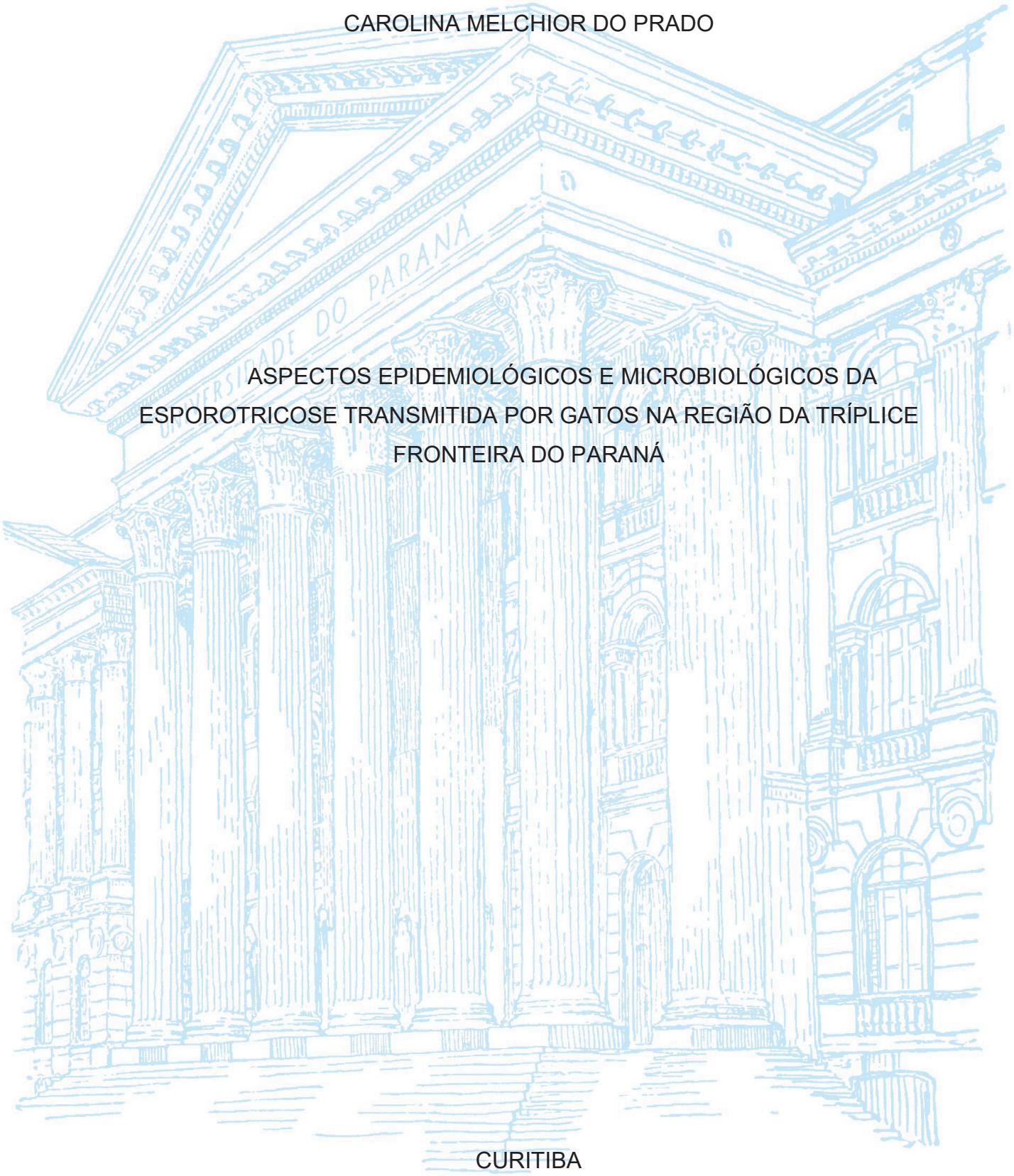
UNIVERSIDADE FEDERAL DO PARANÁ

CAROLINA MELCHIOR DO PRADO



ASPECTOS EPIDEMIOLÓGICOS E MICROBIOLÓGICOS DA ESPOROTRICOSE TRANSMITIDA POR GATOS NA REGIÃO DA TRÍPLICE FRONTEIRA DO PARANÁ

CURITIBA

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Tese apresentada ao Programa de Pós-Graduação em Microbiologia, Parasitologia e Patologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial para obtenção do título de Doutor em Microbiologia.

Orientador: Prof. Dr. Flávio de Queiroz Telles Filho

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação MICROBIOLOGIA, PARASITOLOGIA E PATOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **CAROLINA MELCHIOR DO PRADO**, intitulada: **Aspectos epidemiológicos e microbiológicos da esporotricose transmitida por gatos na Região da Tríplice Fronteira do Paraná.**, sob orientação do Prof. Dr. FLÁVIO DE QUEIROZ TELLES FILHO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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I dedicate this work to my feline patients, the victims of sporotrichosis, who suffer from this disease every day. It is for them that my fight continues in the search for better treatments and prevention solutions.

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Hou je stevig vast aan de mooie dingen in het leven

RESUMO

Sporothrix brasiliensis, um patógeno fúngico emergente na América Latina, é o agente da esporotricose transmitida por gatos, uma epi-zoonose que afeta humanos, gatos e cães. Este estudo apresenta os primeiros casos autóctones de esporotricose felina em Ciudad del Este, Paraguai. Além disso, descreve-se o primeiro caso autóctone de esporotricose humana causada por *S. brasiliensis* no Paraguai, transmitida por um gato paraguaio infectado. O diagnóstico e o tratamento tardios ressaltam a necessidade de maior conscientização sobre a doença. Também descreve-se o surgimento de casos urbanos de esporotricose felina em Puerto Iguazú, Argentina. Para melhor compreender a resistência antifúngica, analisou-se 108 isolados de *S. brasiliensis* de casos felinos na região da tríplice fronteira (2021–2023). Os testes de susceptibilidade antifúngica (AFST) contra nove antifúngicos mostraram baixos níveis de concentração inibitória mínima (MIC) para a maioria dos agentes, exceto por algumas cepas que apresentaram valores anormais (*non-wild type*) de MIC para terbinafina. Notavelmente, a esporotricose cutânea disseminada foi a apresentação clínica predominante (61%) e a única forma associada a alta mortalidade (61%). Nossos achados indicam que os diferentes desfechos clínicos não são causados por resistência pré-existente ao itraconazol. Dado o avanço geográfico da *S. brasiliensis*, uma abordagem de Saúde Única (One Health) é essencial para controlar essa zoonose emergente por meio de vigilância, educação pública e políticas de saúde direcionadas.

