Coxiella and Bartonella spp. in bats (Chiroptera) captured in the Brazilian Atlantic Forest biome

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Abstract

Background: The role of bats as reservoirs of zoonotic agents, especially pathogenic bacteria such as Bartonella and Coxiella, has been discussed around the world. Recent studies have identified bats as potential hosts of species from the proteobacteria phylum. In Brazil, however, the role of bats in the natural cycle of these agents is poorly investigated and generally neglected. In order to analyze the participation of bats in the epidemiology of diseases caused by Bartonella, Coxiella, Rickettsia, Anaplasma and Ehrlichia, we conducted a descriptive epidemiological study in three biogeographic regions of the Brazilian Atlantic Forest.

Results: Tissues of 119 bats captured in preserved areas in the states of Rio de Janeiro, Bahia and Santa Catarina from 2014 to 2015 were submitted to molecular analysis using specific primers. Bartonella spp. was detected in 22 spleen samples (18.5%, 95% CI: 11.9–26.6), whose phylogenetic analysis revealed the generation of at least two independent clusters, suggesting that these may be new unique genotypes of Bartonella species. In addition, four samples (3.4%, 95% CI: 0.9–8.3) were positive for the htpAB gene of C. burnetii [spleen (2), liver (1) and heart (1)]. Rickettsia spp., Anaplasma and Ehrlichia were not identified. This is the first study reporting C. burnetii and Bartonella spp. infections in bats from the Atlantic Forest biome.

Conclusions: These findings shed light on potential host range for these bacteria, which are characterized as important zoonotic pathogens.

Keywords: Coxiella burnetii, Bartonella, Zoonotic bacterial agent, Mammals, Atlantic Forest hotspot, Brazil

Background

Bacteria transmitted by arthropods belonging to the genera Rickettsia, Bartonella, Coxiella, Ehrlichia and Anaplasma are pathogens of domestic and wild animals as well as humans. These agents cause diseases that may be severe and have a widespread geographic distribution, such as bartonellosis, ehrlichiosis, anaplasmosis, spotted fever, and Coxiellosis/Q fever [1–4]. Bartonella spp. (proteobacteria α2 group), an intracellular hemotrophic bacterium that grows fastidiously, is transmitted mainly by flea bites [4]. Coxiella burnetii (proteobacteria γ group), the causative agent of Q fever/Coxiellosis, is a highly infectious zoonotic intracellular bacterium transmitted by inhalation of aerosols or contaminated excreta materials. Ticks are suspected of having a role in the transmission of this pathogen among animals [5]. Rickettsia (proteobacteria α1 group) is a representative genus group of pathogenic or non-pathogenic intracellular bacteria transmitted by ticks, mites, lice and fleas [2]. Ehrlichia and Anaplasma (proteobacteria α1 group), which are known to cause diseases in animals and humans, are kept in the wild in a cycle involving mammals and arthropods.
In recent years, studies have pointed to bats as hosts of these proteobacteria around the world [7–11]. Their increasing diversity and apparent clade-specific association for *Bartonella* spp. [12] encourages increasing inventory and surveillance efforts, especially in sylvatic environments to better understand their natural transmission cycles.

Bats (order Chiroptera) occur in all continents except Antarctica [13]. Among mammals, they are outnumbered only by rodents in species richness but surpass all other groups in dietary diversity, including fruit eaters, nectar feeders, insectivores, carnivores, blood feeders and omnivores. In biodiversity hotspots such as Brazil's Atlantic Forest, bats are the most diverse and abundant mammals, and they represent wild vertebrates that interact with humans [14] especially in urban areas [15]. The role of bats as important hosts for emerging human diseases has gained the attention of the scientific community. They are recognized for harboring viral infectious agents and, less recognizably, bacterial agents of public health importance [16–18].

