

Sporotrichosis in Cats Caused by *Sporothrix brasiliensis* in Two Microregions of Brazil

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ABSTRACT

Background: Sporotrichosis is a subcutaneous mycosis of humans and animals. In recent years, several cities in Brazil have recorded an increase in cases due to zoonotic transmission through cats. The classic clinical presentation is transmitted through plant debris and the 2nd way (zoonotic form) is caused by animal transmission. The alternative zoonotic route has gained attention due to the rapid and significant spread of cat-transmitted sporotrichosis in South America. The aim of this work was to identify the *Sporothrix* species responsible of feline sporotrichosis based on reference standard and molecular methods.

Cases: All isolates were recovered from skin of 27 cats with sporotrichosis between 2020 and 2024. Fifteen cats sampled were from state of Santa Catarina (microregion of Florianópolis) and, 12 from state of Rio Grande do Sul (11 from microregion of Porto Alegre / 1 from Serafina Corrêa city), Brazil. Clinical diagnosis of sporotrichosis was made through examination (inspection and palpation of skin and/or mucous lesions, palpation of superficial lymph nodes and cardio-pulmonary auscultation) and cytopathology. The cytopathological examination was performed either by imprint in ulcerated lesions/swabs. Yeast-like structures were observed in cytopathological exam. Fungal growth in mycological culture (Sabouraud Cycloheximide-Chloramphenicol Agar, incubated at 25°C and 37°C for 10 to 30 days) was monitored by forming dark black colonies. Physiological tests (assimilation of sucrose and raffinose), microscopy of hyphae/conidia and macroscopic colony characteristics (colors and texture) were conducted for the identification of the species. All isolates included in the study exhibited similar macroscopic morphology in their filamentous form. Microscopic observation of the isolates showed presence of septate hyphae showing at the end of conidiophores conidia arranged in a “daisy-like” pattern (sympodial formation). Assimilation tests using sucrose and raffinose were negative. *Sporothrix brasiliensis* was the only agent involved in feline sporotrichosis. The identity of isolates was confirmed using specific primers for the CAL gene and sequencing analyses.

Discussion: According to the literature, our result corroborates reports from other authors who describe cases of sporotrichosis caused by *Sporothrix brasiliensis* in several states in Brazil. Although *S. brasiliensis* is predominant, the identification of fungal species is essential to determine the etiology of sporotrichosis in different areas of the country, and to indicate a possible change in the current scenario of the disease. The transboundary expansion of the species occurs between states and more cases of cat-cat/cat-dog and cat-human transmission may occur; situation described in this work. In the state of Rio Grande do Sul (RS), *S. brasiliensis* was the only isolated species. *Sporothrix brasiliensis* is considered the most virulent species within pathogenic clade and represents an important not only in Brazil, but also in other South American countries; among them are the Paraguay, Argentina and Chile with a description of feline sporotrichosis. In Panama (Central America) the 1st case of zoonotic transmission was reported, however the species of *Sporothrix* genus was not discriminated through molecular diagnosis. In conclusion, the real situation of sporotrichosis in Brazil can be assessed through the clinical diagnosis of sporotrichosis in felines, as well as in humans that have had zoonotic transmission. In addition, laboratory diagnosis, with the respective determination of the species involved. This way the disease will become more visible and effective control measures can be taken within the scope of public health.

Keywords: cats, *Sporothrix brasiliensis*, zoonosis, molecular methods, calmodulin, cutaneous, disseminated sporotrichosis.

DOI: 10.22456/1679-9216.143404

Received: 27 August 2024

Accepted: 30 October 2024

Published: 28 November 2025

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INTRODUCTION

Several species of fungi can infect men and animals in various ways, including traumatic implantation through the skin or mucous membranes. Sporotrichosis is a cutaneous and subcutaneous mycosis caused by *Sporothrix* spp. and cat-transmitted sporotrichosis caused by *Sporothrix brasiliensis* is an endemic neglected subcutaneous mycosis in Brazil [9]. The clinical diagnosis of the disease is made through clinical evaluation and cytology; however, identification of isolates by molecular techniques is still costly and requires human resources specialized in molecular diagnostics (MDx) tools.

