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SHORT COMMUNICATION
Prevalence of *Bartonella* species DNA and antibodies in cats (*Felis catus*) submitted to a spay/neuter program in Rio de Janeiro, Brazil

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The prevalence of *Bartonella* species DNA and antibodies for *Bartonella henselae* were studied in 40 clinically healthy cats (*Felis catus*, Linnaeus 1758) submitted to a spay/neuter program in Rio de Janeiro, Brazil. Additionally, the prevalence of *Bartonella* species DNA was investigated in the fleas found parasitizing the subject cats. For this purpose, blood samples were obtained from all cats, and DNA extraction was performed on the blood, and blood clotted samples, as well as on pools of fleas obtained from them. Antibodies for *B henselae* were detected on serum samples. *Bartonella* species DNA was detected in 17 cats, whereas serum reactivity for *B henselae* was found in 19. A total of 20 cats were flea-infested and nine of these 20 had *Bartonella* species DNA in their blood. In four of the 20 flea-infested cats, *Bartonella* species DNA was detected in the fleas obtained from those cats, but only one of these four cats had *Bartonella* species DNA in its blood.

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The *Bartonella* genus comprises Gram-negative, facultative intraerythrocytic bacteria,[1] belonging to the α2 *Proteobacteria* subgroup.[2] The genus is responsible for causing several diseases of cats and humans.[3] *Bartonella* species are distributed worldwide[4] and are considered emerging pathogens,[3,5] both in veterinary and human medicine.[3] *Ctenocephalides felis* is commonly found infesting cats and is known to harbor bacteria of the genus *Bartonella*.[6] Fleas may transmit *Bartonella henselae* infections amongst cats and may also play a role in the transmission of *Bartonella* species between cats and people.[6,7] *Bartonella* species are arthropod-borne pathogens, favoring high temperature and humidity;[8] therefore, global climatic changes may support the increase and dispersion of vector populations, and consequently alter patterns of infections by arthropod-borne pathogens in susceptible animals. Human bartonellosis is reported worldwide[3,4] and has been specifically reported in Rio de Janeiro.[9] The transmission of this group of bacteria involves arthropods that benefit from the warm and humid environments[10] that are typical of this city.[11] In this study, the circulation of *Bartonella* species was studied in cats (*Felis catus*) living in the city of Rio de Janeiro, Brazil, to generate updated data to support risk analysis.

Forty clinically healthy cats were included in this study, 13 males and 27 females, between 4 and 24 months of age ($\overline{X} = 10.38$ and standard deviation = 7.34), presented to a free spay-neuter program (Desengata program) held in the city of Rio de Janeiro, Brazil. Blood samples were obtained from all cats, both anticoagulated and clotted. The cats were then combed (Fleacomb; Lambert Kay Twinco, USA) for 5 min, over a collecting basin, to retrieve any ectoparasites. All collected fleas were frozen, identified by classical entomological criteria, and kept at −20°C, in pools of one to four fleas until processing. The QIAamp DNA Mini Kit (Qiagen; Hilden, Germany) was used for DNA extraction from all pools of fleas, whole blood and blood

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clots. *Bartonella* genus DNA was identified in blood and blood clots using a polymerase chain reaction (PCR) and the specific primers CAT1 [5′-GATTCAATTGGTTT GAA(G/A)GAGGCT-3′] and CAT2 [5′-TCACATCAC CAGG(A/G)CGTATTC-3′] for selected genes (*htr*A, 60 kDa heat shock protein).[12] DNA fragments were amplified using 8 μl of the eluted DNA solution in a total of 25 μl of PCR mix. A known positive control was used for every PCR and distilled water was used as a negative control. Serum samples were tested for detection of immunoglobulin G (IgG) antibodies for *B henselae* using indirect immunofluorescent assay (IFA) (Bion; Illinois, USA) (cutoff = 1:64). Cats were considered to harbor the bacteria whenever bacterial DNA was detected in blood or clot samples.

Most cats included in this study were shown to have been previously exposed to the genus *Bartonella* by detection of either bacterial DNA in blood or clot samples or by antibody for *B henselae* detection. *Bartonella* species DNA was detected in blood or clot samples of 17 cats (42.5%) whereas serum reactivity for *B henselae* was shown in the serum samples of 19 cats (47.5%). DNA and antibodies were detected simultaneously in samples of nine cats (22.5%). The prevalence of cats harboring bacterial DNA was similar to those found in previous reports,[13] particularly when similar environmental conditions (temperature and humidity) were considered.[8] There were 21 cats (52.5%) in which antibodies for *B henselae* could not be detected; of these 21 cats, bacterial DNA was detected in blood or clot samples of eight (38%), suggesting recent infection, infection by other *Bartonella* species not cross reactive with *B henselae*, immunosuppression or even a result of the low immunostimulatory properties of *Bartonella* species lipopolysaccharide (LPS).[14] Seroreactive animals had titers of 1:64 (1/19, 5.3%), 1:128 (8/19, 42.1%), 1:256 (5/19, 26.3%), 1:512 (3/19, 15.8%), 1:1024 (2/19, 10.5%). The concentration of detectable antibodies should not be interpreted as current *B henselae* infection in cats, given that cross-reactions may occur with other *Bartonella* species.[15] Half of the cats (20) were infested with fleas and nine of these 20 had *Bartonella* species DNA in their blood. Out of those 20 cats, 13 were infested by 1−5 fleas and seven cats by 6−22 fleas. A total of 119 fleas were collected from the cats, and the flea burden varied from 1−22 fleas/animal ($\overline{X} = 5.95$ and standard deviation = 6.44). *Bartonella* species DNA could be detected in the flea pools of four of the 20 flea-infested cats. These four cats presented less than four fleas each, resulting in only one pool per cat. Among the four cats that presented a PCR-positive pool of fleas, only one was shown to have bacterial DNA in its blood. The discrepancy between the prevalence of *Bartonella* species DNA in the blood of cats (42.5%) and the frequency of DNA detection in the fleas found on those cats suggests that the concentration of bacterial DNA in fleas may have been insufficient for its detection by the techniques used in this study. Another possibility is that these cats could have acquired the fleas on location from other uninfected cats, and thus there was not enough time for sufficient bacterial multiplication inside the flea following their recent feeding on infected cats. In conclusion, this pilot study has demonstrated a high prevalence of *Bartonella* species infection in cats, updating information on its circulation in the city of Rio de Janeiro. These results indicate that further studies on larger numbers of animals are needed to determine the overall prevalence of *Bartonella* species in Rio de Janeiro, as this has important implications for veterinary and human health.

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