Sporothrix brasiliensis outbreaks and the rapid emergence of feline sporotrichosis

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Summary

Sporotrichosis is the main subcutaneous mycosis in Brazil, and is caused by Sporothrix schenckii and allied species. Sporothrix propagules present on soil and plant debris may be traumatically inoculated into the cutaneous/subcutaneous tissues of the warm-blooded host. An alternative route involves direct animal–animal and animal–human transmissions through deep scratches and bites of diseased cats. Sporotrichosis is much more common than previously appreciated with several cases emerging over the years especially in South and Southeast Brazil. We conducted an epidemiological surveillance in endemic areas of feline sporotrichosis in the southern region of Rio Grande do Sul state, Brazil. Over the last 5-year period the number of feline sporotrichosis in Rio Grande increased from 0.75 new cases per month in 2010 to 3.33 cases per month in 2014. The wide geographic distribution of diagnosed cases highlights the dynamics of Sporothrix transmission across urban areas with high population density. Molecular identification down to species level by PCR-RFLP of cat-transmitted Sporothrix revealed the emergence of the clonal offshoot S. brasiliensis during feline outbreaks; this scenario is similar to the epidemics taking place in the metropolitan areas of Rio de Janeiro and São Paulo. Controlling and preventing sporotrichosis outbreaks are essential steps to managing the disease among humans and animals.

Key words: Sporothrix brasiliensis, cats, outbreak, sporotrichosis, epidemiology.

Introduction

Sporotrichosis is the main subcutaneous mycosis in Brazil and is caused by dimorphic fungus from the Sporothrix schenckii complex. Species embedded in this complex differ towards ecology, virulence and morphology, which reflect in contrasting epidemics strategies and pathogenicity levels to mammals. The high virulent S. brasiliensis is frequently associated with animal horizontal transmission,1,2 whereas S. schenckii sensu stricto (s. str.) and S. globosa may be involved with the classic sapronotic route of transmission to humans.3–5 Remarkably, on the other extreme of pathogenicity, strictly environmental Sporothrix, i.e. S. mexicana, S. pallida and S. chilensis evolved in association with soil, plants debris and bark-beetles. True environmental species usually lacks pathogenicity to mammals and scarce cases of human infection were described in the literature.6–8
The classic route of transmission, involves contamination of the warm-blooded host via traumatic inoculation of plant matter and soil containing *Sporothrix* propagules. Such route reflects what is commonly observed around the warm areas of the world, especially in the Americas and South Africa involving *S. schenckii s. str.* and in Northeast China and India, involving *S. globosa.* It is suggested that specific conditions in the environment should be met to promote *Sporothrix* expansion in nature to cause sapronotic epidemics. However, in the alternative route of infection, comprising horizontal animal transmission (e.g., cat-cat) in addition to zoonotic transmission (e.g., cat-human), *S. brasiliensis* propagules may be efficiently transferred via deep scratch and bites of infected animals in endemic areas.

Zoonotic sporotrichosis outbreaks have been described since the 1990’s and are emerging as an important infectious disease in South and Southeast Brazil, These cases are mainly associated with *S. brasiliensis,* and domestic cats play key roles in disease epidemiology. Cats are highly susceptible to a great load of yeast-like cells. Consequently, cats present a great potential to spread the disease to other animals and humans in a short period of time due to their mobility and fighting behaviour.

The long-lasting outbreak of cat-transmitted sporotrichosis that originated in Rio de Janeiro presents with epidemic proportions. More recently, the beginning of a similar outbreak was described in Sao Paulo, and *S. brasiliensis* was involved during animal–animal transmission. *Sporothrix* epizootics in cats also occurs through the Rio Grande do Sul state. However, the southern region of Rio Grande do Sul is highlighted due to the expressive number of cases described compared to adjacent areas, with the epicentre around the city of Rio Grande. *Sporothrix* infections may persist in Rio Grande for a long period among symptomatic and asymptomatic animals – a challenge to disease control. Given the importance of feline sporotrichosis, we conducted an epidemiological surveillance study in endemic areas of Rio Grande to investigate the emergence of cat-transmitted disease using classical and molecular tools.