In Brazil, the vespertilionid bat species *Histiotus velatus* (tropical big-eared brown bat), and the phyllostomids *Carollia perspicillata* (Seba's short-tailed bat) and *Desmodus rotundus* (common vampire bat) were considered reservoirs of rickettsiae in an experimental study in the 1950s [19]. After more than 50 years, in the city of São Paulo, molossid, vespertilionid and phyllostomid bats were seroreactive to, at least, one rickettsial antigen of the spotted fever group [20]. In Queensland, Australia, in 2014, the DNA of *C. burnetii* was found in bat urine pools of a *Pteropus* (Pteropodidae) [8]. This finding might be indicative of the potential role of these animals as a source of infection for humans and other animal species through the inhalation of contaminated aerosols. Probably related to their sporulation process, *C. burnetii* survives for long periods in the environment, and inhalation is characterized as the main mechanism by which this microorganism is transmitted [21–23].

In addition, there are studies around the world in which from different species of *Bartonella* has been detected in bats for example, in, Peru [24], Kenya [25], United Kingdom [26], Guatemala [27], Nigeria [7], Puerto Rico [28], Vietnam [9], Costa Rica [10] and Taiwan [29]. More recently, strains of *Bartonella mayotimonensis*, a recognized human pathogen, were identified and isolated from bats in the northern hemisphere [30]. In Brazil, up to now, there is one study associating *Bartonella* with bats [11]. However, the real role of bats as hosts and maintainers of the natural cycle of these bacteria in nature remains unknown. Studies proving associations between bats and *C. burnetii*, *Ehrlichia* and *Anaplasma* have not yet been reported.

Considering the growing importance of bats as potential reservoirs and transmitters of different pathogens around the world as well as the paucity of investigations about the role of bats in the dissemination of proteobacteria pathogens, the present study provides information about the circulation of these zoonotic bacteria in bats captured in three Atlantic Forest localities in Brazil where notifications of Brazilian spotted fever, Q fever and bartonellosis have been reported.

**Methods**

**Study areas and sample collection**

The study was conducted in three Brazil's Atlantic Forest localities: Oswaldo Cruz Foundation (Fiocruz) Atlantic Forest Biological Station (EFMA; 22°56′22.9″S 43°24′12.2″W), Pedra Branca Massif, Jacarepaguá, which is a metropolitan area of the city of Rio de Janeiro (RJ); city of Ilgrapiúna, southern region of Bahia (BA), which is within the Environmental Protection Area (APA) of Pratigi (13°50′43.3″S 39°16′17.0″W); Serra do Tabuleiro State Park (PEST; 27°44′30.8″S 48°48′26.7″W), which is located in the central-eastern region of Santa Catarina state (SC) in the metropolitan area of the city Florianópolis (Fig. 1). The vegetation of the three sampling areas are composed by lowland humid forest areas from three different biogeographic regions of the Atlantic Forest biome.

From December 2013 to May 2015, expeditions were carried out and bats were captured using 10 ground-level mist nets (9 × 3 m) each night in forest edges or along pre-existing trails. Permits for field collection were granted by an Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) license under process numbers 19,037–1; Santa Catarina’s Environment Foundation (FATMA) no. 043/2014/GERUC/DPEC, Chico Mendes Biodiversity Conservation Institute (ICMBio/SISBIO) no. 26934–1 and no. 17131–4 (ICMBio/SISBIO). Adult males and non-pregnant and non-lactating adult females were euthanized. The euthanasia method consisted of cardiac puncture exsanguination performed after the anesthesia procedure of the animal, according to previously established protocols [31]. Efforts were made to minimize animal suffering following protocols approved by the Institutional Ethics Committee on Animal Research (CEUA) of the Oswaldo Cruz Foundation under process numbers CEUA P.62/11–3 (LW-68/12) and P.42/12–1 (LW-81/12).