Cases of sporotrichosis caused by *Sporothrix brasiliensis* were initially described southeastern Brazil (São Paulo and Rio de Janeiro) [8,11,17]. However, in the last decade, cases of sporotrichosis have been identified in more states of Brazil [2,15,16,19].

The aim of this work was to identify the *Sporothrix* species responsible of feline sporotrichosis based on reference standard and molecular diagnostics (MDx) methods.

CASES

A total of 27 cats with cutaneous lesions were diagnosed with sporotrichosis between 2020 and 2024 (Figures 1, 2 & 3) through clinical examination (inspection and palpation of skin and/or mucous lesions, palpation of superficial lymph nodes and cardiopulmonary auscultation) and cytopathology. The cats from Porto Alegre (11) and Serafina Corrêa (1) were referred to Veterinary Medical Teaching Hospital (HCV) of the Federal University of Rio Grande Sul (UFRGS) and the cats from Florianópolis (15) were referred to private veterinary clinics.

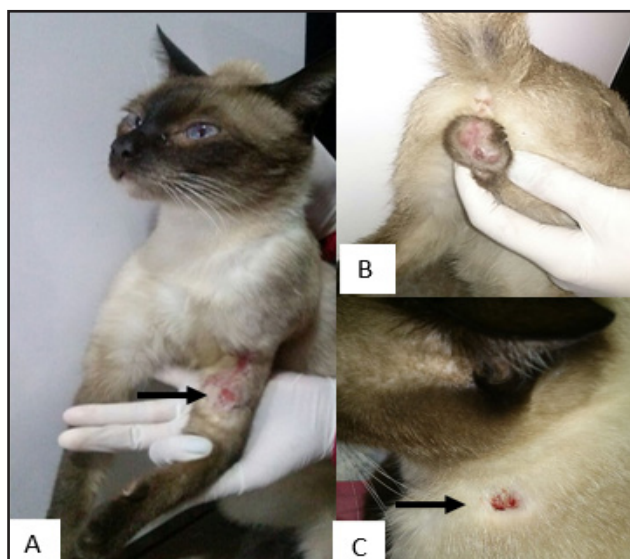


Figure 1. Cat 1: Ulcerative lesions on the pelvic limbs (A), scrotum (B) and cervical region (C).



Figure 2. Cat 22: Ulcerative lesions, with bleeding and signs of inflammation, mainly located in the nasal region.

The cytopathological examination was performed either by imprint in ulcerated lesions/swabs. The samples were air-dried, stained with Quick Panoptic method¹, and examined under a 40× magnification optical microscope. Yeast-like structures were observed in cytopathological exam (Figure 4A-D).

Twenty-nine isolates were recovered from skin of cats with sporotrichosis. Two animals with 2 or more ulcers spread over the head and body had 2 samples collected. Fungal growth in mycological culture (Sabouraud Cycloheximide-Chloramphenicol Agar², incubated at 25°C and 37°C for 10 to 30 days) was monitored by forming dark black colonies. Physiological tests (assimilation of sucrose and raffinose), microscopy of hyphae/conidia and macroscopic colony characteristics (colors and texture) were conducted for species identification [10]. *Sporothrix* isolates were deposited in the culture collection of the Laboratory of Mycology, Veterinary Faculty - UFRGS. All isolates included in the study exhibited similar macroscopic morphology in their filamentous form (Figure 5). After 21 days on Potato Dextrose Agar (30°C), the colonies were glabrous in texture with dark brown to black mycelia. Microscopic observation of the isolates showed presence of septate hyphae showing at the end of conidiophores conidia arranged in a daisy-like pattern (sympodial formation). Assimilation tests using sucrose and raffinose were negative.