Palavras-chave: *Sporothrix brasiliensis*; Esporotricose Felina; Resistência Antifúngica; One Health; Zoonose.

ABSTRACT

Sporothrix brasiliensis, an emerging fungal pathogen in Latin America, is the causative agent of cat-transmitted sporotrichosis, an epi-zoonosis affecting humans, cats, and dogs. This study presents the first autochthonous cases of feline sporotrichosis in Ciudad del Este, Paraguay. Additionally, it describes the first autochthonous case of human sporotrichosis caused by *S. brasiliensis* in Paraguay, transmitted by an infected Paraguayan cat. Late diagnosis and treatment highlight the need for greater awareness of the disease. The emergence of urban cases of feline sporotrichosis in Puerto Iguazú, Argentina, is also described. To better understand antifungal resistance, 108 *S. brasiliensis* isolates from feline cases in the triple border region (2021–2023) were analyzed. Antifungal susceptibility testing (AFST) against nine antifungals showed low minimum inhibitory concentration (MIC) levels for most agents, except for some strains that exhibited abnormal (*non-wild type*) MIC values for terbinafine. Notably, disseminated cutaneous sporotrichosis was the predominant clinical presentation (61%) and the only form associated with high mortality (61%). Our findings indicate that different clinical outcomes are not caused by pre-existing resistance to itraconazole. Given the geographic spread of *S. brasiliensis*, a One Health approach is essential to control this emerging zoonosis through surveillance, public education, and targeted health policies.

Keywords: *Sporothrix brasiliensis*; Feline Sporotrichosis; Antifungal Resistance; One Health; Zoonosis.

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SUMMARY

INTRODUCTION

Sporotrichosis is a neglected disease that primarily affects individuals with low economic and social status, living in precarious conditions and regions lacking basic sanitation. Domestic cats are the most affected victims of the disease, often abandoned or euthanized due to a lack of proper diagnosis and treatment. This factor is a direct consequence of the population's and veterinarians' lack of awareness about the disease. Additionally, an expanding epizootic outbreak continues to affect human and feline patients in various Brazilian regions, with the potential to reach neighboring countries. Therefore, understanding the disease in border regions is crucial for developing a control and prevention program and for constructing specific strategies for these locations.

This thesis addresses, in four chapters, an investigation of cases of Cat-transmitted sporotrichosis (CTS) caused by *Sporothrix brasiliensis* in the triple border region (TBR) between Brazil, Paraguay, and Argentina.

- The first chapter reports the first cases of feline sporotrichosis in Paraguay, where resident cats from Ciudad del Este presented with severe ulcerative lesions, respiratory symptoms, and nasal deformities. Molecular analysis confirmed the presence of *S. brasiliensis*.
- The second chapter describes the first autochthonous case of human sporotrichosis in Paraguay. A 39-year-old woman from Presidente Franco developed progressive skin lesions after being scratched by her cat. The diagnosis was initially mistaken for a bacterial infection, highlighting the need for greater awareness of the disease.
- The third chapter addresses a significant outbreak on the Argentine side, in the city of Puerto Iguazú, Misiones. Surveillance between August 2023 and February 2024 detected *S. brasiliensis* in 42.8% of sampled cats, suggesting a transmission dynamic similar to that observed in the Brazilian epidemic.
- The fourth chapter focuses on the analysis of 108 *S. brasiliensis* isolates from cats before antifungal treatment. Antifungal susceptibility tests indicated that most strains had low minimum inhibitory concentration (MIC) levels for itraconazole, suggesting that treatment failures were not due to pre-existing drug resistance. Clinical outcomes of the cats were also analyzed, revealing that those with disseminated cutaneous sporotrichosis had a 61% mortality

rate, while those with fixed cutaneous or extracutaneous forms did not die from the disease. This suggests that disease severity and progression significantly influence treatment outcomes. The study highlights the rapid spread of *Sporothrix brasiliensis* in the TBR, with cases initially concentrated in the eastern region of Foz do Iguaçu, Brazil, and later spreading to areas near the international bridges. Regarding genetic analysis, genotyping through Short Tandem Repeat (STR) revealed that all isolates from the TBR are closely related to the Rio de Janeiro (RdJ) clade, a dominant *S. brasiliensis* genotype group. This suggests that the pathogen may have been introduced into the region through the movement of infected cats.

1 CHAPTER 1 - First Cases of Feline Sporotrichosis Caused by *Sporothrix brasiliensis* in Paraguay

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1.1 Abstract

Sporothrix brasiliensis is an emerging fungal pathogen causing cat-transmitted sporotrichosis, an epi-zoonosis affecting humans, cats and dogs in Brazil and now spreading to neighboring South American countries. Here, we report the first two autochthonous cases of cat-transmitted sporotrichosis in Paraguay. The first case was a four-year-old male cat showing several ulcerative lesions, nasal deformity and respiratory symptoms. The second case was a one-year-old male cat showing a single ulcerated lesion, respiratory symptoms and nasal deformity. Both cases were admitted to a veterinary clinic in Ciudad del Este, Paraguay. Isolates were recovered from swabs of the two cases. Using molecular methods, the isolates were identified as *S. brasiliensis*.

1.2 Introduction

Sporotrichosis, caused by fungi of the genus *Sporothrix*, is the most prevalent and globally distributed of the implantation mycoses [1]. Transmission is usually by transcutaneous or transmucosal traumatic inoculation of the fungus present in soil, plants and decomposing organic matter (sapronotic), and through enzootic (cat-to-cat/dog) and zoonotic (cat-to-human) routes. *Sporothrix schenckii* and *Sporothrix brasiliensis* are the most common etiological agents in human and animal diseases in Brazil [2,3].