**Materials and methods**

The city of Rio Grande is located on southern Rio Grande do Sul, Brazil, and is surrounded by waters of the Lagoa dos Patos (the largest lagoon in Brazil), Lagoa Mirim and the Atlantic Ocean. The entire municipality lies at a very low altitude, at its highest point only 10 m (33 feet) above sea level. The population estimated by the Brazilian Institute of Geography and Statistics (IBGE) is in 207.036 inhabitants (data from 2014) and it harbours the longest beach in the world (Cassino beach). Cassino beach is approximately 250 km of uninterrupted Atlantic coastline. The biome is pampa, which is restricted in Brazil to the state of Rio Grande do Sul. The climate of Rio Grande is humid subtropical and mild, with a strong oceanic influence and relatively cold winters, warm summers and regular precipitation all year. The average temperature in the city is 18.3 °C and the average annual precipitation is 1.207 mm.

The criteria for feline sporotrichotic infection to qualify as an outbreak case included observing clinical symptoms consistent with *Sporothrix* infection accompanied with isolation of *Sporothrix* from ulcerative lesions. Clinical samples from suspected cases were collected from January 2010 to December 2014 and referred to the Mycology Laboratory of the Faculty of Medicine at Universidade Federal do Rio Grande (FAMED-FURG), which drains cases of human and animal sporotrichosis and other tropical diseases throughout the southern region of Rio Grande do Sul.

Samples were processed by direct mycological examination with Gram stain and culture in duplicate in Sabouraud dextrose agar with and without cycloheximide with incubation of 30 days at 30 °C. The variables studied comprised the year of the diagnosis, the animal origin neighbourhood, gender, location of the lesions (fixed form – only one anatomical site; or disseminated – generalised lesions by skin tissue and /or respiratory signs), animal access to the street (free, limited or none) and period between progression of lesions and diagnosis.

Molecular identification was carried out by PCR-RFLP (Restriction Fragment Length Polymorphism) as described elsewhere. Briefly, DNA was obtained and purified directly from 10-day-old colonies on slants by following the Fast DNA kit protocol (MP Biomedicals, Vista, CA, USA). Calmodulin-encoding gene (CAL) was amplified by PCR using the primers CL1 and CL2A and thereafter digested with the enzyme *HhaI.* The digested products were electrophoresed on 2.5% (w/v) agarose gels for 90 min at 100 V in the presence of GelRed™ (Biotium, Hayward, CA, USA). The bands were visualised using the L-Pix Touch (Lucus Biotecnologia, Sao Paulo, Brazil) imaging system under UV illumination. The reference strain CBS 120339 of *S. brasiliensis* was used as a quality control for CAL-RFLP.
Data were compiled and the descriptive analysis and chi-square test were performed with spss® 20.0 (IBM, Chicago, USA) considering \( P < 0.05 \) statistically significant. Kappa index was calculated to assess the degree of agreement between the diagnostic methods used (direct examination and culture), and sensitivity rates, specificity, positive predictive value and negative predictive value of the direct examination using the mycological culture as the gold standard were also calculated. Kappa values were interpreted as follows: 0.00–0.20, poor agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, good agreement; 0.81–1.00, very good agreement.²⁷

**Results**

Over the last 5-year period, 29 veterinarians joined this study and referred to the Mycology Laboratory at FAMED-FURG a total of 230 clinical samples (swabs from ulcerated lesions) of cats suspected of sporotrichosis. Of them, 129 (56.1%) were positive in culture for *Sporothrix* spp. supporting clinical findings. The positivity percentile of clinical samples did not differ between the years \( (P = 0.387) \) and ranged from 66.7% (24/36) in 2012 to 48.2% (40/83) in 2014 (Fig. 1).

