

## Feline Sporotrichosis: Coinfection with *Toxoplasma gondii*, Feline Immunodeficiency Virus and Feline Leukemia Virus in Cats From an Endemic Area in Brazil

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### ABSTRACT

**Background:** Sporotrichosis is an endemic zoonosis in the metropolitan region of Rio de Janeiro caused by fungi included in the *Sporothrix* complex, in which cats are the main source of infection for humans and animals. Coinfections in cats with sporotrichosis from this region, their risk factors and how they affect the treatment outcome in these animals are little known. The objectives of this study were to determine the coinfections of *Sporothrix* spp. with *Toxoplasma gondii*, Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) and to correlate these infections with risk factors and the outcome of sporotrichosis treatment in cats from an endemic area of sporotrichosis in Rio de Janeiro, Brazil.

**Materials, Methods & Results:** It was conducted a cohort study involving 213 cats with definitive diagnosis of sporotrichosis from the metropolitan area of Rio de Janeiro and assisted in the Laboratory of Clinical Research on Dermatозoonosis in Domestic Animals (LAPCLIN-DERMZOO)/Evandro Chagas National Institute of Infectious Diseases (INI)/Oswaldo Cruz Foundation (Fiocruz), in Rio de Janeiro, RJ, Brazil, from November 2007 until February 2011. These animals were monthly evaluated due to sporotrichosis treatment until their sporotrichosis treatment outcomes. In every clinical evaluation, 5 mL of blood were collected in order to obtain the serum, which was stored at -20°C. Information from the animal's medical records have also been collected, such as sex, eating habits, living with other cats, access to the streets, castration, age and the outcome of sporotrichosis treatment. Serological follow-up of anti-*T. gondii* antibodies were performed through indirect hemagglutination assay (IHA) and indirect fluorescent antibody test (IFAT) in all clinical evaluations. The FIV and FeLV antibody detection were made through a rapid immunoassay using the cats' serum samples from the first clinical evaluation. Fisher's exact test was applied to verify associations between *T. gondii*, FIV and FeLV coinfections, the outcome of sporotrichosis treatment and risk factors. To compare IHA and IFAT, the values of total, positive and negative concordances were evaluated. A *P*-value < 0.05 indicated significant associations in the statistical tests. Of the 213 cats, fourteen (6.6%) showed antibodies anti-*T. gondii*, twelve (5.6%) anti-FIV and thirty-five (16.4%) anti-FeLV. There was a concordance of 100% between IFAT and IHA for the serological diagnosis of *T. gondii* infection. No statistical difference was observed between the presence of anti-*T. gondii* antibodies with the FIV and FeLV infections and with the outcome of sporotrichosis treatment (*P* > 0.05). Furthermore there was no significant statistical difference between the presence of anti-*T. gondii* antibodies and the variables sex, eating habits, living with other cats, free access to the street, castration and age (*P* > 0.05). The follow-up of anti-*T. gondii* antibodies showed that in two cats there was a fourfold rise in the titers between two consecutive follow-ups and in one there was seroconversion, which were indicative of acute infection.

**Discussion:** The occurrence of coinfections of sporotrichosis with *T. gondii*, FIV and FeLV was low in cats from the metropolitan region of Rio de Janeiro, Brazil, where sporotrichosis is endemic. This was the first study that determine and follow-up the frequency of anti-*T. gondii* antibodies in a group of cats diagnosed with sporotrichosis. The fact that cats were domiciled with adequate feeding and management, the low frequency of *T. gondii* and the rare cases indicative of acute infection in the study population indicate that these animals are not highly exposed to infection by this protozoan.

**Keywords:** cats, sporotrichosis, toxoplasmosis, retrovirus, risk factors, immunodiagnostics.

## INTRODUCTION

Sporotrichosis is a subcutaneous mycosis caused by the dimorphic fungi included in the *Sporothrix* complex, which affects humans and animal species [9]. *Sporothrix brasiliensis* is the main causative agent in Brazil [7]. An endemic form of zoonotic sporotrichosis in which the cats are the main source of infection for humans occurs in Rio de Janeiro since 1998 [7].

Toxoplasmosis is an important protozoosis whose etiologic agent is *Toxoplasma gondii* [1]. Cats and other felines play an important role in the epidemiology of the infection, since they eliminate oocysts in the environment that may infect humans and warm-blooded animals [6].

The feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) belong to the *Retroviridae* family and affect the immune system of cats, rendering them susceptible to secondary infections [10]. Some authors report that the severity of feline sporotrichosis may be related to immunosuppression caused by coinfection with FIV and FeLV, but it was not confirmed [8,9]. Furthermore, both the seroprevalence and the magnitude of *T. gondii* antibodies were higher in FIV-infected cats [4].