Cat-transmitted sporotrichosis (CTS) is a neglected disease. Since the 1990s, this important zoonosis has emerged as a public health problem in Brazil, where it has been responsible for thousands of associated human and feline cases, which almost exclusively involve infections with *S. brasiliensis* [4]. Cases have quickly spread throughout Brazil and to other countries in South America [5]. In the enzootic (cat-to-cat, cat-to-dog) and zoonotic (cat-to-human) routes, transmission can occur through bites, scratches, contact with exudate from skin lesions and probably through respiratory droplets during cat sneezing [6,7].

In cats, clinical manifestations may include single or multiple ulcerative lesions on the skin, associated with enlarged lymph nodes and respiratory signs (mainly sneezing). Most commonly, cats can present a disseminated disease with multiple cutaneous lesions and mucosal involvement, especially the nasal mucosa.

Conjunctival, oral and genital mucosa may also be affected [8]. Culture isolation is the gold standard technique for the diagnosis of sporotrichosis in animals. But since infected cats display a high fungal burden, the direct examination of yeast cells isolated from skin lesions is frequently used by veterinarians and microbiologists in clinical practice [6]. Molecular identification methods based on the sequence analysis of the calmodulin gene are required to identify the *Sporothrix* species [9].

In Paraguay, the only reported CTS cases so far occurred in 2017 among relatives who moved from Brazil with an infected cat. Laboratory tests performed identified the etiological agent as *Sporothrix* spp. (without molecular identification). This was the first record of a travel-associated case of *Sporothrix* outside Brazil or Argentina, documenting the potential for spread to new areas through the transport of infected cats [10]. Other cases supporting CTS's potential to spread were observed in the U.S. and U.K. In 2020, a Brazilian woman was diagnosed with CTS in Boston, U.S., after being bitten by a sick cat in Minas Gerais, Brazil [11]. More recently, researchers reported the first human and feline *S. brasiliensis* cases in the U.K., both involving people who had moved from Brazil [12]. In 2019, the World Health Organization published an international alert on the potential risk of transmission of infection by *S. brasiliensis* in neighboring countries [13]. Besides the spread of CTS in Brazil, sporotrichosis cases caused by *S. brasiliensis* have been observed in cats in other Latin American countries without previous exposure to Brazil [14,15]. In Argentina, for example, *S. brasiliensis* has already been described in cats from the provinces of Buenos Aires and Santa Cruz, located in the Argentinean Patagonian region. In Misiones, a province bordering Brazil, there was a single description of *S. brasiliensis* isolated from a human in 1986 [15]. In 2022, researchers from Chile described three cases of domestic and feral cats with *S. brasiliensis* in the Magallanes region of southern Chile. Those cases were the first isolation reports of *S. brasiliensis* in cats from Chile. Cases 1 and 2 came from the Río Verde County, a small rural village, and case 3 came from the city of Punta Arenas [14]. Here, we report the first autochthonous feline cases of CTS by *S. brasiliensis* in Paraguay (PY).

1.3 Case Presentation

This study was approved by the Committee for Ethics in Research of the Federal University of Parana (number—CAAE 52726021.8.0000.0102) and by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Federal University of Parana.

Ciudad del Este (CDE) (25°29′36″ S and 54°40′18″ W) is a city and district of PY, located in the extreme east of the country on the banks of the Paraná River. More specifically, it is situated on the Triple Border with Argentina and Brazil and has approximately 300,000 inhabitants. It is the capital of the Alto Paraná department, located 327 km from the country's capital—Asunción.

The first case observed in this city was a four-year-old mixed-breed male cat admitted on June 22 to a veterinary clinic in CDE, with numerous ulcerated lesions and respiratory symptoms. The animal had a history of respiratory signs (sneezing, nasal secretion and respiratory difficulty) for about twenty-one days and ulcerated lesions for about two months (beginning with lesions on the tail and later involving lesions on the face and paws). After a physical examination, four lesions in the cranial region (Figure 1A), a small nasal deformity (Figure 1A), one lesion in the left hind limb (Figure 1B) and four ulcerated lesions in the initial and medial portion of the tail (which had been amputated due to tissue necrosis) were found (Figure 1C).