Considering the whole study period (60 months), an average of about two new cases were diagnosed per month. However, we found a remarkable increase over the years of study regarding the number of samples received and the number of confirmed cases. In 2010, only 15 samples were received, confirming nine cases of sporotrichosis. Subsequently, the number of positive samples increased in 2013–2014 to 85 animals (65.9%) with confirmed sporotrichosis (Fig. 1). Thus, if we consider the number of positive diagnosis for the years of the study, it increased from 0.75 new cases per month in 2010 to 3.33 cases per month in 2014.

The cases of feline sporotrichosis were scattered among 30 different areas of Rio Grande, especially from Cassino beach, accounting for 25% of cases \( (n = 28) \), followed by Parque Marinha \( (n = 13/10.9\%) \) and Cidade Nova \( (n = 12/10.1\%) \) (Fig. 2a and Fig. 2b). Approximately half of the animals with sporotrichosis developed single lesion (56.9%), and the majority were located at the cephalic region. Strikingly, the disease was significantly more diagnosed in male animals \( (P = 0.007) \) which accounted for 74% of cases, and with free access to street \( (P < 0.001) \) as described in 86.2% of sporotrichotic patients. In addition, the period between lesions appearing and diagnosis ranged from 3 days to 3 years, but only 21.2% of the animals were early diagnosed in less than 30 days to the onset of lesions.

Of the 129 animals that the culture techniques allowed the isolation of *Sporothrix*, 61 (47.3%) were positive in the direct mycological examination, demonstrating the presence of elongated and oval yeast-like cells. Moderate agreement was found between these two diagnostic methods \( (P < 0.001; \text{Kappa} = 0.441) \). Judging from the mycological culture as the gold standard, sensitivity, specificity, positive predictive value and negative predictive value of the direct mycological examination were 47.2%, 100%, 100% and 59.8%, respectively.

We employed PCR-RFLP to identify 35 *Sporothrix* isolates down to species level. Cat-transmitted isolates were submitted to CAL-RFLP and after electrophoresis fragments were 251, 232, 198, 96 and 85 bp in length, which are compatible with the restriction pattern of *S. brasiliensis* (CBS 120339).

**Discussion**

The Rio Grande do Sul State is an endemic area of sporotrichosis with several studies highlighting the
threat of animal–animal and animal–human transmis-
sions.\textsuperscript{17,18,21–24} Eco-epidemiological characteristics of
the \textit{Sporothrix}-associated outbreaks in the Southern
region of the state are of interesting, due to the emerg-
ing number of infected animals. Judging from previous
records of feline sporotrichosis, we observed a signifi-
cant increase in disease incidence rising from 92 cases
among seven municipalities over a 10-year period,\textsuperscript{18}
to 129 cases in only 5 years and in a single munici-
pality, of which 85 cases were diagnosed during the
last 2 years of our study (2013–2014). \textit{Sporothrix} is
unique in the fungal kingdom due to its frequent
occurrence as outbreaks. Indeed, the high number of
cases in a short period is compatible with the ongoing
situation reported earlier in other endemic areas of
Brazil.\textsuperscript{1–3,6,12,14,15,19,20,28,29}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{(a) Geographic localization of Rio Grande do Sul state from Brazil, highlighting Rio Grande city. (b) Aerial view of Rio Grande city (32°04'28"S 52°08'32'W); each yellow point represents confirmed cases of feline sporotrichosis included in the study, highlighting the dynamics of \textit{Sporothrix} transmission across urban areas with high population density. (Accessed in: October 28, 2014).}
\end{figure}
The real prevalence of feline sporotrichosis is likely to be underestimated in Southern Brazil, since it is not an obligatorily reported disease. We considered only the cases referred to FAMED-FURG and with confirmed laboratorial diagnosis of sporotrichosis, i.e., by isolation of *Sporothrix* in culture. Cases diagnosed in other laboratories, or cases with clinical suspect, were not included. Moreover, feline sporotrichosis is a disease that classically occurs in urban areas characterised by poor sanitation, substandard housing and little or no access to health services, what leads us to believe, that most of the cases may be neglected by pet owners that do not have access to veterinary care.

