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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

Mariana Campos Fontalvo[a], *Alexsandra Rodrigues de Mendonça Favacho*[b], *Andreina de Carvalho Araujo*[a], *Naylla Mayana dos Santos*[a], *Glauber Meneses Barboza de Oliveira*[a], *Daniel Moura Aguiar*[c], *Elba Regina Sampaio de Lemos*[b], *Mauricio Claudio Horta*[a,*]

[a] *Universidade Federal do Vale do São Francisco (Univasf), Laboratório de Doenças Parasitárias, Petrolina, PE, Brazil*
[b] *Instituto Oswaldo Cruz (FIOCRUZ), Laboratório de Hantaviroses e Rickettsioses, Rio de Janeiro, RJ, Brazil*
[c] *Universidade Federal de Mato Grosso, Laboratório de Virologia e Rickettsioses, Cuiabá, MT, Brazil*

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ABSTRACT

This study verified the occurrence of *Bartonella* spp. in dogs, cats, wild mammals and their ectoparasites in Petrolina and Lagoa Grande Counties, Pernambuco, located in a semi-arid region in Northeastern Brazil. Anti-*Bartonella* spp. antibodies were detected by indirect immunofluorescence assay (IFA) in 24.8% of dogs (27/109) and in 15% of cats (6/40). *Bartonella* sp. DNA was identified by PCR performed on DNA extracted from blood and ectoparasites using primers targeting *Bartonella* sp. *gltA* and *ribC* genes in 100% (9/9) of *Pulex irritans* from *Cerdocyon thous*, 57.4% (35/61) of *P. irritans* from dogs, 2.3% (1/43) of *Ctenocephalides felis felis* from dogs, 53.3% (24/45) of *C. felis felis* from cats, and 10% (1/10) of *Polyplax* spp. from *Thrichomys apereoides*. DNA sequencing identified *Bartonella clarridgeiae* and *Bartonella henselae* in *C. felis felis* from cats, *Bartonella rochalimae* in *P. irritans* from dog and *C. thous*, and *Bartonella vinsoni berkhofii* in *P. irritans* from dog.

* *Corresponding author.*
E-mail address: horta.mc@hotmail.com (M.C. Horta).
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Introduction

Bartonella spp. comprises a group of emerging pathogens that are prevalent in a large variety of vertebrates and cause diversified symptoms and clinical manifestations.[1] Transmission predominantly occurs via a vector, and fleas, hematophagous lice, and ticks have confirmed vector competence.[2] *Bartonella* DNA has also been isolated from mites.[3]

Bartonella henselae is the main causal agent of bartonellosis in humans. Although cats are a major reservoir for human infections with these *Bartonella* species, this agent can infect many mammalian hosts. Thus, rodents and other wild mammals can act as natural reservoirs for numerous species of these bacteria and the presence of the bacteria have been described in these animals on five continents.[3–7]

In Brazil, the circulation of the agent has been demonstrated in humans, cats, dogs, and wild animals using serological and molecular techniques. However, little information is available about the occurrence of *Bartonella* in this country, and to date no study has verified the occurrence of *Bartonella* spp. in the Caatinga biome. Therefore, in the present study we investigated the circulation of *Bartonella* spp. in domestic mammals (dogs and cats), free-ranging small wild mammals (rodents, marsupials, and canids) and their ectoparasites (fleas, ticks and hematophagous lice) in the Caatinga biome.

Materials and methods

Study site

This study was conducted in the Petrolina (9°19′41″ S, 40°33′30″ W) and Lagoa Grande (8°40′1″ S, 40°8′42″ W) municipalities, State of Pernambuco, Brazil (Fig. 1). This region has semi-arid tropical weather and is part of the Caatinga biome in Northeastern Brazil.[8] The municipalities are located in the region of the São Francisco Valley and represent two distinct areas within a degraded environment.

Sample collection

A total of 77 small mammals were trapped in eight trials from August 2014 to May 2015. Each trial was in a different area, with four in each municipality and two in each season of the year (spring, summer, autumn, and winter). Animals were chemically immobilized as recommended by Mares-Guia.[9] Blood samples were collected from these animals via the caudal vein or intra-cardiac puncture. Additionally, ectoparasites were collected from the animals and stored in 1.5 mL microtubes containing absolute ethyl alcohol (C_2H_5OH) at −20 °C prior to laboratory analysis. After collection of biological materials, the animals were marked by cutting the hair from the sacral region and were set free in the same point of capture after full recovery of consciousness.

Domicile dogs and cats were analyzed in rural dwellings around each collection area. Information including age, gender, county of origin, tick, flea and/or louse presence, history of ectoparasitism, and access to the forest was obtained. Blood samples were collected from the cephalic vein in tubes containing EDTA, properly identified, and centrifuged at 3000 rpm for 15 min. Plasma and whole blood were stored in 1.5 mL microtubes at −20 °C prior to analysis.

Ticks, fleas, and lice were collected and conserved in absolute ethanol and stored at room temperature until identification, according to Linardi and Guimaraes,[10] Barros-Battesti, Arzua and Bechara,[11] and Pereira et al.,[12] and molecular testing.

All procedures followed the ethical standards of animal experimentation established by the Committee on Ethics and Studies and Research at the Federal University of São Francisco Valley – CEDEP/Univasf (protocol number 9/021014) and by the Brazilian Institute of Environment and Renewable Natural Resources - IBAMA (protocol number 45764-1) according to the recommendations and laws regarding the maintenance of animal welfare.

Indirect immunofluorescence assay (IFA)

Plasma samples from dogs and cats were subjected to indirect immunofluorescent antibody assay (IFA) (Bion, IL, USA) to detect anti-*Bartonella* sp. antibodies as recommended by the manufacturer. A cutoff value of 1:64 was used.[13]

Polymerase chain reaction (PCR)

DNA was extracted from whole blood of dogs, cats, and wild animals and from individual ticks using a commercial kit (Promega, Madison, WI, USA) as recommended by the manufacturer. A negative DNA extraction control consisting of 100 μL of sterile distilled water was included in each batch of samples. Whole fleas and lice were individually subjected to DNA extraction by boiling at 100 °C for 20 min.[14]

All samples were individually processed by PCR using the primers Bhcs.781p (5′-GGG GAC CAG CTC ATG GTG G-3′) and Bhcs.1137n (5′-AAT GCA AAA AGA ACA GTA AAC A-3′), which amplified a 380-bp fragment of the citrate synthase gene (*gltA*) of *Bartonella* spp., and BARTON-1 (5′-TAA CCG ATA TTG GTT GTG TTG AAG-3′) and BARTON-2 (5′-TAA AGC TAG AAA GTC TGG CAA CAT AAC G-3′), which amplified a 580-bp fragment of the riboflavin synthase C gene (*ribC*). The PCR reactions were performed as described previously.[15,16] The PCR products were stained with ethidium bromide and visualized by electrophoresis in a 1.5% agarose gel.

PCR products of the expected amplicon size were purified, and their forward and reverse nucleotide sequences were subjected to direct sequencing using the ABI Prism BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, CA, USA). Partial sequences obtained from this study were submitted to BLAST (Basic Local Alignment Search Tool) analysis to determine similarities to other *Bartonella* species sequences available in the GenBank database.[17]

Statistical analysis

Categorical variables are described as proportions with the respective 95% confidence interval, and compared by the Chi-square test (X^2) using the software Epi Info 7.1.