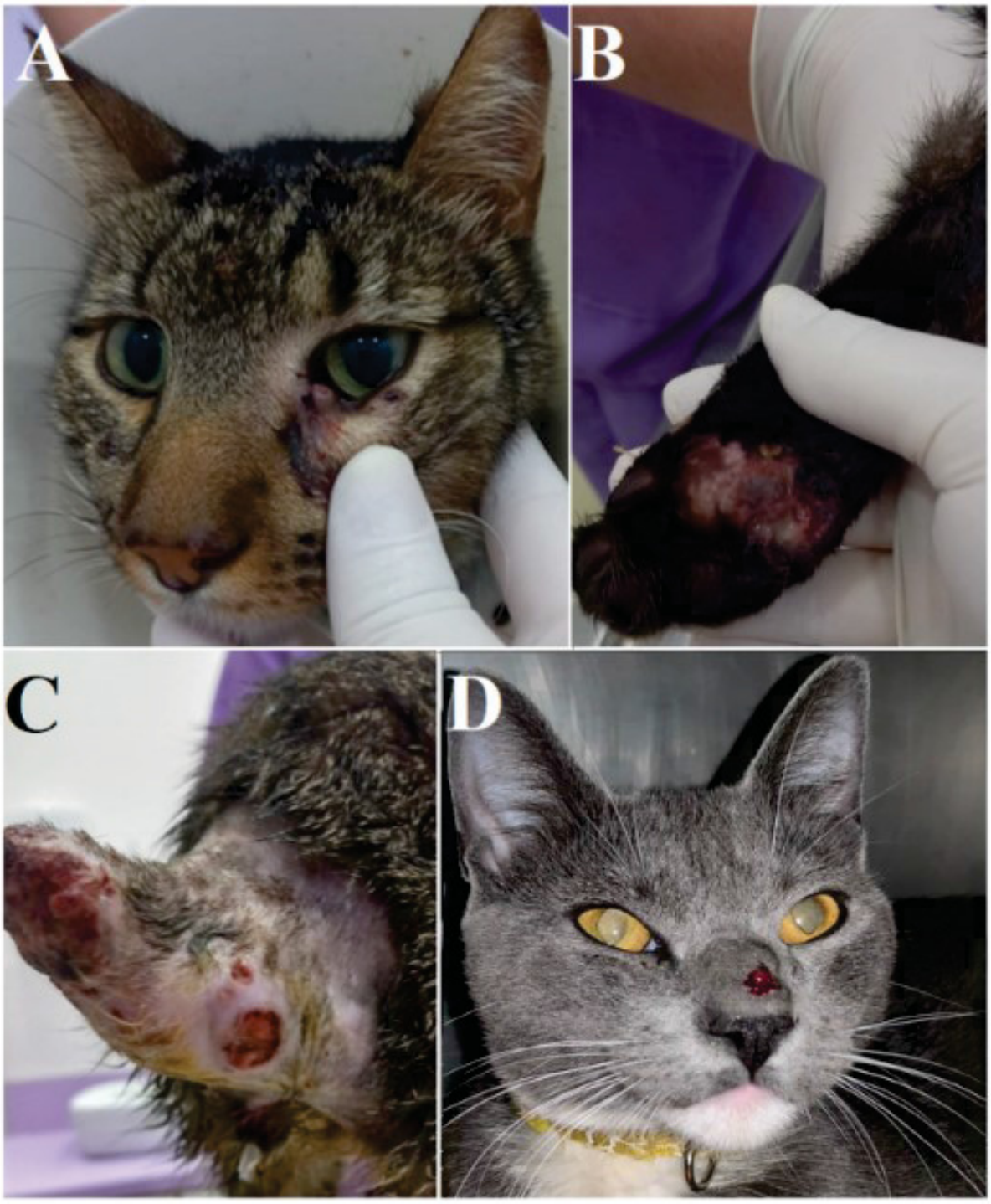

Figure 1. Lesions in the cranial region and a small nasal deformity (A), ulcerated lesion in the left hind limb (B) and ulcerated lesions in the initial and medial portion of the tail (C) found after physical examination of animal 1. Nasal deformity and ulcerated lesion (D) found after physical examination of the animal 2.

Two months later (on August 22), a second case was admitted to the same veterinary clinic in CDE. It was a one-year-old mixed-breed male cat showing a single ulcerated lesion in the nose, respiratory symptoms (sneezing, nasal secretion and

respiratory difficulty) and nasal deformation. The onset of clinical signs occurred eight days before the presentation at the clinic. After a physical examination, the only ulcerated lesion was confirmed, together with a significant nasal deformity (Figure 1D). This patient, who presented symptoms two months after the diagnosis of the first case, was from the same residence as the previous one.

In both cases, feline sporotrichosis was determined as the main diagnostic suspicion. Relevant clinical and epidemiological data were collected through clinical–epidemiological questionnaires applied to the owners (Figure A1 and Figure A2). The animals had a history of climbing and scratching trees and burying their feces outside. They were neutered and had no history of other previous illnesses. Both had had only their first rabies vaccine and access to the yard, the street and other residences. The owners reported that both animals had always lived in CDE and had no previous travel history. The geographic coordinates of the cats' residence were established using Google Earth® software. Cartographic bases from the Brazilian Institute of Geography and Statistics (IBGE) were used for georeferencing the coordinates. Quantus Gis software (QGIS) was used to assemble the map (Figure 2). The animals had not received any previous treatment with antibiotics and antifungals.

