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Case report

FIRST ALLOCHTHONOUS RECORD OF FELINE AND HUMAN SPOROTRICHOSIS IN THE CITY OF CUIABÁ, MATO GROSSO, CENTRAL-WEST REGION OF BRAZIL

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Sporotrichosis is a subcutaneous mycosis caused by fungi of the genus *Sporothrix*, commonly found in tropical regions. This report describes the first documented case of feline and human sporotrichosis in Cuiabá, Mato Grosso. A male cat developed progressive skin lesions, and its owner was also infected after being scratched. Tissue samples confirmed the presence of *Sporothrix* spp. through cytology, histopathology, fungal culture, and PCR. The initial treatment with fluconazole was switched to itraconazole after diagnostic confirmation. This report highlights the importance of rapid diagnosis and containment measures to control the spread of this zoonosis.

Keywords: *Sporothrix brasiliensis,* zoonosis, molecular diagnosis, transmission.

INTRODUCTION

Sporotrichosis, caused by dimorphic fungi of the genus *Sporothrix*, is a subcutaneous mycosis with a worldwide distribution [1]. Transmission generally occurs through the traumatic implantation of the fungus present in plants, soil, and organic matter, as well as through enzootic and zoonotic transmission. Human-to-human transmission is considered rare [2]. For many years, the infection was associated with a single fungal species, *Sporothrix schenckii*. However, molecular analyses have revealed the existence of other species, such as *S. brasiliensis*, *S. globosa*, *S. mexicana*, and *S. luriei* [1]. *Sporothrix brasiliensis* is the primary agent associated with zoonotic transmission by cats [1] and

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has been the main driver of sporotrichosis spread in Brazil since the epidemic that began in the 1990s in Rio de Janeiro [2].

Cats are the most susceptible hosts for *Sporothrix brasiliensis* infection and can develop both the cutaneous and severe disseminated forms of the disease. The condition is more common in free-roaming, unneutered male cats [1,3]. Cats with sporotrichosis initially present isolated skin lesions that manifest as nodules and ulcers, primarily in the nasal region. These lesions progress to multiple lesions in various areas. The nodules may ulcerate and drain serosanguinous exudate. There is typically mucocutaneous involvement, primarily nasal, but it can also affect the conjunctival, oral, and genital regions, as well as cause lymph node enlargement [4,5]. The most common extracutaneous signs are sneezing, dyspnea, and nasal discharge, and these are strongly associated with *Sporothrix brasiliensis* [5].

The spread of sporotrichosis, transmitted by cats and associated with *S. brasiliensis* throughout Brazil, has become a public health threat. In the last decade, the disease, which was previously confined to regions of southeastern Brazil, has expanded to the northeastern, northern, and central-western regions of the country [6]. Cases of zoonotic sporotrichosis caused by *S. brasiliensis* and transmitted by cats have been reported in other South American countries, such as Argentina, Paraguay, and Chile, as well as outside South America [2].

In light of the alarming spread of zoonotic sporotrichosis across Brazil, even though the disease in animals is not a mandatory reportable condition in the country, in 2023 the Ministry of Health issued a technical note recommending that all suspected or confirmed cases of sporotrichosis in cats and dogs be reported and investigated [7]. Considering this, this report describes the first allochthonous case of feline and human sporotrichosis caused by *Sporothrix brasiliensis* in Cuiabá, Mato Grosso, in the central region of Brazil.

CASE PRESENTATION

A male, neutered, mixed-breed cat was seen at a university veterinary hospital with a history of progressively enlarging, ulcerated skin lesions on the pelvic limbs, perineal region, and tail, as well as occasional episodes of epistaxis. According to the report, the cat had relocated with its owners from the state of Pará (northern Brazil) to the state of Mato Grosso (central-western Brazil), where it began showing the skin signs 15 days after the move. The owners reported that, in the previous residence, the cat had access to the street and had been involved in a fight with a street cat. Upon physical examination, the cat presented with multiple ulcerated skin lesions and edema in the pelvic limbs, perineal region, and tail. During a more thorough skin examination, hematomas were identified in the perineal region and pelvic limbs, along with areas of necrosis adjacent to the lesions (Figure 1 A and B). Lesional tissue samples were collected for cytology, histopathology, fungal culture, and PCR. The owner also

presented skin lesions at the site where she was scratched by the animal (Figure 1 D) and was referred to a medical service, where a tissue sample from the skin lesion was collected for histopathology, fungal culture, and PCR.