There are few studies regarding coinfections in cats with sporotrichosis from the endemic region of Rio de Janeiro, their risk factors and how they affects the outcome of sporotrichosis. These studies evaluated only coinfections with FIV, FeLV and *Bartonella* species [8,14,15].

The objectives of this study were to determine the coinfections of *Sporothrix* with *T. gondii*, FIV and FeLV and to correlate these infections with risk factors and the outcome of sporotrichosis treatment in cats from an endemic area of sporotrichosis in the state of Rio de Janeiro, Brazil.

## MATERIALS AND METHODS

### Target population

This work involved a cohort study consisting of a convenience sample comprising the serum of 213 cats with sporotrichosis from metropolitan Rio de Janeiro, Brazil, and treated at Laboratory of Clinical Research in Dermatозoonosis in Domestic Animals (LAPCLIN-DERMZOO), Evandro Chagas National Institute of Infectious Diseases (INI)/ Fiocruz in Rio de Janeiro, RJ, Brazil, from November 2007 to February 2011. The animals were treated with ketoconazole (13.5 to 27.0 mg/

kg/day) or itraconazole (8.3 to 27.7 mg/kg/day), underwent monthly clinical and laboratory examinations until the outcome. The treatment outcome was classified as favorable (cats with clinical cure, this means complete remission of cutaneous and extracutaneous clinical signs of sporotrichosis) or unfavorable (cats that showed stagnation, worsening of cutaneous and extracutaneous clinical signs within two consecutive monthly rechecks or death).

### Collection of biological samples

In each monthly clinical follow-up, 5 mL of blood were collected by venopuncture of the jugular vein. After coagulation, the blood samples were centrifuged at 1125 g for 5 min and the serum was separated and stored at -20 C until the time of analysis. These procedures were performed after obtaining the owners' consent. The number of clinical evaluations per animal varied from one to eleven. Of the 213 animals that initially participated in this study, 170 returned for the second clinical follow-up, 136 in the third, 102 in the fourth, 69 in the fifth, 38 in the sixth, 23 in the seventh, 13 in the eighth, 9 in the ninth, 3 in the tenth, and only one in the eleventh follow-up.

### Variables

The following variables were obtained from the animals' medical records: sex, eating habits, living with other cats, access to the streets, castration, age and the outcome of sporotrichosis treatment (favorable and unfavorable). Two hundred and twelve of the 213 cats of this study were analyzed based on their coexistence with other cats, 207 based on age, due to insufficient information in their medical records and 195 based on the outcome of sporotrichosis treatment, because 18 animals lost to follow-up at the beginning of the study. To reduce the inequality of the age variable, the stratification was made based on the small number of cats older than three years, which were allocated to a single category.

### Laboratory techniques

For the serological follow-up of anti-*T. gondii* IgG and IgM antibodies, the sera collected from the cats in each clinical evaluation were examined by the indirect fluorescent antibody test (IFAT). The IFAT was performed as described previously [3] using *T. gondii* RH strain tachyzoites as antigen. Goat anti-cat polyclonal IgG and IgM conjugated to fluorescein isothiocyanate<sup>1</sup> were used. The sera were diluted to 1:16, 1:64, 1:256, 1:1024, and 1:4096 in 0.01 M PBS (pH 7.2). The indirect

hemagglutination assay (IHA) for detection of anti-*T. gondii* IgG antibodies in cat serum was performed with the Imuno-HAI Toxoplasmos@kit<sup>2</sup> according to manufacturer instructions. Cats that showed antibody titers higher than or equal to 16 in IHA and 64 in IFAT were defined as positive. These tests were performed at the Laboratory of Toxoplasmosis of the Oswaldo Cruz Institute/Fiocruz.

Testing for FIV and FeLV infections was performed at LAPCLIN-DERMZOO, INI/Fiocruz, using the Snap Combo FIV/FeLV Test<sup>3</sup>, a fast immunoassay, using the cats' serum samples from the first clinical evaluation.

*Statistical analysis*

Fisher's exact test was applied to verify associations between the outcome of sporotrichosis treatment (favorable and unfavorable) and *T. gondii*, FIV and FeLV coinfections. In addition, this test was used to verify association between *T. gondii* infection with the categorical variables (sex, eating habits, living with other cats, access to the streets, castration and age) and FIV/FeLV coinfections. A *P*-value < 0.05 indicated significant associations in the statistical tests. To compare the diagnostic tests (IHA and IFAT), the values of total, positive and negative concordances were evaluated. The Statistical Package for Social Science (SPSS) software, version 16.0, was used in this analysis.

**RESULTS**

Fifty-four (25.3%) of the 213 cats with sporotrichosis showed coinfections with *T. gondii*, FIV or FeLV (Table 1).

