

Molecular evidence of *Bartonella* spp. in free-living opossums (Didelphimorphia: Didelphidae) from peri-urban Atlantic Forest fragments of Brazil

Amanda Alcantara¹, Helena Thoma², Sabrina Campos¹, Rosemeri Teixeira¹, Hans Lima¹, Jeferson Pires³, Renata Moraes⁴, and Aline Souza¹

¹Universidade Federal Fluminense

²Universidade Federal do Rio de Janeiro

³Universidade Estacio de Sa - Campus Reboucas-Rio Comprido

⁴Universidade de Vassouras

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Abstract

Many emerging infectious diseases are zoonotic and transmitted by an arthropod vector; thus, the Global One Health perspective is necessary for a better understanding of these diseases. Bartonellosis, one of those emerging infectious diseases, is caused by bacteria of the genus *Bartonella*, and can have different clinical manifestations in humans and animals. Opossums and their ectoparasites have been linked to human-wildlife conflicts. Knowing this, we aimed to evaluate infection by *Bartonella* spp. in free-living opossums seized or rescued from Atlantic Forest biome of Rio de Janeiro (southeastern Brazil). Whole blood was obtained from seven *Didelphis aurita* and eight *D. albiventris*, and DNA was assessed by conventional and quantitative polymerase chain reactions (PCR and qPCR). Positivity was achieved in 40.0% (PCR) and 46.7% (qPCR) of the samples, revealing, possibly for the first time, the presence of *Bartonella* spp. DNA in opossums from peri-urban areas of Atlantic Forest biome. Studies should continue, in order to elucidate synanthropic mammals importance in bartonellosis epidemiology.

Main document

Introduction

Opossums (*Didelphis* spp.) are neotropical marsupials of the order Didelphimorphia, family Didelphidae. Among the species that occur in Brazil, two can be easily found in the great Brazilian biomes, *D. aurita* (black-eared opossum) and *D. albiventris* (white-eared opossum) (Aléssio et al., 2005; Moura et al., 2009; Paglia et al., 2012). These mammals have opportunistic habits, and can live in wild, rural and urban environments, maintaining contact with other animals and human beings (Lucheis et al., 2009; Tarragona et al., 2011). Notably, white-eared opossum is widely distributed in Brazilian biomes and is strongly adapted to highly degraded areas, as well as urban forest fragments (Aléssio et al., 2005).

This type of behavior, called synanthropic, places opossums as a key group in the connection between wild and domestic epidemiological cycle of emerging parasites and microorganisms (Forzanari et al., 2011; Tarragona et al., 2011). For example, opossums and its most common ectoparasite, *Ctenocephalis felis* flea, have been epidemiologically linked to flea-borne pathogens transmission and human-wild life conflicts in residential environments (Krueger et al., 2016).

Studies support that cat flea (*C. felis*) is the major competent vector for *Bartonella henselae*, *B. clarridgeae*, *B. koehlerae*, and potentially *B. quintana* (Pennisi et al., 2013; Zangwill, 2013). Those species among others

belong to the α -proteobacteria, are hemotropic, short and pleomorphic Gram-negative bacteria that cause intra-erythrocytic infections in animals and humans (Jacomó et al., 2002; Deng et al., 2012). *Bartonella* species appear to be highly adapted to one or few reservoir hosts, within which these bacteria have coevolved and thence remain for long periods in bloodstream (Kaiser et al., 2011; Harms and Dehio, 2012).

Until 1990, only two diseases were linked to *Bartonella* species, Carrión disease (*B. bacilliformis*) and Trench fever (*B. quintana*) (Karem et al., 2000). Since then, many other Bartonellae have been identified as causative agents of diseases in animals and humans (Iannino et al., 2018). Importantly, the list of mammalian-adapted *Bartonella* species continues to grow, as well as the number of human disease associated with genus *Bartonella* (Kaiser et al., 2011; Harms and Dehio, 2012; Buffet et al., 2013).

Currently, Cat-scratch disease (CSD) is the most common human disease caused by *Bartonella* species, including *B. henselae*, and *B. clarridgeiae* (Iannino et al., 2018). Clinical manifestation of CSD depends on patient immune status, and pet cats and dogs often develop similar manifestations as humans (Chomel et al., 2009; Breitschwerdt et al., 2010; Beerlage et al., 2012). Typical CSD denotes a self-limiting illness characterized by fever and lymphadenopathy; however, atypical manifestations can occur (Jacomó et al., 2002; Breitschwerdt et al., 2010; Zangwill, 2013).

Bartonella genus has already been isolated or detected in domestic and wild animals worldwide, including bats, birds, canids, cattle, deer, felids, horses, marine mammals, rodents, sheep and reptiles (Deng et al., 2012). Little is known about wild reservoirs of these bacteria in Brazil, but recently, a study reported *B. henselae* and *B. clarridgeiae* in domestic cats from an Atlantic Forest area of Rio de Janeiro state (RJ), where humans, domestic, synanthropic and wild animals can easily interact (Silva et al., 2019). Opossums are often identified as likely maintainers of infectious agents with zoonotic potential (Abel et al., 2000; Ruiz-Piña and Cruz-Reyes, 2002; Fornazari et al., 2011; Pena et al., 2011).