Cytological examination of the feline lesion material revealed the presence of fungal structures (Figure 1 E). Histological examination using Periodic Acid-Schiff (PAS) staining and Grocott's methenamine silver stain (both positive) resulted in a diagnosis of possible infection by *Histoplasma* sp. or *Sporothrix* sp., based on the lesion pattern and the morphological characteristics of the yeast. Lesional tissue samples from the cat and its owner were cultured on Sabouraud agar with chloramphenicol for 7 days at 25°C. Dimorphism was demonstrated by subculturing the fungus on Brain Heart Infusion (BHI) agar at 37°C for 7 days.. The macromorphological and micromorphological characteristics of the colony allowed for the presumptive identification of *Sporothrix* spp. (Figure 1 F).

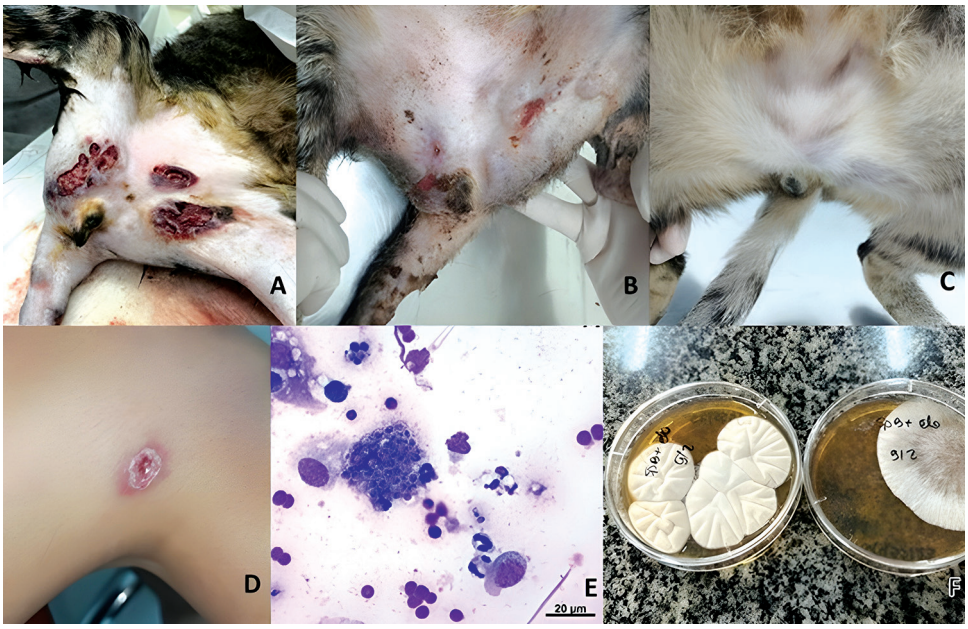

Figure 1. (A) Ulcerative lesion caused by *Sporothrix brasiliensis* on the day of presentation. **(B)** Lesion after 20 days of treatment with fluconazole. **(C)** Recovery after 30 days of treatment with itraconazole. **(D)** Lesion on the lateral aspect of the contact human's leg. **(E)** Cytology of feline lesion material showing *Sporothrix* sp. **(F)** Fungal culture of *Sporothrix* after inoculation of lesion material.

DNA extraction was performed from the fungal isolate. The colony was resuspended in lysis buffer (100 mM NaCl, 25 mM EDTA, 100 mM Tris-HCl pH 8.0, 0.5% SDS, 0.1 mg/mL Proteinase K) and glass beads, and incubated with agitation at 56 °C overnight. DNA was then extracted using the phenol-chloroform method, as described by Green and Sambrook [8]. The DNA was resuspended in ultrapure

water and stored at –20 °C until use. The extracted DNA was tested for *Sporothrix* spp. by PCR, using the primers SSHF31 (5'-GCAGCCCACGTCCAACAAGACT-3'), SSLF64 (5'-CATTAGATATTATCGGGGCCCAAGT-3'), and SSHR97 (5'-GTCAGAGGTCTTATTGGACGTGA-3'), which are specific for the topoisomerase II gene and amplify products ranging from 677 to 817 bp [9]. PCR reactions were carried out in a final volume of 25 µL, containing 10 ng of DNA, 1 U of Taq DNA polymerase (Invitrogen®), 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 1× PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), and 20 pmol of each primer. The thermal cycling conditions consisted of an initial denaturation at 96 °C for 2 minutes, followed by 35 cycles of 96 °C for 30 seconds, 70 °C for 3 seconds, 74 °C for 30 seconds, and a final extension at 72 °C for 2 minutes. Amplification products were analyzed by 1.5% agarose gel electrophoresis, stained with GelRed™ (Biotium®), and visualized using a ChemiDoc™ XRS system (Image Lab™), with a 100 bp DNA Ladder (Fermentas®). PCR products were purified using the GFX™ PCR DNA and Gel Band Purification Kit and used in sequencing reactions with the BigDye™ Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems®) on an ABI 3500 Genetic Analyzer (Applied Biosystems®). The obtained sequences were compared with the GenBank database using the BLAST tool available at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST). PCR amplification confirmed the infection by *Sporothrix* sp. Sequencing of the fungal isolates from both the human and feline samples revealed a 99.8% identity with *S. brasiliensis* with the GenBank sequence: NW_024467137.1. The sequenced nucleotides were deposited and are identified by the accession numbers PP341571.1 (human) and PP341570.1 (cat).