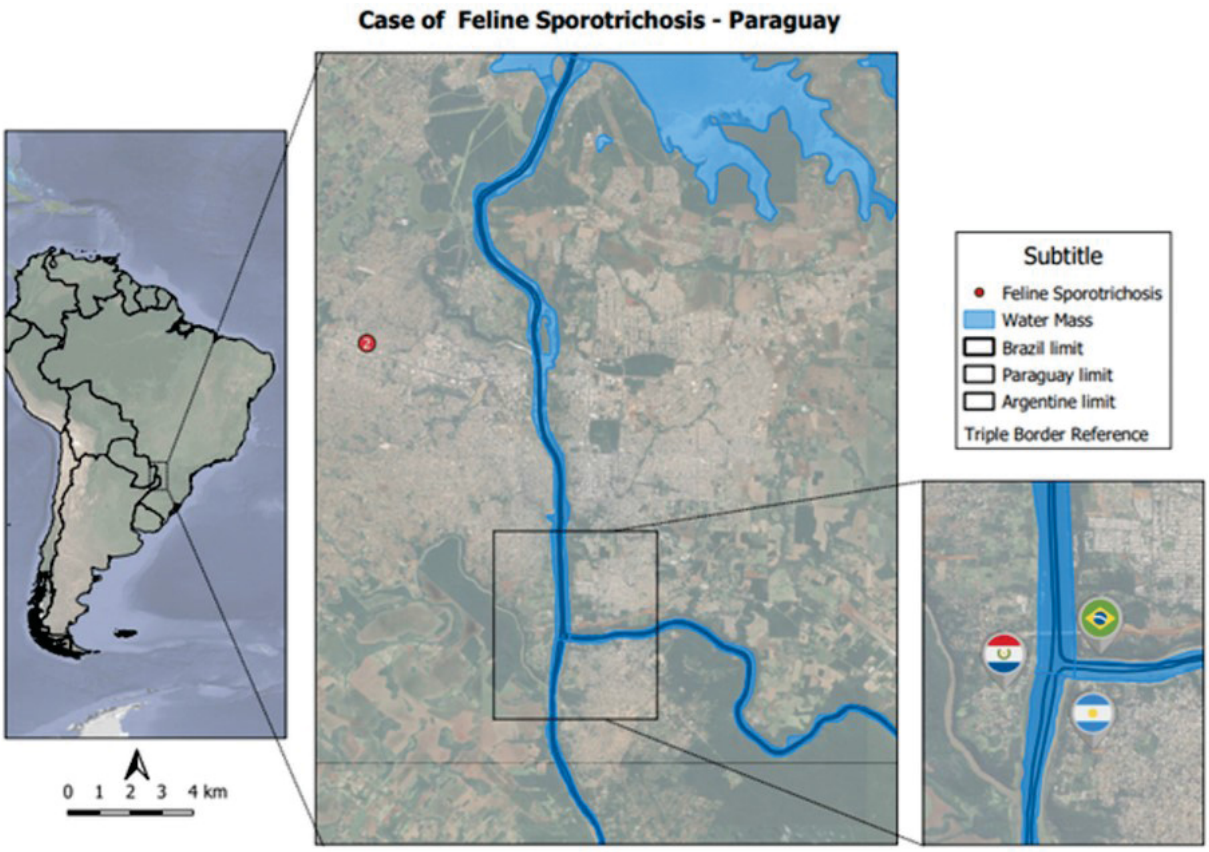

Figure 2. Spatial distribution of the two animals with a positive diagnosis for sporotrichosis in Ciudad del Este, Paraguay.

In the first case, the patient was referred for euthanasia due to his poorly clinical status. In the second case, 100 mg/day itraconazole and 30 mg/day potassium iodide therapy were applied for about eight months until the clinical signs disappeared and were maintained for another two months to minimize the risk of recurrence.

For both cases, swab samples of the lesions and slides with an imprint of the lesions were collected to perform the definitive diagnosis. Mycological examination of the slide samples was performed based on direct Gram-stain microscopy, which enabled visualizing cigar-shaped yeast structures in the feline samples (Figure 3A). Considering that culture is the gold standard for the diagnosis of sporotrichosis [6], swabs collected from the wounds were placed in Stuart's medium and sent to the laboratory for mycological cultures. The samples were cultivated on Sabouraud dextrose agar (SDA) (KASVI, ttps://www.kasvi.com.br, accessed on 9 June 2022) containing chloramphenicol and incubated at 25 °C for the growth of colonies for up to 10 days.

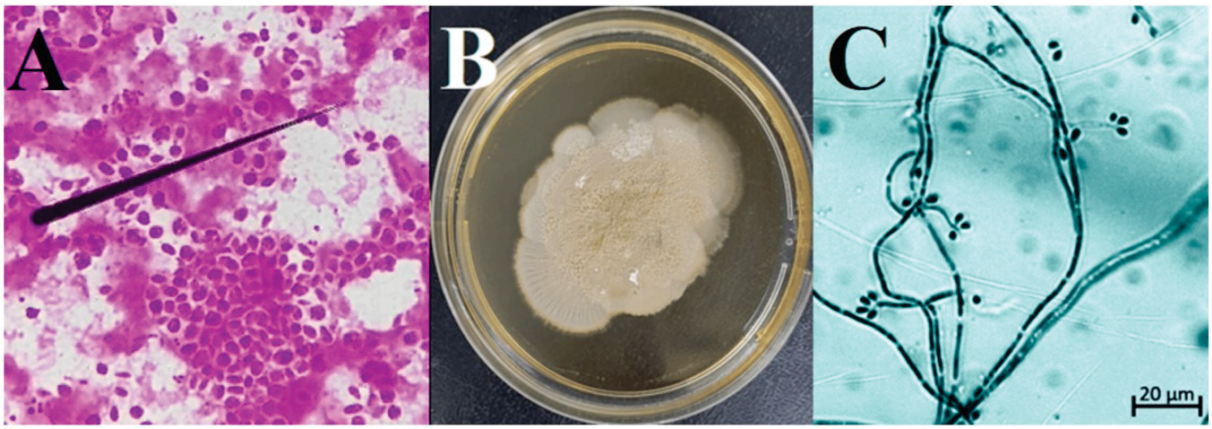

Figure 3. (A) Cigar-shaped yeast structures (black arrow) observed in feline samples (Gram-stain, 1000×). (B) *Sporothrix* colony after growth on Sabouraud agar with chloramphenicol. (C) Micromorphology of *Sporothrix* spp. colonies (black arrow) (lactophenol cotton blue ×400).

Macroscopic and microscopic characteristics of the colonies were observed and confirmed the fungi of the genus *Sporothrix* identity. The clinical samples showed growth of a white, creamy colony at 25 °C (Figure 3B). For the identification

of the mycelial phase, monosporic cultures were grown on SDA at room temperature. We used the slide culture method to characterize microscopic filamentous features of the *Sporothrix* spp. isolates. We inoculated mycelia fragments into 1 × 1 cm SDA blocks, which were then mounted on a slide, covered with a coverslip and incubated for 14–21 days at room temperature. The mycelial preparation was stained with lactophenol blue and visualized with a Zeiss AxioObserver Z1 microscope equipped with a 40× objective. The microscopical analysis showed hyaline hyphae and septate with rounded unicellular conidia (Figure 3C).

