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Zoonotic pathogens in Atlantic Forest wild rodents in Brazil: *Bartonella* and *Coxiella* infections

Tatiana Rozental[a,*], Michelle Santos Ferreira[a], Alexandro Guterres[a], Maria Angélica Mares-Guia[a], Bernardo R. Teixeira[b], Jonathan Gonçalves[b], Cibele Rodrigues Bonvicino[b,c], Paulo Sergio D'Andrea[b], Elba Regina Sampaio de Lemos[a]

[a] *Laboratório de Hantaviroses e Rickettsioses, Instituto Oswaldo Cruz (IOC), Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil*
[b] *Laboratório de Biologia e Parasitologia de Mamíferos Silvestres e Reservatórios, Fundação Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil*
[c] *Instituto Nacional do Câncer (INCA), Rio de Janeiro, Brazil*

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ABSTRACT

Zoonotic pathogens comprise a significant and increasing fraction of all emerging and re-emerging infectious diseases that plague humans. Identifying host species is one of the keys to controlling emerging infectious diseases. From March 2007 until April 2012, we collected a total of 131 wild rodents in eight municipalities of Rio de Janeiro, Brazil. We investigated these rodents for infection with *Coxiella burnetii*, *Bartonella* spp. and *Rickettsia* spp. In total, 22.1% (29/131) of the rodents were infected by at least one pathogen; co-infection was detected in 1.5% (2/131) of rodents. *Coxiella burnetii* was detected in 4.6% (6/131) of the wild animals, 17.6% of the rodents harbored *Bartonella* spp. No cases of *Rickettsia* were identified. *Bartonella doshiae* and *Bartonella vinsonii* were the species found on the wild mammals. This report is the first to note *C. burnetii*, *B. doshiae* and *B. vinsonii* natural infections in Atlantic Forest wild rodents in Brazil. Our work highlights the potential risk of transmission to humans, since most of the infected specimens belong to generalist species that live near human dwellings.

1. Introduction

Wild rodents, which are important hosts of a large number of zoonotic pathogens, are involved in the natural cycle of various bacterial diseases (Bengis et al., 2004; Meerburg et al., 2009). Rodentia is the most abundant and diversified order of living mammals, representing approximately 40% of mammalian species (Huchon et al., 2002; Wilson and Reeder, 1993). Within the last few decades, the incidence of human diseases caused by zoonotic bacteria associated with small mammal hosts appears to have increased, seriously threatening public health (Meerburg et al., 2009). This situation may be due to the peridomestic habits of some rodent species that often live in close proximity to human settlements. This situation implies that rodents may be an important link for transmitting infections among wildlife, humans and livestock (Mills, 2011).

Rickettsia spp., a representative genus group of pathogenic or non-pathogenic agents, are intracellular bacteria. *Rickettsia rickettsii*, a bacterium belonging to the *Rickettsiaeceae* family, is the causative agent of Brazilian spotted fever, the most severe and most frequently reported rickettsial illness in Brazil. Rickettsial illness is transmitted to humans by tick bites, and the primary vector is *Amblyomma sculptum*. In a few areas in southeastern Brazil, *Amblyomma aureolatum* replaces *A. sculptum* as the primary vector (Katz et al., 2009; Ogrzewalska et al., 2012). Since the first studies of *Rickettsia* in wild mammals published in the 1950s, publications about these proteobacterial infections have been largely restricted to domestic animals and arthropods (Dantas-Torres et al., 2012; Horta et al., 2004; Lemos et al., 1996; Magalhães, 1953; Milagres et al., 2010; Soares et al., 2015).

Bartonella spp., an intracellular hemotropic bacterium that grows fastidiously, is transmitted mainly by flea bites for a range of mammalian hosts, including rodents, cats, dogs, humans and bats (Chomel and Kasten, 2010; Harms and Dehio, 2012). In Brazil, little information is available about the diversity of *Bartonella*. Up until now, *B. henselae, B. quintana, B. clarridgeiae, B. vinsonii* subsp. *berkhoffii* and *B. vinsonii subsp. arupensis* were detected infecting humans and animals (Costa et al., 2005; Diniz et al., 2007; Favacho et al., 2015; Gonçalves et al., 2016; Pitassi et al., 2015).