There was no statistical significant association between these coinfections with the outcome of sporotrichosis treatment (*P* > 0,05) [Table 1]. In addition, there was no statistical significant association between the presence of anti-*T. gondii* antibodies and the variables of this study (sex, eating habits, living with other cats, free access to the street, castration and age) and FIV/FeLV coinfections (*P* > 0,05) [Table 2].

There was a concordance of 100% between IFAT and IHA for the serological diagnosis of *T. gondii* infection. All 14 cats positive for anti-*T. gondii* antibodies had IHA titers of 64.

Among the seropositive cats for *T. gondii* infection, 13 (92.9%) were detected in the first clinical evaluation. There was only one case of seroconversion (7.1%), which was detected in the fourth clinical evaluation (Table 3). Anti-*T. gondii* IgM antibodies were not detected in this animal's serum samples.

Anti-*T. gondii* IgM antibodies were only found in a cat (#10), which was also positive for anti-*T. gondii* IgG antibodies. In this cat, the anti-*T. gondii* IgM antibodies were detected only in the first clinical evaluation, with a titer of 64.

**Table 1.** Frequency of coinfection of *Sporothrix* spp. with *Toxoplasma gondii*, Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) of 213 cats with sporotrichosis from the metropolitan are of Rio de Janeiro, according to sporotrichosis treatment outcome, between November 2007 and February 2011.

Coinfection with <i>Sporothrix</i> spp. (n = 213)	Frequency n (%)	Outcome of treatment (n=195) <sup>1</sup>	
		Favorable n (%)	Unfavorable n (%)
FeLV	Total	11/31 (35.5%)	20/31 (64.5%)
	+FIV	2/3 (66.7%)	1/3(33.3%)
	+ <i>T. gondii</i>	0/3 (0%)	3/3 (100.0%)
	Single <sup>2</sup>	9/25 (36.0%)	16/25 (64.0%)
<i>T. gondii</i>	Total	4/12 (33.3%)	8/12 (66.7%)
	+FIV	0/1 (0%)	1/1(100.0%)
	Single	4/8 (50.0%)	4/8 (50.0%)
FIV	Total	4/10 (40.0%)	6/10 (60.0%)
	Single	2/6 (33.3%)	4/6 (66.7%)

<sup>1</sup>Only 195 out of 213 cats were analyzed based on the outcome of sporotrichosis treatment, because 18 animals lost follow-up at the beginning of the study; <sup>2</sup>Single= single coinfection; n=number of cats; += coinfecting with.

**Table 2.** Variables of cats obtained from the animals' medical records, coinfections with FIV/FelV and the results of serological tests for the diagnosis of *T. gondii* infection in 213 cats with sporotrichosis from the metropolitan area of Rio de Janeiro examined between November 2007 and February 2011.

Variable	<i>T. gondii</i> infection		P-value	
	Negative	Positive		
Sex	Female	38 (95.0%)	02 (5.0%)	1.00
	Male	161 (93.1%)	12 (6.9%)	
Food	Homemade and commercial	57 (93.4%)	04 (6.6%)	1.00
	Commercial	142 (93.4%)	10 (6.6%)	
Living with other cats	Yes	66 (90.4%)	07 (9.6%)	0.37
	No	133 (95.0%)	07 (5.0%)	
Access to street	Yes	173 (92.5%)	14 (7.5%)	0.37
	No	26 (100%)	0 (0%)	
Castration	Yes	67 (95.7%)	3 (4.3%)	0.55
	No	132 (92.3%)	11 (7.7%)	
Age	Under 3 years	140 (95.9%)	06 (4.1%)	0.06
	Over 3 years	54 (88.5%)	07 (11.5%)	
FIV	Positive	11 (91.7%)	1 (8.3%)	0.54
	Negative	188 (93.5%)	13 (6.5%)	
FelV	Positive	32 (91.4%)	3 (8.6%)	0.45
	Negative	167 (93.8%)	11 (6.2%)	

**Table 3.** Monthly serological follow-up of the 14 cats with sporotrichosis and reactive to indirect fluorescent antibody test (IFAT) for IgG anti-*T.gondii* antibody from the metropolitan area of Rio de Janeiro, which were examined between November 2007 and February 2011.