The Qiagen DNeasy® plant mini-DNA extraction kit³ protocol was used to extract DNA from mycelial colonies of *Sporothrix* according to manufacturer instructions. Molecular identification was performed using sequences that targeted the calmodulin gene (CAL) [14]. The PCR assay was performed as previ-

ously described by Spanamberg *et al.* [19]. PCR product was separated on a 2% agarose gel (Figure 6). PCR product was purified using PuriLink™ PCR Purification Kit⁴, and sequencing to confirm the identity. The isolates were confirmed as *S. brasiliensis* following the amplification of the CAL gene and sequence analyses.



Figure 3. Multiple ulcerated skin lesions. A- On the head, ears and cervical region (Cat 5). B- Extensive ulcerative lesion in the pelvic limb (Cat 10). C&D- Ulcers on the face and skin belly (Cat 21).

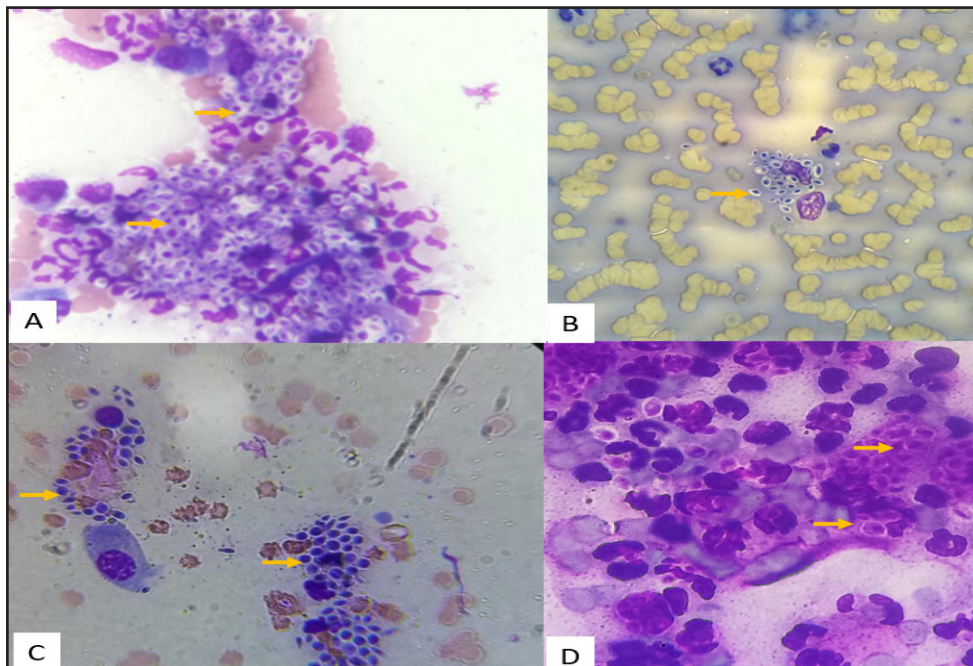


Figure 4. Yeast-like structures in cytopathological exam (arrows) of skin lesions [Quick Panoptic stain]. A- Cat 3; B- Cat 9; C- Cat 21; D- Cat 26.



Figure 5. Growth of filamentous colonies on Sabouraud dextrose agar plate incubated at 30°C.

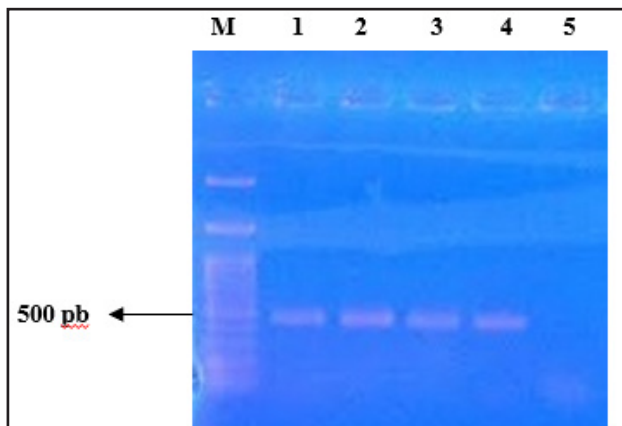


Figure 6. PCR amplification of fragments of approximately 500 bp generated by primers (*Sporothrix brasiliensis*) from purified DNA from the isolate obtained from cats with sporotrichosis. M: molecular size marker (100bp ladder); Lane 1,2,3 is *Sporothrix brasiliensis* (isolates); Lane 4: positive control. Lane 5: negative control.