Molecular analysis of the two isolates was based on calmodulin (CAL) gene sequences. For molecular identification, colonies cultivated on Sabouraud glucose agar (SGA) were used for DNA extraction. The fungi mycelium was transferred to a 1.5 mL tube containing 100 mg of a silica celite mixture (2:1, w/w) and 300 µL CTAB buffer [CTAB 2% (w/v), NaCl 1.4 M, Tris-HCl 100 mM, pH 8.0; EDTA 20 mM]. Fungi cells were disrupted with a pestle for 5 min and incubated for 40 min at 65 °C. Then, 500 µL 24:1 chloroform: isoamyl alcohol (CIA) was added and the solution was centrifuged for 10 min at 20,500× g. The supernatant was collected in a new tube with 2 vols of ice-cold 96% ethanol. The DNA was precipitated for 2 h at −20 °C and then centrifuged for 10 min at 20,500× g, washed with cold 70% ethanol, dried at room temperature and resuspended in 100 µL in ultrapure water [16]. DNA integrity was assessed by running a 5 µL aliquot of the extraction on a 0.8% agar agarose-gel electrophoresis stained with 0.5 µg/ mL ethidium bromide. To estimate the DNA yield, we used a NanoDrop 1000c Instrument (Thermo Fisher Scientific, Waltham, MA, USA). Amplification of the CAL was performed using CL1 and CL2A primers [17]. PCR reaction mixtures were made with a total volume of 12.5 µL (1× PCR buffer, 2.0 mM MgCl2, 25 µM deoxynucleotide triphosphates (dNTPs)), 0.5 µM of each forward and reverse primer, 1 U of Taq DNA polymerase (Ludwig Biotec, Bela Vista, Brazil) and 10 ng of genomic DNA. The PCR parameters for amplification were 94 °C for 3 min, followed by 35 cycles consisting of 94 °C for 35 s, 58 °C for 30 s and 72 °C for 1 min, and a delay at 72 °C for 1 min, performed in an ABI Prism 2720 thermocycler (Applied Biosystems, Foster City, CA, USA). Amplicons were sequenced with BigDye Terminator cycle sequencing kit v. 3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, using the same primers of the PCR, and the amplification conditions were as follows: 95 °C for 1 min, followed by 30 cycles consisting of 95 °C for 10 s, 50 °C for 5 s and 60 °C. The consensus sequence of the

CAL regions was visually inspected using MEGA v.7 software [18] and compared to the GenBank Blast (NCBI). The sequences of CAL were aligned with reference strains (Table A1) using the online MUSCLE interface and the substitution model was selected for each genus using the MEGA software (GTR + G). The chosen index was the one that presented the lowest AIC. The phylogeny was constructed based on the CAL gene. Maximum likelihood analysis was performed in the RaxML-HPC2 [19] implemented in the CIPRES server using the 1000 bootstrap to reach split frequencies of ≤0.01. The resulting trees were plotted in FigTree v.1.4.2 [20]. The results showed that the clinical strains were *Sporothrix brasiliensis* (Figure **3**4).

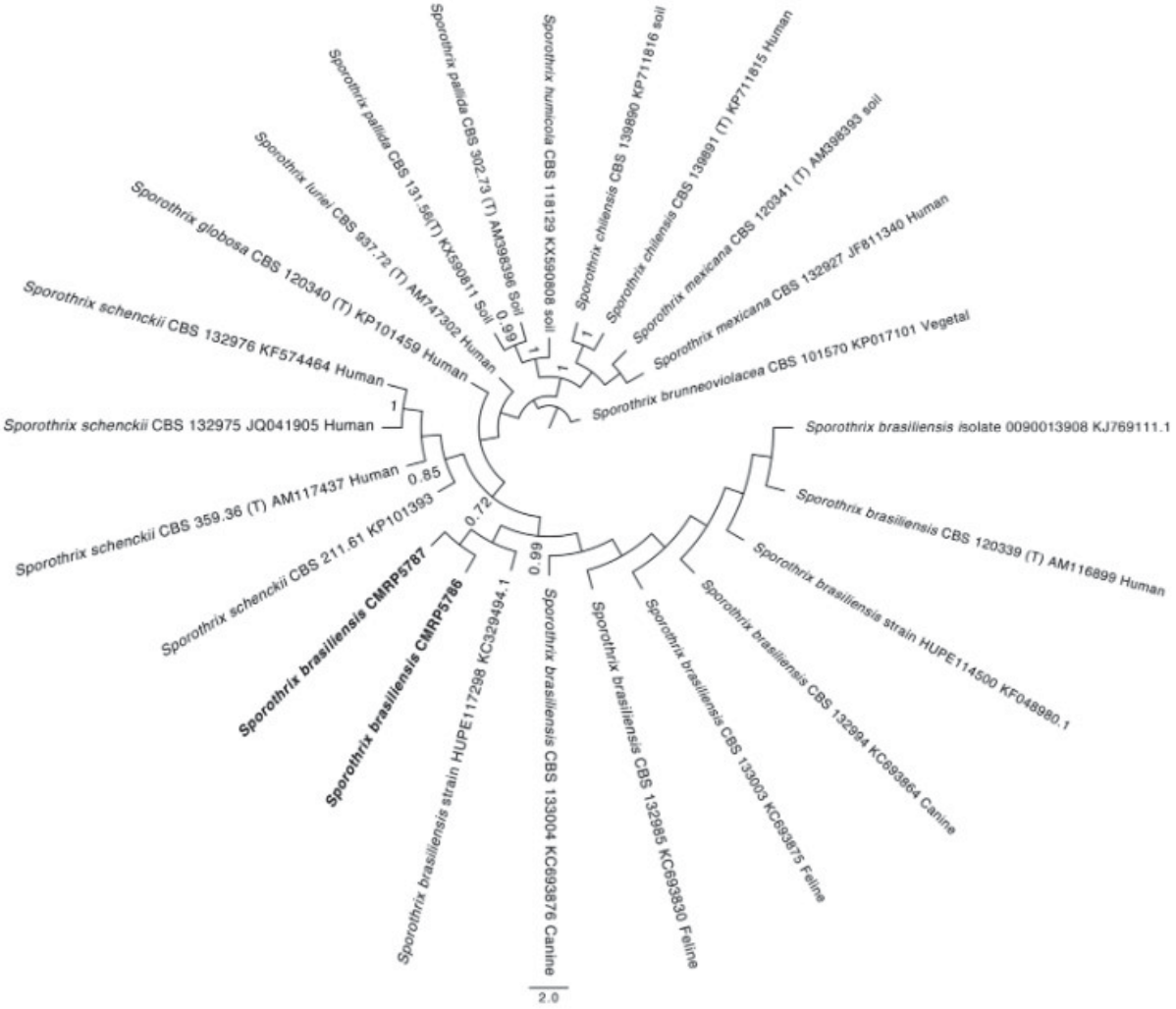

Figure 4. Phylogenetic tree of *Sporothrix brasiliensis*, obtained by ML analysis based on partial sequences of the CAL. The isolates CMRP5786 and CMRP5787 represent the isolates of Paraguay. Reference strains used can be seen in Table A1.

