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Detection of serum antibodies against *Bartonella* species in cats with sporotrichosis from Rio de Janeiro, Brazil

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Abstract

Cat scratch disease is a zoonosis caused by *Bartonella* species, transmitted to humans through scratches or bites from infected cats and via direct contact with infected feces. Sporotrichosis, caused by the fungal complex *Sporothrix*, is transmitted by traumatic inoculation of the fungus. Cats are important in zoonotic transmission. Serum samples from 112 domestic cats with sporotrichosis and 77 samples from healthy cats were analyzed by indirect immunofluorescence assay (IFA), using the commercial kit *Bartonella henselae* IFA IgG (Bion). The presence of antibodies against feline leukemia virus (FeLV) and of feline immunodeficiency virus (FIV) core antigens was detected using the commercial kit Snap Combo FIV–FeLV (Idexx). The group of animals with sporotrichosis contained 93 males with a median age of 22 months, eight (7.1%) of which were positive for FIV and 15 (13.4%) for FeLV. The group of animals without sporotrichosis contained 36 males with a median age 48 months, 10 (13.0%) of which were positive for FIV and eight (10.4%) for FeLV. Of the 112 cats with sporotrichosis and 77 cats without mycosis, 72 (64.3%) and 35 (45.5%), respectively, were IFA reactive. No association was found between age, sex, FIV/FeLV and the presence of antibodies to *Bartonella* species. The results suggest that the study population can be considered a potential source of zoonotic infection for both diseases.

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Introduction

Cat scratch disease (CSD) is a zoonosis caused by members of the genus *Bartonella*, which are small fastidious Gram-negative, rod-shaped bacteria that parasitize mammalian erythrocytes and endothelial cells.[1,2] *Bartonella* species are distributed worldwide and are responsible for several diseases in humans. More than 30 species and subspecies have been identified to date. Although *Bartonella henselae* is the predominant cause of CSD in South America,[3] there is increasing evidence that cats may be involved in the transmission of *Bartonella quintana* to humans.[4] The cat flea *Ctenocephalides felis* is an important vector of these agents.[5] Cats do not generally exhibit clinical signs during infection.[3,6] The clinical spectrum of human infection has expanded continually and includes fever, lymphadenopathy, ocular manifestations, and neurological and cardiovascular complications.[3,7–9]

Sporotrichosis, which is caused by a fungus of the genus *Sporothrix*,[10] is transmitted by traumatic inoculation of soil, plants and organic matter contaminated with the fungus.[11] Zoonotic transfer has been on the rise in Rio de Janeiro since 1998, through contact with lesion

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exudates, bites or scratches from sick cats.[12] Feline sporotrichosis has a broad clinical spectrum ranging from subclinical infection to fatal systemic forms.[13] Humans present four different clinical forms of sporotrichosis: fixed cutaneous, lymphocutaneous, extracutaneous and disseminated. The last form is rare, but closely associated with immunocompromised individuals.[14]

In Brazil, the number of humans and cats reported to suffer from bartonellosis and sporotrichosis has increased in recent years.[12,15,16] In the case of humans, a differential diagnosis is needed owing to the lymphocutaneous form and reports of previous contacts with cats common to both diseases.

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are among the most common infectious diseases of cats. The two viruses depress the immune system, favoring infections by other agents.[17] *Bartonella* species infection in cats does not seem to be influenced by immunosuppressive viral infections in general, but latent FeLV infection may predispose cats to *B henselae* infection or persistence.[18] There are few studies regarding the association between infection by FIV/FeLV and sporotrichosis, and no association has been established.[13,19]

The main objective of this study was to investigate the presence of antibodies against *Bartonella* species in cats with sporotrichosis in Rio de Janeiro, Brazil.

Materials and methods

Serum samples of 112 domestic cats diagnosed with sporotrichosis at the Laboratory for Clinical Research in Domestic Animal Dermatozoonoses (Lapclin-Dermzoo), Evandro Chagas Clinical Research Institute (IPEC), Oswaldo Cruz Foundation (FIOCRUZ) in Rio de Janeiro, Brazil were collected between October 2007 and August 2011. These cats were part of a clinical trial for the therapeutic treatment of sporotrichosis, the main exclusion criteria of which were that the cats were younger than 6 months and older than 9 years, pregnancy, and prior use of systemic antifungal or steroids agents. A group of 77 cats without sporotrichosis from the same region was also included.

The samples were aliquoted, placed in small plastic vials, and stored at −20ºC until required for analysis. The serological analysis of *Bartonella* species was performed using a *B henselae* immunofluorescence assay (IFA) IgG commercial kit (Bion) at the Laboratory of Hantaviruses and Rickettsioses (LHR), Oswaldo Cruz Institute (IOC)/FIOCRUZ. A screening cut-off titer of 1:64 was used.[20]

The detection of FIV antigens and FeLV antibodies was performed at Lapclin-Dermzoo, using a Snap Combo FIV/FeLV commercial kit (Idexx), following the manufacturer's instructions.

Frequencies of categorical variables and the median age variable, which did not show normality in the Kolmogorov–Smirnov test, were calculated.