* Corresponding author at: Laboratório de Hantaviroses e Rickettsioses, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Avenida Brasil 4365, Manguinhos, Rio de Janeiro, 21045-900, Brazil.
E-mail address: rozental@ioc.fiocruz.br (T. Rozental).

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Coxiella burnetii, the causative agent of Q fever, is transmitted to both human and animals by inhalation of aerosols or contaminated excreta materials as milk, feces, urine, saliva and products of conceptions (placenta, vaginal secretions, amniotic fluids) derived from infected animals. Ticks are suspected having a role in the transmission of the bacteria among wild vertebrates, specially rodents (Porter et al., 2011). There is a hypothesis that ticks could transmit *Coxiella* from infected wild animals to domestic naive animals (Hirai and To, 1998). In Brazil, despite the existence of serologic evidence of Q fever since the 1950s, reports of this zoonosis have been rare (Borges, 1962; Brandão et al., 1953; Epelboin et al., 2016; Lemos et al., 2011; Mares-Guia et al., 2016; Ribeiro do Valle et al., 1955; Rozental et al., 2012; Travassos et al., 1954).

Therefore, *Bartonella* spp., *C. burnetii* and *Rickettsia* spp. infect a wide range of arthropods species and domestic and wild vertebrates, including mainly small, wild rodents. The diseases caused by these pathogens in humans may be severe and characterized by a widespread geographic distribution. Here, we investigate the presence of these emerging zoonotic bacteria in rodent spleen tissue samples trapped in eight municipalities of the state of Rio de Janeiro, Brazil.

2. Materials and methods

2.1. Study area and sample collection

This study was conducted in the state of Rio de Janeiro in southeastern Brazil. Between March 2007 and April 2012, we trapped rodents in eight municipalities in Rio de Janeiro state: Angra dos Reis (23°0′36″S, 44°19′6″W), Cantagalo (21°58′52″S, 42°22′05″W), Itaboraí (22°44′50″S, 42°51′31″W), Paraty (23°13′0″S, 44°43′4″W), Piraí (22°38′1″S, 43°54′17″W), Teresópolis (22°24′44″S, 42°57′59″W), Três Rios (22°7′6″S, 43°12′34″W) and Valença (22°14′44″S, 43°42′01″W) (Fig. 1).

At each collection site, wild rodents were live-trapped for five consecutive nights. The identification of wild-caught rodents was based on external and cranial morphology, karyotype analysis or mitochondrial DNA sequencing of the *cytochrome b* (*cyt b*) gene (Bonvicino et al., 1996; Bonvicino and Moreira, 2001; Oliveira and Bonvicino, 2006; Patton et al., 2015). Tissue samples were obtained in accordance with recommended safety procedures and followed previously established standard protocols (Lemos and D'Andrea, 2014; Mills et al., 1995). Voucher specimens were deposited at the National Museum of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil and the Laboratory of Biology and Parasitology of Wild Mammals Reservoirs IOC/FIOCRUZ, Rio de Janeiro, Brazil.

2.2. Nucleic acid extraction

Bacterial DNA was extracted from spleen samples using a commercial kit (QIAamp DNA Mini Kit, QIAGEN, Valencia, CA, USA), according to the manufacturer's instructions; handling was performed in a laminar flow biosafety cabinet (Veco Biosafe, Veco, Campinas, SP, Brazil). A portion of each animal's spleen (10 mg) was individually crushed in a sterile Eppendorf tube. DNA from each sample was eluted in a final volume of 100 μl AE Buffer. Genomic DNA was stored at −20 °C until analysis.