1.4 Discussion

These are the first feline cases of CTS in PY. Human sporotrichosis is a deep mycosis, relatively common in PY. In PY, this disease is still mostly related to the classic form of transmission, the sapronotic route, and has the greatest effect on certain professions such as carpenters, gardeners and farmers [10].

Ciudad del Este is situated near the Iguazu Falls at the busiest frontier in Brazil and in the context of the triple international border between Brazil, Paraguay and Argentina. The flow of people, animals, plants and microorganisms, sometimes without proper inspection and monitoring, is constant. And in terms of the flow of people, Foz do Iguaçu, as an important tourist destination, presents other peculiarities, occupying the third place in the preference of foreign tourists arriving in the country. Therefore, it is necessary to reflect on how these international borders can contribute to the dissemination of microorganisms such as *S. brasiliensis* in view of the lack of specific public health policies in this region [21].

In recent decades, the domestic cat (*Felis catus*) has played an important role in the epidemiology of sporotrichosis due to its increased susceptibility to this disease and its role as a vehicle of transmission to humans through bites, scratches, sneezing and contact with secretions from infected animals [8]. Sporotrichosis in cats is generally systemic, showing disseminated lesions with a high burden of infectious yeast-like cells and which, without treatment, cause death [22]. Infected animals show disseminated cutaneous lesions characterized by ulcers and nodules with exudate, especially on the face. Upon licking or touching with the extremities, the agent is transferred to the paws or the mouth, facilitating contagion. This behavior, together with the high burden of mycotic elements in the wounds, facilitates contagion among cats and other animals such as dogs and humans [23].

Although the first animal was euthanized, it is important to note that sporotrichosis is treatable. The drugs indicated for sporotrichosis treatment are itraconazole, potassium iodide, terbinafine and amphotericin B. Itraconazole is the first choice for treatment, at a dose of 100 mg/day until the complete resolution of the lesions [24]. Treatment duration varies in the literature and is determined by the patient's clinical response. Furthermore, it is important to point out that the capacity for transmission by the infected cat reduces exponentially with the implementation of the treatment [15].

Furthermore, the animal's history of access to the streets should always be considered during the period of treatment, especially in endemic regions [23]. Therefore, it is worth highlighting the importance of considering sporotrichosis as a differential diagnosis for animals showing skin lesions due to the potential zoonotic risk of *S. brasiliensis* transmission [25].

Animals under suspicion of sporotrichosis should be isolated from other animals. Decontamination of the environment can be carried out with sodium hypochlorite (bleach) [8]. Injuries resulting from the typical behavior of cats in distress should be avoided through the application of correct animal restraint techniques and the use of personal protective equipment, such as procedure gloves, aprons and goggles [26]. This is especially important as this fungal species has a high transmission potential due to contact with secretions and exudates from the lesions of sick cats during treatment and handling procedures. Feline management methods based on the "Cat-Friendly Practices" philosophy can help prevent injuries, as they aim to keep the patient calm so that their handling is peaceful and safe [27].

Author Contributions

Conceptualization, C.M.d.P., V.A.V., W.K.S. and F.Q.-T.; writing—original draft preparation, C.M.d.P. and F.Q.-T.; writing—review and editing, C.M.d.P., V.A.V., W.K.S. and F.Q.-T.; investigation and data curation, C.M.d.P., E.R., G.S., L.O., M.R.G., N.S. and J.P.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Committee for Ethics in Research of the Federal University of Parana (number—CAAE 52726021.8.0000.0102, 28 December 2021) and by the Animal Use Ethics Committee of the Agricultural Sciences Campus of the Federal University of Parana (protocol code 028/2021, 7 June 2021).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are openly available in NCBI, accession number OR501574 and OR501573.

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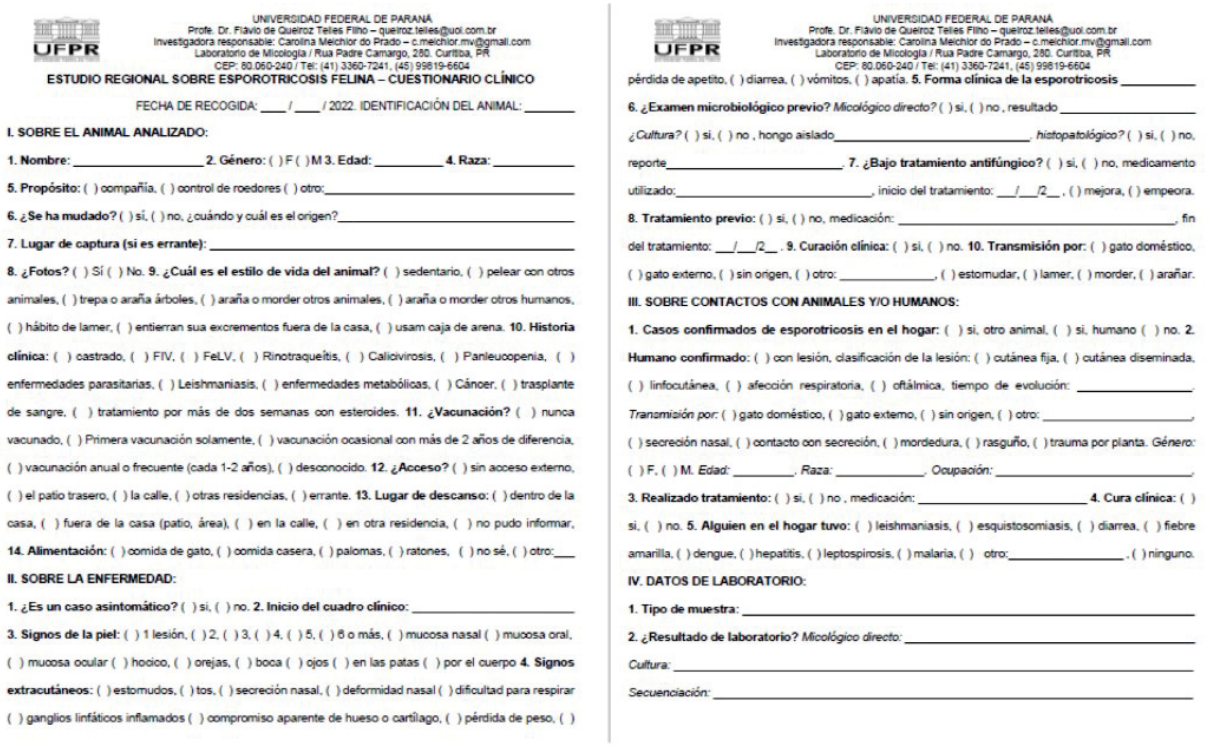
Conflicts of Interest

The authors declare no conflict of interest.