DISCUSSION

In this study all isolates were *S. brasiliensis*, confirmed by sequencing analysis of calmodulin gene. These results suggest that, as in other states, *S. brasiliensis* is the predominant species in the study area. The identification of fungal species is fundamental to specify the etiology of sporotrichosis in different areas

from Brazil. Despite the efficiency of conventional and molecular diagnosis, sporotrichosis is still underestimated, is sub-notified, and remains as a neglected disease [8].

The transboundary expansion of the species occurs between states and more cases of cat-cat/cat-dog and cat-human transmission may occur; situation described in this work. *Sporothrix brasiliensis* is considered the most virulent species within pathogenic clade and represents an important not only in Brazil, but also in other South American countries [7]; among them are the Paraguay, Argentina and Chile with a description of feline sporotrichosis [3,4,12]. In Panama (Central America) the 1st case of zoonotic transmission was reported, however the species of *Sporothrix* were not discriminated through molecular diagnosis [13].

The diagnosis of sporotrichosis is based on cytopathological examination carried out together with the clinical evaluation [8,18]. Most cats had skin lesions in one region of the body and only 2 had widespread lesions (head and body). In this context, more than 1 sample was collected from each animal to exclude coinfections with another fungus, such as *Cryptococcus neoformans* and/or another species of *Sporothrix*. Differential diagnosis must also be explored and mycotic diseases like cryptococcosis, histoplasmosis, phaeohyphomycosis must be considered. In addition, squamous cell carcinoma, eosinophilic dermatosis, bacterial pyoderma among others must be investigated by history, areas of endemicity, and lab tests [9].

The reference standard for the diagnosis of sporotrichosis is the fungal culture obtained from the lesion. In 2017, a case of sporotrichosis in a cat with infection osteoarticular and extensive bone lysis and absence of external skin lesions was identified by traditional and molecular methods [6]. Regarding feline patients with lytic bone lesions, it's extremely important the culture of samples because *Sporothrix brasiliensis* may be involved, even when cytological and histopathological evaluation are inconclusive. The literature suggests that implantation of the agent may occur by traumatic inoculation into the tarsal region in animals with bone and joint injuries with absence of cutaneous presentations and systemic signs [5]. Interesting situation was described for the 1st time in the United Kingdom, were 3 cases of cat-transmitted sporotrichosis caused by *S. brasiliensis* occurred

through indoor domestic cat without skin lesions that lived in South-Eastern Brazil 3 years previously and the authors suggested that *S. brasiliensis* can lay dormant for many years [1]. The enzootic (cat-to-cat) transmission, the large cat population abandoned in several cities, the lack of public policies and the evolutionary adaptation of *S. brasiliensis* configure the disease as a serious public health problem [9].

In conclusion, the real situation of sporotrichosis in Brazil may be assessed through the clinical diagnosis of sporotrichosis in cats, as well as in humans in contact with infected animals. Also, in relationship to the laboratory diagnosis, it is important to emphasize the necessity of determination of the respective species involved. With this approach, measures for effective control and continued surveillance can be taken in order to avoid the spread of this mycotic zoonosis,

which concerns the Public Health, since cat-transmitted sporotrichosis has emerged as a zoonotic epidemic in many microregions of Brazil.

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Acknowledgments. The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq): grant #306685/2015-9 (PQ 1D) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for supporting this study. R.A. was financed by Fundação para a Ciência e a Tecnologia, Portugal, by the project 10.54499/CEEC-IND/01070/2018/CP1558/CT0001.

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

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