2.3. PCR amplification

Once the DNA was extracted, it was used in conventional PCR template assays to detect *Bartonella* spp., *C. burnetii* and *Rickettsia* spp. (Table 1). The DNA samples were screened by PCR targeting the *gltA* and *rpoB* gene to detect *Bartonella* spp. *Coxiella burnetii* bacterial DNA was detected with *C. burnetii*-specific primers designed to amplify the IS1111 gene, derived from a transposon-like repeat region of the genome of the bacteria. *Rickettsial* DNA was detected using a genus-specific PCR targeting the partial sequence of the *gltA* gene. Samples yielding visible PCR products were further tested with primers targeting the rickettsial 190-kDa outer membrane protein gene (*OmpA*), specific for spotted fever-group rickettsiae.

DNA amplification was carried out using Taq Platinum DNA Polymerase for *C. burnetii* and *Bartonella* spp.; for *Rickettsia* spp., AmpliTaq Gold DNA Polymerase was performed. The size of the DNA sample varied as a function of the primer used (3 μl *Bartonella* spp.; 3 μl for PCR 1 and 2 μl for PCR 2, *Rickettsia* spp.; 4 μl for PCR 1 and 2 μl for PCR 2, *C. burnetii*). The reaction contained 2.5 μl of 10× PCR buffer, 0.6 μl of 10 mM of each primer, 0.75–2 μl of 50 mM $MgCl_2$, 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). PCR amplification success was verified by migration in 1.5% agarose gel, stained with GelRed™ (Biotium, Hayward, CA, USA).

2.4. DNA sequencing

After DNA purification, using Illustra GFX PCR DNA and Gel Band Purification® kit (GE Healthcare, Buckinghamshire, UK), direct nucleotide sequencing amplicon was performed using BigDye Terminator v3.1 Cycle Sequencing kit and for purification 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, 96 capillaries (Applied Biosystems) and the nucleotide sequences were analysed using MEGA6 software (downloaded from www.megasoftware.net). A consensus sequence for each bacterial genome was derived from contiguous sequences assembled with the same software.

3. Results

3.1. Rodent trapping

A total of 131 rodents belonging to 18 species were captured. The Cursorial Grass mouse, *Akodon cursor*, was the most abundant species (32 specimens), followed by the House mouse, *Mus musculus*, (19), the Black-footed Colilargo, *Oligoryzomys nigripes* (16), the Striped Delomys, *Delomys dorsalis* (14), the Atlantic Forest Hocicudo, *Oxymycterus dasytrichus* (12), the Russet Euryoryzomys, *Euryoryzomys russatus* (7), the São Paulo Spiny rat, *Trinomys iheringi* (6), the Atlantic Water rat, *Nectomys squamipes* (5), the Lindberghís Grass mouse, *Akodon lindberghi* (5), the Rio de Janeiro Spiny rat, *Trinomys dimidiatus* (4), the Montane Grass mouse, *Akodon montensis* (3), the Flavescent Colilargo, *Oligoryzomys flavescens* (2), the Iheringís Brucie, *Brucepatersonius iheringi* (1), the Delicate Vesper mouse, *Calomys tener* (1), the Hairy-tailed Akodont, *Necromys lasiurus* (1), the Atlantic Forest Oecomys, *Oecomys catherinae* (1), the Spy Hocicudo, *Oxymycterus delator* (1) and the Sky Rhipidomys, *Rhipidomys itoan* (1) (Table 2) (Patton et al., 2015). All species were known to inhabit the Atlantic Forest, and these rodents occupied different habitats and had different habits (terrestrial, scansorial, semiaquatic, semifossorial). Many of the rodents were found frequently in remnant forest fragments and disturbed areas near human dwellings.

3.2. Detection of Bartonella spp.

Bartonella DNA was detected in 23 animals (17.6%) in six different municipalities with the highest prevalence in Valença (8/23; 34.8%). Although *A. cursor* was the most frequent host of *Bartonella*