslandensis
127298	Blood clot		
127297	Blood clot		
127296	Blood clot		
127301	Blood clot		
127300	Blood clot		
127299	Blood clot		
127304	Blood clot		
127303	Blood clot		
127302	Blood clot		
127307	Blood clot		

Table I. Continuation.

127306	Blood clot		
127305	Blood clot		
127310	Blood clot		
127309	Blood clot		
127308	Blood clot		
127313	Blood clot		
127312	Blood clot		
127311	Blood clot		
127315	Blood clot		
127314	Blood clot		
130932	Blood clot		
130931	Blood clot		
130930	Blood clot		
130929	Blood clot	pos	100% JF429580, 96.5% similarity to B. queenslandensis
130928	Blood clot		
130927	Blood clot		
130919	Blood clot		
130918	Blood clot		
130917	Blood clot		
130916	Blood clot		
130914	Blood clot		
130913	Blood clot		
130912	Blood clot		
130911	Blood clot	pos	100% JF429580, 96.5% similarity to B. queenslandensis
130910	Blood clot		
130909	Blood clot	pos	100% JF429580, 96.5% similarity to B. queenslandensis
130908	Blood clot	pos	100% JF429532, 99.7% similarity to B. tribocorum
130907	Blood clot	pos	100% JF429580, 96.5% similarity to B. queenslandensis
130906	Blood clot		
130905	Blood clot		
130904	Blood clot	pos	100% JF429580, 96.5% similarity to B. queenslandensis
130903	Blood clot		
130902	Blood clot		
141244	Blood clot		

Table I. Continuation.

141243	Blood clot		
141242	Blood clot		
141241	Blood clot		
141240	Blood clot		
141239	Blood clot	pos	100% JF429532, 99.7% similarity to B. tribocorum
141238	Blood clot		
141237	Blood clot		
141236	Blood clot		
141235	Blood clot		
141234	Blood clot		
141233	Blood clot		
141248	Blood clot		
141247	Blood clot		

DISCUSSION

This is the second study testing urban rodents for *Bartonella* in Brazil, and it is a step to address a significant gap on the epidemiology of these bacterial infections that remain understudied, especially in urban settings. The present results indicate that *Bartonella* circulates in urban rodents at appreciable levels in Salvador. Our results are similar to a previous study conducted in the city (Costa et al. 2014a), reporting 19.2% positivity; however, unlike the present results, all their immature individuals tested negative. Gonçalves et al. (2016) found an overall positivity of 25.6%, and 2/29 (6.8%) for *Rattus rattus* across five Brazilian biomes. In contrast, in 2020, he observed only 3.6% positivity in rats in southern Brazilian, as well as no positive ectoparasites collected from tested rats (Gonçalves et al. 2020). In similar studies testing for other synanthropic or potentially synanthropic species, Braga et al. (2012) detected 4.5% positivity in pet cats in São Luiz, Ferreira et al. (2018) detected 18.5% positivity for bats in the Atlantic Forest, and Gonçalves-Oliveira et al. (2020) observed 4.34% positivity across several taxa of mammals in peri urban forest fragments in Rio de Janeiro.

The lack of studies on the subject in Brazil, although not totally unexpected given Bartonellosis' status as neglected infections (Lins et al. 2019), is cause for concern given the absence of basic epidemiological data for the country on an infection that is under-reported Silva et al. 2019, Lins et al. 2019). The problem of rodent-borne diseases mainly affects the most vulnerable of society, that are susceptible to disease transmission due to the environmental conditions of poor urban areas. These populations disproportionately carry a higher burden of disease, which is one mechanism that leads to the poverty trap. (Garchitorena et al. 2017, Costa et al. 2014b, 2017). This is particularly true for *Bartonella* infections, commonly more prevalent in marginalized or underserved populations (Chamberlin et al. 2002). The present results can be considered a sign for concern regarding a neglected group of potentially pathogenic bacteria circulating in a synanthropic reservoir already implicated in the endemic transmission of zoonotic pathogens in

Salvador (Costa et al. 2014a, b, Felzemburgh et al. 2014). However, further studies are necessary to properly characterize the situation of *Bartonella* circulation in order to inform an action plan.

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CGZ: field work, first draft, final version; DO: sample management, laboratory analysis, first draft, final version; MYK: field work coordination, data analysis (taxonomic expertise), final version; MGR: data curation, laboratory supervision, final draft; AIK: study design (laboratory), first draft; JEC: study design (field work), first draft, final version; FC: project supervision, data curation, first draft, final version.