The wide geographic distribution of diagnosed cases highlights the dynamics of *Sporothrix* transmission (e.g. cat–cat transmission) among the feline population across the urban areas of Rio Grande as well as the Cassino beach. The scenario is aggravated by the fact that most cases occurs in male animals, with free access to the street and that developed ulcerated lesions within more than 30 days before diagnosis. Indeed, male cats tend to engage in disputes over territory or females, and once infected they can easily transmit the disease to other animals or humans by deep scratch and bites. Thus, cats remain the main source of infection of *S. brasiliensis* in South and Southeast Brazil.

It is remarkable that close related *Sporothrix*, i.e. *S. schenckii s. str.* and *S. globosa* follows different strategies during outbreaks. Therefore, dissimilar routes of transmission requires different approach to tackle epidemic due to *Sporothrix* spp. Judging from the animal–animal transmission as in *S. brasiliensis*, it is unlikely that the epidemic will end spontaneously, what is in accordance with our data reporting the increasing number of feline sporotrichosis in Southern Brazil.

The first outbreak of feline sporotrichosis in Rio Grande occurred in 2000, affecting 30 animals. Afterwards, a considerable increasing in the number of confirmed cases in Rio Grande and Cassino beach was observed between 2008 and 2010. Montenegro et al. [2] also describes the emerging character of feline sporotrichosis in the metropolitan region of São Paulo, with 163 cases diagnosed over the last 3-year period. In addition, the long-lasting outbreak of cat-transmitted sporotrichosis in Rio de Janeiro started in 1990’s decade and now is accounting for over 4000 human cases and 3800 in felines. Rio Grande do Sul, São Paulo and Rio de Janeiro states present different prevalence of cat infections, and ecological biomes, although they have in common the presence of urban areas with high population density, which is a major contributing factor to epizootics. In addition, all these regions described an early outbreak episode triggering the continued transmission, which points to negligence of the epidemic.

The gold standard to diagnosis sporotrichosis is mycological culture; however, it can take up to 30 days to conclusive results. In this sense, it is recommended to perform the direct mycological examination concomitantly to culture. In cats, whose injuries often develop a large number of fungal propagules, direct examination has a great value, allowing the presumptive diagnosis in a few minutes and can help to guide specific treatment and consequently improve clinical outcome. Although the yeast cells of *Sporothrix* spp. observed in the direct examination are not pathognomonic and can be confused with other microorganisms, such as *Candida glabrata* and *Histoplasma capsulatum*, in our study the specificity of this test was 100%, specially taking into consideration the association with clinical and epidemiological data of the patient. Thus, a positive result in the direct examination of a clinical sample taken from a feline with suggestive lesions of sporotrichosis and in areas of high endemicity of the disease, can safely be used to aid diagnosis.

Molecular characterisation of *Sporothrix* isolates is now an essential tool in our attempts to understand the epidemiology of feline sporotrichosis. *Sporothrix brasiliensis* is the etiological agent of all cases in our study identified down to the species level, corroborating previous studies. *Sporothrix brasiliensis* is a clonal offshoot of the classical species *S. schenckii s. str.* and is considered the most virulent pathogen in the *S. schenckii* complex. Moreover, *S. brasiliensis* is associated with zoonotic transmission and atypical clinical presentations in humans. However, the isolates of *S. brasiliensis* from Rio Grande do Sul differ molecularly of those from Rio de Janeiro, São Paulo and Minas Gerais, which deserves more investigation.

In conclusion, this study demonstrated the presence of *Sporothrix* infection among cats in the Southeast Brazil, indicating a high prevalence of the clonal offshoot *S. brasiliensis*. The emerging nature of feline sporotrichosis alerts to the need for constant surveillance programs and to develop measures to prevention and control sporotrichosis in endemic areas.

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Conflicts of interest

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

References