The sex, age class and biometry of each bat were registered. Bats tissues (i.e., kidney, liver, spleen, lung and heart) were sampled and preserved in absolute ethanol. Tissue samples were obtained in accordance with recommended safety procedures and followed previously established standards protocols [32]. Bat species was identified following the identification keys available in Gardner [33] and nomenclature in Nogueira et al. [34]. Voucher specimens were deposited at scientific collections from each region where the study was performed: Federal University of Rio de Janeiro, for bats collected in Rio de Janeiro; State University of Santa
Cruz, for bats from Bahia; Foundation University of Blumenal and in the Collection of Mammals of the Federal University of Paraíba, for bats collected in Santa Catarina state. Prevalences and approximated confidence intervals (CIs) were calculated using the package “binom”. To evaluate the influence of the sex-ratio on the positivity we use a Fisher exact test.

Nucleic acid extraction
The DNA extraction procedures were performed in laminar flow biosafety cabinet in a Biosafety Level 3 laboratory (Vecbiosafe, Veco, Campinas, SP, Brazil). DNA was extracted from 10 mg of each bat tissue using the commercial QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer’s instructions. The final volume of 100-μl obtained after elution in AE buffer (QIAGEN, Valencia, CA, USA). Negative controls using nuclease-free water were included in each extraction to check for DNA contamination.

Spleen tissues were investigated for all agents covered in this study; however, in an attempt to identify
complementarity of information for Coxiella burnetii research, other tissues were tested as well.

**PCR amplification**

Conventional polymerase chain reaction (PCR) assays was used to detect the target genes. The gene for which each agent investigated in this study was tested is listed below and demonstrated in Table 1. Bartonella spp., gltA gene [35]. Coxiella burnetii, bacterial-specific primers designed to amplify the IS1111 gene [36, 37]. Rickettsia spp., a partial sequence of the gltA gene [35]. Ehrlichia and Anaplasma bacterial, 16S rRNA gene [38].

The mixture to each reaction contained 2.5 μl of 10X PCR buffer, 0.6 μl of 10 mM of each primer, 0.75–2 μl of 50 mM MgCl2, 0.25 μl deoxynucleotides (20 mM of each deoxynucleotide triphosphate), 0.1 μl Taq Platinum DNA polymerase (5 U/μl Invitrogen, Carlsbad, CA, USA) and nuclease-free water (Promega, Madison, WI, USA) to obtain a final volume of 25 μl. The volumes of the DNA sample varied as a function of the primer used. To Bartonella spp. was used 3 μl; to Rickettsia spp., 3 μl for PCR 1 and 2 μl for nested PCR; to C. burnetii, 4 μl for PCR 1 and 2 μl for nested PCR; to Ehrlichia and Anaplasma spp., 2.5 μl.

Volumes pre-established in a previous study [35]. Negative controls using nuclease-free water were included in each PCR assay to check for possible DNA contamination. Genomic DNA extracted from positive clinical samples from the National Reference Laboratory for Rickettsioses were used as positive controls. PCR amplification was subjected to a 1.5% agarose gel, stained with GelRed™ (Biotium, Hayward, CA, USA).

**DNA sequencing and phylogenetic analyses**

Appropriately sized fragments were purified using Illustra GFX PCR DNA and Gel Band Purification™ kit (GE Healthcare, Buckinghamshire, UK), direct nucleotide sequencing amplicon was performed using the BigDye Terminator v3.1 Cycle Sequencing kit, and purification was performed using the BigDye® X-Terminator Purification kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer’s recommendations. The analyses of the amplicons were performed in an ABI Prism 3730XL with 96 capillaries (Applied Biosystems) and the nucleotide sequences were analyzed using MEGA7 software (downloaded from www.megasoftware.net). A consensus sequence for each bacterial genome was derived from contiguous sequences assembled with the same software.

Multiple sequence alignments were done with sequences obtained from this study and sequences from the GenBank using MUSCLE in the SeaView v.4 program [39].