Treatment was initiated with fluconazole at a dose of 10 mg/kg twice daily, based on the initial suspicion of *Histoplasma* sp. in the cytology. The treatment continued for 30 days, during which there was significant improvement in the skin lesions and the episodes of epistaxis. After the confirmation of *Sporothrix* sp. by PCR, the antifungal medication was switched to itraconazole at a dose of 50 mg/cat once daily, resulting in complete resolution of the clinical signs (Figure 1 C). The animal continued treatment for two months after clinical recovery. Simultaneously, the owner was treated with itraconazole, leading to resolution of the skin lesions, and was discharged from medical care.

DISCUSSION

Zoonotic sporotrichosis associated with cats has become increasingly significant, particularly in the state of Rio de Janeiro in recent decades [4]. The expansion of the disease has been observed through outbreaks and epidemics, primarily due to the lack of disease control, which is exacerbated by challenges in treatment (high cost, prolonged therapy) and abandonment by owners [10]. The lack of recognition of sporotrichosis as a disease with significant impact on the country's development results in limited investment in prevention and control measures [10]. The disease

has expanded to states in the northeastern, northern, and central-western regions over the past five years [5], even causing outbreaks in the Northeast, such as in the capital city of Salvador. There, the large number of cases and the rapid spread across neighborhoods were only properly documented after the implementation of mandatory reporting [5]. In Cuiabá, Mato Grosso, there is a reported case of the disease in a cat [11]; however, the *Sporothrix* genus was not confirmed by PCR, and zoonotic transmission was not observed in that case. Comparing with international studies that analyzed sporotrichosis outbreaks in Asia [12], it becomes evident that an integrated approach between public health and veterinary medicine is crucial. Similarly, in Brazil, collaboration between human and veterinary health professionals may be the key to more effective disease control.

Despite the growing spread of the disease across the country, sporotrichosis remains a neglected disease, and in most states, it is still not a mandatory reportable condition. This lack of mandatory reporting makes it difficult to identify cases, monitor the disease, record epidemiological data, and implement control measures. Public policies focused on epidemiological surveillance and the nationwide implementation of mandatory disease notification are urgently needed.

It is important to highlight that, although itraconazole remains the primary therapeutic option for sporotrichosis in cats and humans, cases of antifungal resistance to this drug have been reported in *Sporothrix brasiliensis* isolates in Brazil, albeit rarely. Such resistance may compromise treatment efficacy and contribute to disease progression [13]. Furthermore, the discussion on surveillance strategies could be expanded by emphasizing the importance of molecular typing tools, which enable strain differentiation, support outbreak detection, and assist in tracing infection sources. These approaches are essential for guiding more effective control measures. Finally, it is appropriate to acknowledge some limitations of the present study, such as the absence of antifungal susceptibility testing and molecular characterization of the isolates. Future studies should consider investigating the genetic diversity and resistance profiles of S. brasiliensis, particularly in recently affected regions, to better understand the dynamics of disease spread and to adapt therapeutic and epidemiological surveillance strategies accordingly.

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Authors' contributions

ACS, ELJC and ABPFA conceived of the study, and participated in its design and coordination and helped to draft the manuscript. NDGV and MAS performed the histopathological analysis and review the manuscript. VD and LN carried out the molecular genetic studies, fungal culture and review the manuscript. VLR and FKSFA carried out clinical care, diagnosis and monitoring of the human case. All authors read and approved the final manuscript.

Declaration of conflicting of interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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PRVI SLUČAJ SPOROTRIHOZE KOD MAČKE I ČOVEKA U OBLASTI MATO GROSO, CENTRALNO-ZAPADNI REGION BRAZILA

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Sporotrihoza je supkutana mikoza koju izazivaju gljivice iz roda *Sporothrix*, koje se često nalaze u tropskim regionima. Ovaj izveštaj opisuje prvi dokumentovani slučaj sporotrihoze kod mačaka i ljudi u Kujabi, Mato Groso. Mačor je razvio progresivne kožne

lezije, a i njegov vlasnik je bio zaražen nakon što je ogreban. Uzorci tkiva potvrdili su prisustvo *Sporothrix* spp. nakon dijagnostike primenom citologije, histopatologije, gljivične kulture i PCR-a. Početni tretman flukonazolom je prebačen na terapiju itrakonazolom nakon dijagnostičke potvrde. Ovaj izveštaj ističe važnost postavljanja brze dijagnoze kao i mera suzbijanja, kako bi se kontrolisalo širenje ove zoonoze.