Animal	Serological follow-up: titers of IgG anti- <i>T. gondii</i>					
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>
01 <sup>1</sup>	256	4096	4096	4096	4096	1024
02	256	256	4096	1024	- <sup>2</sup>	-
03	1024	-	-	-	-	-
04	256	256	256	-	-	-
05	256	-	-	-	-	-
06	NR <sup>3</sup>	NR	NR	64	256	64
07	1024	-	-	-	-	-
08 <sup>1</sup>	256	256	-	-	-	-
09	256	256	64	-	-	-
10 <sup>4</sup>	1024	256	1024	-	-	-
11	256	256	64	256	256	-
12	1024	256	-	-	-	-
13 <sup>1</sup>	1024	1024	-	-	-	-
14 <sup>5</sup>	256	-	-	-	-	-

<sup>1</sup>FelV-infected; <sup>2</sup>"-"= not performed; <sup>3</sup>NR = non-reactive; <sup>4</sup>Animal with positive IgM (1:64) in the 1<sup>st</sup> clinical follow-up; <sup>5</sup>FIV-infected.



## DISCUSSION

The occurrence of coinfections of *Sporothrix* spp. with *T. gondii*, FIV and FeLV were low in cats from the metropolitan region of Rio de Janeiro, Brazil, where sporotrichosis is endemic. These findings are similar to that reported by others authors [15] who studied an other population of cats with sporotrichosis in the same region, observing that antibodies against FIV were detected in 28 (19.7%) cats, FeLV antigen was detected in 2 (1.4%) cats, and both FIV antibodies and FeLV antigen were detected in 1 of 142 (0.7%) cats tested. There were no significant clinical or laboratory differences between FIV-FeLV-positive and FIV-FeLV-negative cats [15]. However, in cats with sporotrichosis from the same region the coinfection with *Bartonella* species that cause the cat scratch disease was high (64%) and thus this population of cats was considered a potential source of zoonotic infection by both diseases [8].

This was the first study that determine and follow-up the frequency of antibodies against *T. gondii* in a group of cats diagnosed with sporotrichosis. The frequency of *T. gondii* antibodies observed in this study (6.6%) was similar to the frequency of 5.6% reported in cats from Rio de Janeiro, using the HAI [2]. This low rate can be explained by the fact that the cats in both studies were household pets whose owners appeared to be responsible, since the great majority of them fed the cats only with commercial pet food and also showed concern for the treatment and welfare of their pets. However, higher frequencies of 24.4% [13] and 72% [11] were detected by IFAT in stray cats from the metropolitan region of the state of Rio de Janeiro, probably because this kind of feline population is more exposed to infection with *T. gondii* by hunting birds and rodents and ingesting water contaminated by oocysts [19]. Although the *T. gondii* infection rate was low in the cats under study, they should be considered a potential source of infection by this zoonosis. The reason is that seronegative cats are more susceptible to oocysts shedding into the environment after becoming infected with *T. gondii* for the first time [5].

The follow-up of anti-*T. gondii* antibodies showed that the great majority of infections were chronic, but in two cases there was a fourfold rise in the titers between two consecutive follow-ups and in one there was seroconversion, which were indicative of acute infection [20]. In addition, the only anti-*T. gondii* IgM seropositive animal (1:64) also showed a titer for IgG (1:1024) in the same evaluation, indicating the transition from a recent

infection or the acute phase of the illness to a latent or chronic infection.

The 100% of concordance in the *T. gondii* diagnostic test in this study, indicates that it would be advantageous to make greater use of the IHA, which is a practical and low cost diagnostic technique for detecting *T. gondii* infection mainly for screening and elimination of sera nonreactive.

The lack of a statistically meaningful correlation between the coinfections of *Sporothrix* with *T. gondii*, FIV and FeLV, risk factors and the outcome of sporotrichosis treatment was probably due to the low occurrence of these coinfections. The absence of association between FIV/FeLV infection and the presence of antibodies against *Bartonella* species was also attributed to the small number of cats that tested positive for these retroviruses [8]. A similar result has been reported by other researchers attempting to establish the association between FIV/FeLV infection and sporotrichosis [8,14]; FIV/FeLV and *T. gondii* infection [18]; FIV, *Leishmania* and *T. gondii* infection [12,16]. However, some authors [17] reported a strong association when studying *T. gondii* and *Leishmania* coinfections in stray cats, which was demonstrated by the finding that 33.3% of the cats with anti-*T. gondii* antibodies were also infected with *L. chagasi*.

## CONCLUSIONS

There was a low occurrence of infection by FIV, FeLV and *T. gondii* in cats with sporotrichosis of the endemic region of Rio de Janeiro and a lack of correlation of these coinfections with outcome of sporotrichosis treatment and risk factors. The fact that cats were domiciled with adequate feeding and management, the low frequency of anti-*T. gondii* antibodies and the rare cases indicative of acute infection by *T. gondii* in the study population indicate that these animals are not highly exposed to infection by this protozoan.

## MANUFACTURERS

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**Ethical approval.** For this study, a serum bank was created using serum from the animals whose protocol was approved by the Ethics Committee on Animal Use of Fiocruz (Permit No. L-041/06).

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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