Considering the growing spectrum of animal reservoir hosts for *Bartonella* species, and given the presence of *Bartonella* spp. in RJ state, this study aimed to evaluate natural infections by those bacteria in free-ranging opossums that lived in peri-urban areas of the Atlantic Forest of RJ, Brazil.

Materials and Methods

This cross-sectional study was approved by Ethics Committee on Animal Use (CEUA) of Universidade Federal Fluminense (UFF) (8405150718) and licensed by Biodiversity Information and Authorization System (SISBIO/IBAMA63464-2).

Adult and young free-living opossums were included in this study. Animals were seized or rescued by environmental authorities and samples were collected at wild life rehabilitation facilities of Universidade Estácio de Sá (22° 58' 46" S, 43° 27' 24" W) (eight *D. aurita*) and Universidade de Vassouras (22° 24' 14" S, 43° 39' 45" W) (seven *D. albiventris*), both located in RJ state, Brazil. Animals were physically restrained and venipuncture was proceeded from ventral caudal vein, obtaining up to 1.0mL of blood, which was immediately placed in ethylenediaminetetraacetic acid (EDTA) tubes.

DNA extraction was performed with the Master Pure™ DNA Purification Kit for Blood Version (Epicentre®), Madison, Wisconsin, USA). In order to avoid false negative results, DNA samples were tested for presence of amplifiable DNA using the primer set GAPDH-F (5'-CCTTCATTGACCTCAACTACAT-3') and GAPDH-R (5'-CCAAAGTTGTCATGGATGACC-3'), resulting in amplification of a 399 bp fragment of the housekeeping *gapdh* gene, encoding glyceraldehyde-3-phosphate dehydrogenase enzyme, which is present in all mammals (Birkenheuer et al., 2003).

Subsequently, presence of bacterial DNA was tested by conventional and quantitative polymerase chain reactions (PCR and qPCR) targeting a 365 bp fragment of *Bartonella* spp. citrate synthase (*gltA*) gene with primers BhCS781p (5' GGGGACCAGCTCATGGTGG 3') and BhCS1137n (5' AATGCAAAAAGAA-CAGTAAACA 3'), as previously proposed (Norman et al., 1995). Positive (purified DNA of *B. henselae* extracted from a naturally infected cat) and negative (nuclease-free water) controls were used for each reaction. Amplicons underwent electrophoresis on 1.5% agarose gel stained with ethidium bromide.

Results and discussion

Conventional PCR identified *Bartonella* spp. DNA in 40% of opossums (6/15). All positive samples belonged to *D. aurita* species, representing 85.7% among evaluated samples of this species (6/7). Using the qPCR assays, we found 46.7% (7/15) of positive results. It is important to note that simultaneous positivity in both PCR and qPCR assays was observed in the same specimens of *D. aurita*. One animal, of *D. albiventris* species, was negative in conventional PCR and positive in qPCR.

This is, for the best of author's knowledge, the first report of natural *Bartonella* spp. infection in opossums in areas of Atlantic Forest biome at RJ state. Although not expected, the high frequency of PCR positive animals may be justified, since other studies have successfully demonstrated presence of *Bartonella* spp. in wild mammals and ectoparasites in different Brazilian biomes (Favacho et al., 2015; Sousa et al., 2018). In addition, *C. felis*, the main vector involved in *Bartonella* spp. transmission among cats, has been observed in opossums from RJ (Oliveira et al., 2010). It is also necessary to emphasize the occurrence of *B. henselae* and *B. vinsoni* subsp. *arupensis* in *C. felis* collected from the Virginia opossum, *D. virginiana*, from California, USA (Reeves et al., 2005; Krueger et al., 2016). According to Reeves et al., 2005, the presence of *B. henselae* in cat fleas collected from opossums, implicates both *C. felis* and opossums in maintaining this pathogen.

Recently, Silva et al., 2019 revealed the natural infection by *B. henselae* and *B. clarridgeiae* in stray cats that illegally inhabited a preserved area of Atlantic Forest in RJ State. According to the authors, study area harbored a wide variety of fauna and flora, as well as allowed human visitation and ecotourism. Although cats are important hosts for *B. henselae*, *B. clarridgeiae*, and *B. koehlerae*, there are CSD patients that deny the occurrence of bite, scratch or even contact with cats, making the possibility of transmission from other animal hosts more and more probable (Breitschwerdt, 2014).

In this context, roaming animal population, such as that found by Silva et al., 2019, can be a source of infection for pet cats. Added to this, we suggest that in epidemiological cycle of bartonellosis, cat flea is probably the bridge that links wild mammals (such as *D. aurita* and *D. albiventris*) to domestic or stray cats and these latter to humans.

Temperature and relative humidity are relevant factors for successful reproduction, development, and survival of fleas (Dryden et al., 1994), and, apparently, cats are more parasitized by fleas during summer and autumn months (Farkas, 2009). Given that, several places of Atlantic Forest biome in RJ may provide a favorable scenario for interaction among *Bartonella*-infected cats, their fleas and susceptible opossums.

We concluded that Bartonellae have many important human-animal-environment aspects. For this reason, it is crucial to adopt the One Health strategy for emerging zoonosis management in stray animal populations and synanthropic animals, involving collaborative work of environmentalists, medical and veterinary researchers. The study continuity is necessary to identify *Bartonella* species involved, potential risk factors and epidemiological function of synanthropic mammals (including opossums) as reservoirs in maintenance and transmission of these bacteria.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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