### Table 1

| Pathogen             | Target gene | Oligonucleotide primer | Primer sequence (5′ – 3′) | Amplicon size (bp) | Cycling conditions | Reference |
|----------------------|-------------|------------------------|---------------------------|--------------------|--------------------|-----------|
| Coxiella burnetii    | IS1111      | Outer primer F         | TATGTATCCACCGTGAGCCAG     | 687 bp             | 95 °C for 5 min, 40 cycles of 95 °C for 30s, 60 °C for 30s, 72 °C for 1 min, final extension of 72 °C for 7 min 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 66 °C for 30 s, 72 °C for 30 s, final extension of 72 °C for 5 min | [36, 37] |
|                      |             | Outer primer R         | CCCACCCACGCCTTGATTCCTTATTC | 440 bp             |                    | [35, 37] |
|                      |             | Nested primer F        | AAGCCGTTTGGAGGAGCGAACCC   |                    |                    | [35]      |
|                      |             | Nested primer R        | CTCGTAATGACCAAATCGCTGTC   |                    |                    |          |
| Bartonella spp.      | gltA        | Outer primer F         | GCTATGCTGCTTCTGTTCT       | 731 bp             | 95 °C for 10 min, 35 cycles of 95 °C for 30s, 58 °C for 30s, 72 °C for 1 min, final extension of 72 °C for 8 min | [35]      |
|                      |             | Outer primer R         | ATCAYGA                   |                    |                    |          |
|                      |             | Nested primer F        | AGAAACAGTAAACATTCN        |                    |                    |          |
|                      |             | Nested primer R        | GTHGGG                    |                    |                    |          |
| Rickettsia spp.      | gltA        | Outer primer F         | CATCCTATGCTTATTATCTGTGC   | 885 bp             | 95 °C for 10 min, 30 cycles of 95 °C for 30 s, 55 °C for 40 s, 72 °C for 55 s, final extension of 72 °C for 10 min 95 °C for 7 min, 25 cycles of 95 °C for 30 s, 63 °C for 30 s, 72 °C for 35 s, final extension of 72 °C for 10 min | [35]      |
|                      |             | Outer primer R         | TTACCTCTCTATG(T)/AAC(G)/ T(A)/GACCC | 572 bp             |                    | [35]      |
|                      |             | Nested primer F        | CTTACCGCATATAGAATGAGTGC   |                    |                    |          |
|                      |             | Nested primer R        | GAGGCA(T/G)AGCTCAAG(T)/ C(T)CTAT |                    |                    |          |
| Ehrlichia spp.       | 16 s rRNA   | Outer primer F         | GGTACCCAGAAAGAGGTTACAG    | 345 bp             | 95 °C for 3 min, 35 cycles of 95 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s, final extension of 72 °C for 5 min | [38]      |
| Anaplasma spp.       |             | Outer primer R         | TGCACTCATCTGGTACAG        |                    |                    |          |
best-fit evolutionary model was determined using MEGA version 7 by the Bayesian Information Criterion [40]. The phylogenetic tree was estimated using two methods: (a) Maximum Likelihood using PhyML implemented in SeaView v.4 [41], where the statistical support of the clades was measured by a heuristic search with 1000 bootstrap replicates; and (b) a Bayesian Markov Chain Monte Carlo (MCMC) method implemented in MrBayes v.3.2.6 [42]. The Bayesian analysis consisted of two simultaneous independent runs of 10 million MCMC generations (burn-in of 25%).

Results

Bat sampling

A total of 119 adult bats belonging to 21 species were sampled; n = 44 from EFMA/RJ; n = 47 from APA Pratigi/BA and n = 28 from PEST/SC (Table 2). The species sampled and their abundances are as follows: Carollia perspicillata (n = 34), Desmodus rotundus (15), Artibeus lituratus (14), Sturnira lilium (12), Artibeus fimbriatus (7), Rhinophylla pumilio (7) Artibeus planirostris (5), Dermanura cinerea (4), Phyllostomus discolor (4), Artibes obscurus (2), Glossophaga soricina (2), Myotis nigricans (2), Sturnira tildae (2), Vampyressa pusilla (2), Anoura caudifer (1), Chiropodomus doriae (1), Lonchophylla peracchii (1), Micronycteris minuta (1), Micronycteris sp. (1), Phyllostomus hastatus (1), and Trinecteris nicefori (1).

Detection of Bartonella spp.