Possible associations between age (≤24 months and >24 months), sex, and status for FIV, FeLV and *Bartonella* species among groups (with or without sporotrichosis) were investigated using Pearson's χ^2 test. Also, the association between these variables and the status of *Bartonella* species in each group was investigated using Pearson's χ^2 test or Fisher's exact test. The difference in median age as a function of seropositivity for *Bartonella* species was verified by the non-parametric Mann–Whitney test, using the statistical program SPSS version 16.0 and a 5% level of significance.

Results

In the association among variables as a function of the group (with or without sporotrichosis), significant differences were observed with regard to age (P <0.001), age group (P <0.001), sex (P <0.001) and serostatus for *Bartonella* species (P = 0.012) (Table 1). Based on these results, we chose to examine the associations with serostatus for *Bartonella* species in the stratified group.

Among the 112 animals with sporotrichosis, 83.03% were males, ranging in age from 6 to 96 months, with a median age of 22 months. In this group, 72 (64.3%) were reactive by IFA for detection of antibodies against *Bartonella* species, eight (7.1%) were positive for FIV and 15 (13.4%) were positive for FeLV. No association was found between the presence of antibodies against *Bartonella* species and sex (P = 0.602), age group (P = 0.349), or serostatus for FIV (P = 0.453) and FeLV (P = 0.710).

In the group of animals without sporotrichosis (n = 77), 46.75% ranged in age from 6 to 96 months, with a median age of 48 months. Thirty-five samples (45.5%) were found to be reactive in the IFA for the detection of antibodies against *Bartonella* species — most of them were females (65.7%, n = 23; P = 0.045). Ten (13.0%) were positive for FIV, and eight (10.4%) for FeLV. No association was found between the presence of antibodies against *Bartonella* species and age group (P = 0.451), or serostatus for FIV (P = 0.497) and FeLV (P = 0.285).

Discussion

This was the first study to investigate the presence of antibodies against *Bartonella* species in a group of cats diagnosed with sporotrichosis. The frequency of reactive animals by IFA was high in both groups, with or without sporotrichosis (64.3% and 45.5%, respectively) when compared with the results of studies conducted in other regions in Brazil.[21–23] In Rio de Janeiro, a high prevalence of *Bartonella* species infection in cats was identified by serological and molecular tests.[16,20]

Cats younger than 6 months have the highest prevalence of infection.[24] The difficulty in obtaining blood samples from healthy and sick cats, especially kittens, was a limitation of this study. This may explain the

Table 1 Association of age, age group, sex and status for feline immunodeficiency virus (FIV), feline leukemia virus (FeLV) and *Bartonella* species infection in groups of cats with or without sporotrichosis

Variables	Categories	Group with sporotrichosis		Group without sporotrichosis		*P*
		n		n		
Age in months, median (IQR)	–	106	22 (12–30)	48	48 (36–60)	<0.001*
Age groups	≤24 months	78	73.6%	8	16.7%	<0.001*
	>24 months	28	26.4%	40	83.3%	
Sex	Male	93	83.0%	36	46.8%	<0.001*
	Female	19	17.0%	41	53.2%	
FIV	Negative	104	92.9%	66	86.8%	0.209
	Positive	8	44.4%	10	13.2%	
FeLV	Negative	97	86.6%	68	89.5%	0.653
	Positive	15	13.4%	8	10.5%	
Bartonella species	Negative	40	35.7%	42	54.5%	0.012*
	Positive	72	64.3%	35	45.5%	

IQR = Interquartile ranges (Q1–Q3)
*P <0.05

absence of association between the presence of antibodies to *Bartonella* species and different age groups.

The IFA is considered the reference test for the diagnosis of previous infection by *Bartonella* species[16] Despite the possibility of cross-reaction with other agents, the high titers of anti-*Bartonella* IgG that were identified in this study reinforce the belief that this is not a heterologous reaction occurring in early infection, while the detection of IgM may be associated with a false-positive result.[16,25,26]

Molecular tests are increasingly used for the diagnosis of infections caused by *Bartonella* species.[21–23] The polymerase chain reaction technique offers advantages in the serological detection of recent infection, when insufficient time may have passed for the induction of antibody synthesis. We cannot ignore the possibility that this occurred with the serum of the animals evaluated here; however, molecular tests could not be performed in this study.

The lack of association between FIV/FeLV infection and the presence of antibodies against *Bartonella* species may have been owing to the small number of animals that tested positive for these retroviruses in the samples under study. The same problem has been reported by other researchers attempting to establish the association between FIV/FeLV infection and sporotrichosis.[13,19]

Conclusions

Bartonella species infection should be included in the differential diagnosis of febrile illnesses in humans associated with lymphadenopathy, particularly with sporotrichosis, a highly prevalent zoonotic disease in Rio de Janeiro.[12] In addition, the cat population of this study should be considered a potential source of human infection for both diseases. Human patients with cutaneous lesions caused by cat scratches or bites should be carefully examined to ensure an accurate diagnosis and, particularly in Rio de Janeiro, doctors should be on the alert for the possibility of co-infection by these two important zoonoses.

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Conflict of interest The authors do not have any potential conflicts of interest to declare.

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