Bartonella DNA was detected in 22 animals (18.5%, 95% CI: 11.9–26.6) (Table 3) collected from the three regions of this study (Table 2): Jacarepaguá/RJ (10/44; 23.0%, 95% CI: 11.4–37.8); APA Pratigi/BA (7/47; 15%, 95% CI: 6.2–28.3) and PEST/SC (5/28; 18%, 95% CI: 6.0–36.8). Although S. lilium (6/22 Bartonella-positive bats) and D. rotundus (6/22) were the most frequent hosts of Bartonella, six other bats species (i.e., C. perspicillata, A. lituratus, A. fimbriatus, A. obscurus, P. pumilio and P. discolor) were also positive (Table 3). Contrasting the pool of males against the pool of females per locality, in all study areas we found more positive samples for males than females, but only in APA Pratigi/BA this difference was significant (Fisher exact’s test \( p = 0.0027 \)). Due to the small sample size per species/locality, we did not run this analysis per species.

However, in this study, we opted to work with sequences that presented fragment sizes that would allow reliable results when submitted to phylogenetic analyses. Thus, of the 22 samples that were positive for the Bartonella gltA gene, 11 were included in these analyses. The phylogenetic inference based on the gltA gene sequences revealed two different clusters for the new sequences of this study (Fig. 2). In the gltA gene tree, the first cluster is composed of two groups; one of them comprised our sequence (EM 209) found in P. discolor in the state of Bahia as well as the sequence Bartonella sp. clone SJ114 (KJ816690) found in Costa Rica; the second group is composed of the sequence Bartonella sp. clone SJ131 (KJ816670) found in Costa Rica as a stem lineage in the clade (1/ 85). The next node (1/ 100) within the cluster contained our sequences (EM 805, RM 525) found in S. lilium from the states of Santa Catarina and Rio de Janeiro (1/ 83), in a sister relation to the clade of our sequences found in A. fimbriatus and A. obscurus (RM 524, RM 529) from Rio de Janeiro (0.79/ *).

The second weakly supported cluster comprises two sister groups. One (1/ 99) includes our three sequences (RM 512, RM 534, RM 564) found in D. rotundus from the state of Rio de Janeiro, and the other includes the sequence Bartonella sp. clone SJ117 (KJ816691) found in C. perspicillata from Costa Rica and our two sequences (EM 185, EM 199) found in C. perspicillata from Bahia state (0.93/ 95). The other group is composed of the sequence Bartonella sp. clone SJ128 (KJ816692) found in P. discolor from Costa Rica as a stem lineage in the group (1/ 94). The next bifurcation within the cluster contained our sequence (EM 819) found in S. lilium from Santa Catarina state as well as the sequence of Bartonella sp. clone 1 (KY356753) found in an undescribed bat species from Brazil and the sequence Bartonella sp. clone SJ130 (KJ816674) found S. lilium from Costa Rica (1/ 99).

Detection of Coxiella burnetii

Coxiella burnetii DNA was detected in four specimens from two bat species (3.4%, 95% CI: 0.9–8.3), A. lituratus (3/4 Coxiella-positive bats) and A. fimbriatus (1/4) from two different regions: Jacarepaguá/RJ (3/4; 7%, 95% CI: 1.4–18.6) and PEST/SC (1/28; 4%, 95% CI: 0.1–18.3) (Tables 2 and 3). No differences between positivity in males and females were observed. Coxiella DNA sequences showed 100% identity to the complete genome of C. burnetii [GenBank CP018005, CP020616, AE 016828, LA 937696]. In our survey, co-infection was detected in one bat sample of A. fimbriatus from the Jacarepaguá/RJ region (Table 2). Rickettsia spp., Ehrlichia spp. and Anaplasma spp. DNA was not detected in any of the bat samples tested.

Nucleotide sequence accession numbers

All sequences obtained including 11 for Bartonella spp. gltA (MH204887-MH204897) and 4 for Coxiella burnetii IS1111 (MH229948-MH229951) have been deposited in GenBank.
Considering the growing importance of bats as potential hosts of zoonotic agents of human disease, our findings reveal that bats of different species are infected with *Bartonella* spp. and *C. burnetii* in the Atlantic Forest regions of Rio de Janeiro, Bahia and Santa Catarina. In this study, we found a prevalence of 3.4% of bats positive for *C. burnetii* DNA, all belonging to the genus *Artibeus*. Characteristics of this genus, such as the formation of colonies grouping dozens of individuals of reproductive age [43] can contribute to a rapid and widespread transmission of *C. burnetii* among these animals, especially considering the high resistance of these proteobacteria, which can survive for several weeks in the environment where the

### Table 2

| Family: Sub-family | Localities          | PCR assay                                      |
|--------------------|--------------------|------------------------------------------------|
|                    | Locality           | Bartonella positive p/N(%;CI)                  |
|                    |                    | Coxielia positive p/N(%; CI)                   |
|                    |                    | Bartonella and Coxiella positive p/N(%; CI)    |
| Phyllostomidae: Carolliniae |                |                                                 |
| Carollia perspicillata | 4/34(11.8)        | 1/34(0.3–31.0)                                  |
| Rhinophylla pumilio   | 0/7(0)             | 1/7(0.3–57.8)                                   |
| Phyllostomidae: Desmodontinae |        |                                                 |
| Desmodus rotundus    | 15/15(100)         |                                                 |
| Phyllostomidae: Glossophaginae |         |                                                 |
| Anoura caudifer      | 0/1(0)             |                                                 |
| Glossophaga soricina | 2/2(100)           |                                                 |
| Lonchophylla peracchii | 1/1(100)         |                                                 |
| Phyllostomidae: Glyphonycterinae |            |                                                 |
| Trinycteris niceroxii | 0/1(0)             |                                                 |
| Phyllostomidae: Micronycterinae |            |                                                 |
| Micronycteris minuta  | 1/1(100)           |                                                 |
| Micronycteris sp.     | 1/1(100)           |                                                 |
| Phyllostomidae: Phyllostominae |          |                                                 |
| Phyllostomus discolor | 0/4(0)             |                                                 |
| Phyllostomus hastatus | 0/1(0)             |                                                 |
| Phyllostomidae: Stenodermatinae |        |                                                 |
| Artibeus fimbriatus   | 3/7 (42.9)         | 1/7(14.3; 0.3–57.8)                             |
| Artibeus lituratus    | 4/14(28.6)         | 1/14(7.1; 0.1–33.8)                            |
| Artibeus obscurus     | 1/2(50)            | 1/2(50; 1.2–98.7)                               |
| Artibeus planirostris | 0/5(0)             | 5/5(100)                                       |
| Chiroderma donae      | 0/1(0)             | 1/1(100)                                       |
| Dermarura cinea       | 0/4(0)             | 4/4(100)                                       |
| Stenura lilium        | 6/12(50)           | 6/12(50)                                       |
| Stumira tildae        | 2/2(100)           | 2/2(100)                                       |
| Vampyressa pusilla    | 2/2(100)           | 2/2(100)                                       |
| Vespertilionidae: Myotinae |            |                                                 |
| Myotis nigricans      | 2/2(100)           |                                                 |
| TOTAL                | 44                 | 47                                             |

NS negative sample, NA not applicable

**Discussion**

Considering the growing importance of bats as potential hosts of zoonotic agents of human disease, our findings reveal that bats of different species are infected with *Bartonella* spp. and *C. burnetii* in the Atlantic Forest regions of Rio de Janeiro, Bahia and Santa Catarina. In this study, we found a prevalence of 3.4% of bats positive for *C. burnetii* DNA, all belonging to the genus *Artibeus*. Characteristics of this genus, such as the formation of colonies grouping dozens of individuals of reproductive age [43] can contribute to a rapid and widespread transmission of *C. burnetii* among these animals, especially considering the high resistance of these proteobacteria, which can survive for several weeks in the environment where the
animals were present [22]. Our results as well as recently published findings [35, 44] suggest the existence of a complex C. burnetii transmission cycle involving a large number of wild and domestic animals in Rio de Janeiro. In addition, the presence of C. burnetii DNA in bats captured in the region of Santa Catarina state is the first evidence of the circulation of this agent in the state.

Our prevalence results (18.5%) corroborate other studies worldwide showing the prevalence of Bartonella spp. in bats between 18.0–33.3% [10, 24, 27]. In our study, S. lilium (27.3%), D. rotundus (27.3%) and C. perspicillata (22.7%) were the species that presented the highest prevalence of Bartonella infection. In South America, similar results were obtained in Peru, with a prevalence of 15% for C. perspicillata (4/27), 37% for D. rotundus, (10/27) and 4% for S. lilium (1/27) [24]. In Brazil, in a study recently carried out in 5 different states, positive samples for Bartonella spp. were found in S. lilium, C. perspicillata, P. disolor, Glossophaga soricina and Natalus espiritosantensis (Natalus macrourus [45]) [11]. Although we have found more positive samples for males than females in all studied areas, the evidence available, including the established knowledge on transmission routes and the role of bats in the circulation of these pathogens, do not allow us to speculate on the pathogen prevalence in males. Further analyses comparing males and females per species are necessary for better understanding the role of sexes in the pathogen circulation.

The phylogenetic tree revealed the formation of independent clades when compared to Bartonella species reported in the literature, which may indicate that previously unknown genotypes of Bartonella are infecting these bats. This is a common finding with studies of this agent in bats from other regions of the world (e.g., in Guatemala, Nigeria, Costa Rica and China) [7, 10, 27, 46]. Some of our obtained sequences showed a clear separation of the group formed in Bartonella sequences found in wild rodents of the Atlantic Forest in the state of Rio de Janeiro (LBCE- Laboratory of Biology and Control of Schistosomiasis), reinforcing that a new genotype may be circulating among bats and that these strains differ between rodents and bats [35]. Interestingly, our sequences were most closely related to others identified in bats from Costa Rica and related only to a single sequence found in a Brazilian bat. Furthermore, positive samples belonging to the S. lilium species were grouped into two distinct clades, suggesting that a single bat species is host to two different species of Bartonella. Similarly, sequences generated from Bahia, Rio de Janeiro and Santa Catarina states subjected to phylogenetic analyses were divided into two clades and suggested that the circulation of more than one species of Bartonella may be associated with bats in each state. Co-infection of different Bartonella species in a single bat species was also observed in Kenya, Guatemala, China and Georgia [25, 27, 46, 47]. A recent study demonstrated that Bartonella strains tend to cluster according to families, super-families and suborders of bats [48]. In addition, a co-infection with different species of Bartonella in a single species of bat may imply a change of bacteria via recombination, as shown by Bartonella in rodents [49]. The presence of divergent sequences in the analyzes of this study suggests the presence of more than one Bartonella lineage, since the divergence of the sampled sequences of the gltA gene varied from 0.0 to 20.9% in sequences of the same clade, the sequence divergence among Bartonella species suggested for this fragment is about 30% [50] (Additional file 1). To characterize the Bartonella species, it is necessary to sequencing other housekeeping genes (ie, rpoB, ftsZ, groEL and its) to recognize the diversity of lineages found in bats and to determine the structure of populations and phylogenetic data [51, 52]. Sequencing of the additional genes for improved taxonomic resolution was beyond the scope of this paper.

### Table 3 Specimens infected by Bartonella spp. and Coxiella burnetii.

| Field Number | Species                  | Sex      | Locality           |
|--------------|--------------------------|----------|--------------------|
| RM 510       | Desmodus rotundus        | Female   | Jacarepaguá – RJ  |
| RM 512       | Desmodus rotundus        | Male     | Jacarepaguá – RJ  |
| RM 517       | Desmodus rotundus        | Female   | Jacarepaguá – RJ  |
| RM 523       | Desmodus rotundus        | Male     | Jacarepaguá – RJ  |
| RM 524       | Antibeus fimbriatus      | Male     | Jacarepaguá - RJ  |
| RM 525       | Sturnira lilium          | Female   | Jacarepaguá - RJ  |
| RM 529       | Antibeus obscurus        | Male     | Jacarepaguá - RJ  |
| RM 532       | Sturnira lilium          | Male     | Jacarepaguá - RJ  |
| RM 534       | Desmodus rotundus        | Male     | Jacarepaguá - RJ  |
| RM 564       | Desmodus rotundus        | Female   | Jacarepaguá – RJ  |
| EM 179       | Carollia persicillata    | Male     | APA do Pratigi - BA |
| EM 185       | Carollia persicillata    | Male     | APA do Pratigi - BA |
| EM 186       | Antibeus lituratus       | Male     | APA do Pratigi - BA |
| EM 189       | Rhinophylla pumilio      | Male     | APA do Pratigi - BA |
| EM 199       | Carollia persicillata    | Male     | APA do Pratigi - BA |
| EM 209       | Phyllostomus discolor    | Male     | APA do Pratigi - BA |
| EM 217       | Carollia persicillata    | Male     | APA do Pratigi – BA |
| EM 795       | Sturnira lilium          | Male     | PEST - SC          |
| EM 800       | Carollia persicillata    | Male     | PEST - SC          |
| EM 803       | Sturnira lilium          | Male     | PEST - SC          |
| EM 805       | Sturnira lilium          | Male     | PEST - SC          |
| EM 819       | Sturnira lilium          | Female   | PEST – SC          |
| RM 514       | Antibeus lituratus       | Female   | Jacarepaguá/RJ     |
| RM 524       | Antibeus fimbriatus      | Male     | Jacarepaguá/RJ     |
| RM 557       | Antibeus lituratus       | Male     | Jacarepaguá/RJ     |
| EM 817       | Antibeus lituratus       | Female   | PEST /SC           |
Our study has identified a co-infection with *C. burnetii* and *Bartonella* spp. in an individual of *A. fimbriatus*. Although a pattern cannot be established, dual infection can reinforce the potential of bats to host these bacterial pathogens. Many bat species are gregarious and can form small groups of a few individuals to large colonies of up to 20 million individuals, such as the species *Tadarida brasiliensis* in Bracken Cave in Texas, USA [53]. Furthermore, different bat species can cohabit in the same shelter, allowing the possibility for interspecific transmission and a high rate of contact within these colonies that can lead to rapid transmission of pathogens [54]. Bats have been identified as potential natural reservoirs of a number of high-impact zoonotic agents. Recently, a study provided evidence that bats are indeed special in hosting more zoonotic viruses and more total viruses per species than rodents [55].

The absence of *Rickettsia* in bat samples corroborate the literature demonstrating that a lack of rickettsial amplification in wild animal is an expected result, since vertebrates act as amplifiers and food sources for ticks, which are in fact the true reservoirs of these proteobacteria in nature [35]. Besides, this reinforces that the role of bats as carriers of *Rickettsia* is still unknown, despite reports in the 1950s that bats harbor pathogenic rickettsial [19, 56, 57]. No samples tested in our study were positive for *Ehrlichia* and *Anaplasma* infections. Although bats have been found infected with proteobacteria of the families Anaplasmataceae [58], there is still no record of DNA amplification of *Ehrlichia* spp. or *Anaplasma* spp. in these mammals. Regardless of which other prior studies have used a similar method, it seems plausible that some negative detections may have resulted from a less sensitive PCR approach (i.e., conventional rather than nested).
Conclusions
This study confirms the presence of infected bats with C. burnetii and Bartonella spp. in the Atlantic Forest biome.

Additional file

Additional file 1: Estimates of Evolutionary Divergence between Bartonella gibr partial sequences. There were a total of 512 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. Presentation of the PCR positive bats species for Bartonella spp. in this study with their respective GenBank accession numbers and the estimated divergence found between Bartonella gibr partial sequences of the gene deposited in GenBank. (DOCX 25 kb)

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Competing interests
The authors declare that they have no competing interests.

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