Appendix A



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ESTUDIO REGIONAL SOBRE ESPOROTRICOSIS FELINA – CUESTIONARIO CLÍNICO

FECHA DE RECOGIDA: ___ / ___ / 2022. IDENTIFICACIÓN DEL ANIMAL: _____

I. SOBRE EL ANIMAL ANALIZADO:

1. Nombre: _____ **2. Género:** ( ) F ( ) M **3. Edad:** _____ **4. Raza:** _____

5. Propósito: ( ) compañía, ( ) control de roedores ( ) otro:_____

6. ¿Se ha mudado? ( ) sí, ( ) no, ¿cuándo y cuál es el origen?_____

7. Lugar de captura (si es errante): _____

8. ¿Fotos? ( ) Sí ( ) No. **9. ¿Cuál es el estilo de vida del animal?** ( ) sedentario, ( ) pelear con otros animales, ( ) trepa o araña árboles, ( ) araña o morder otros animales, ( ) araña o morder otros humanos, ( ) hábito de lamer, ( ) entierran sua excrementos fuera de la casa, ( ) usam caja de arena. **10. Historia clínica:** ( ) castrado, ( ) FIV, ( ) FeLV, ( ) Rinotraqueítis, ( ) Calicivirosis, ( ) Panleucopenia, ( ) enfermedades parasitarias, ( ) Leishmaniasis, ( ) enfermedades metabólicas, ( ) Cáncer, ( ) trasplante de sangre, ( ) tratamiento por más de dos semanas con esteroides. **11. ¿Vacunación?** ( ) nunca vacunado, ( ) Primera vacunación solamente, ( ) vacunación ocasional con más de 2 años de diferencia, ( ) vacunación anual o frecuente (cada 1-2 años), ( ) desconocido. **12. ¿Acceso?** ( ) sin acceso externo, ( ) el patio trasero, ( ) la calle, ( ) otras residencias, ( ) errante. **13. Lugar de descanso:** ( ) dentro de la casa, ( ) fuera de la casa (patio, área), ( ) en la calle, ( ) en otra residencia, ( ) no pudo informar. **14. Alimentación:** ( ) comida de gato, ( ) comida casera, ( ) palomas, ( ) ratones, ( ) no sé, ( ) otro:___

II. SOBRE LA ENFERMEDAD:

1. ¿Es un caso asintomático? ( ) si, ( ) no. **2. Inicio del cuadro clínico:** _____

3. Signos de la piel: ( ) 1 lesión, ( ) 2, ( ) 3, ( ) 4, ( ) 5, ( ) 6 o más, ( ) mucosa nasal ( ) mucosa oral, ( ) mucosa ocular ( ) hocico, ( ) orejas, ( ) boca ( ) ojos ( ) en las patas ( ) por el cuerpo **4. Signos extracutáneos:** ( ) estornudos, ( ) tos, ( ) secreción nasal, ( ) deformidad nasal ( ) dificultad para respirar ( ) ganglios linfáticos inflamados ( ) compromiso aparente de hueso o cartílago, ( ) pérdida de peso, ( ) pérdida de apetito, ( ) diarrea, ( ) vómitos, ( ) apatía. **5. Forma clínica de la esporotricosis** _____

6. ¿Examen microbiológico previo? *Micológico directo?* ( ) si, ( ) no , resultado _____

¿Cultura? ( ) si, ( ) no , hongo aislado_____ *histopatológico?* ( ) si, ( ) no, reporte_____. **7. ¿Bajo tratamiento antifúngico?** ( ) si, ( ) no, medicamento utilizado:_____, inicio del tratamiento: ___/___/2__ , ( ) mejora, ( ) empeora.

8. Tratamiento previo: ( ) si, ( ) no, medicación: _____, fin del tratamiento: ___/___/2__ . **9. Curación clínica:** ( ) si, ( ) no. **10. Transmisión por:** ( ) gato doméstico, ( ) gato externo, ( ) sin origen, ( ) otro: _____, ( ) estornudar, ( ) lamer, ( ) morder, ( ) arañar.

III. SOBRE CONTACTOS CON ANIMALES Y/O HUMANOS:

1. Casos confirmados de esporotricosis en el hogar: ( ) si, otro animal, ( ) si, humano ( ) no. **2. Humano confirmado:** ( ) con lesión, clasificación de la lesión: ( ) cutánea fija, ( ) cutánea diseminada, ( ) linfocutánea, ( ) afección respiratoria, ( ) oftálmica, tiempo de evolución: _____

Transmisión por: ( ) gato doméstico, ( ) gato externo, ( ) sin origen, ( ) otro: _____, ( ) secreción nasal, ( ) contacto con secreción, ( ) mordedura, ( ) rasguño, ( ) trauma por planta. *Género:* ( ) F, ( ) M. *Edad:* _____. *Raza:* _____. *Ocupación:* _____.

3. Realizado tratamiento: ( ) si, ( ) no , medicación: _____ **4. Cura clínica:** ( ) si, ( ) no. **5. Alguien en el hogar tuvo:** ( ) leishmaniasis, ( ) esquistosomiasis, ( ) diarrea, ( ) fiebre amarilla, ( ) dengue, ( ) hepatitis, ( ) leptospirosis, ( ) malaria, ( ) otro:_____, ( ) ninguno.

IV. DATOS DE LABORATORIO:

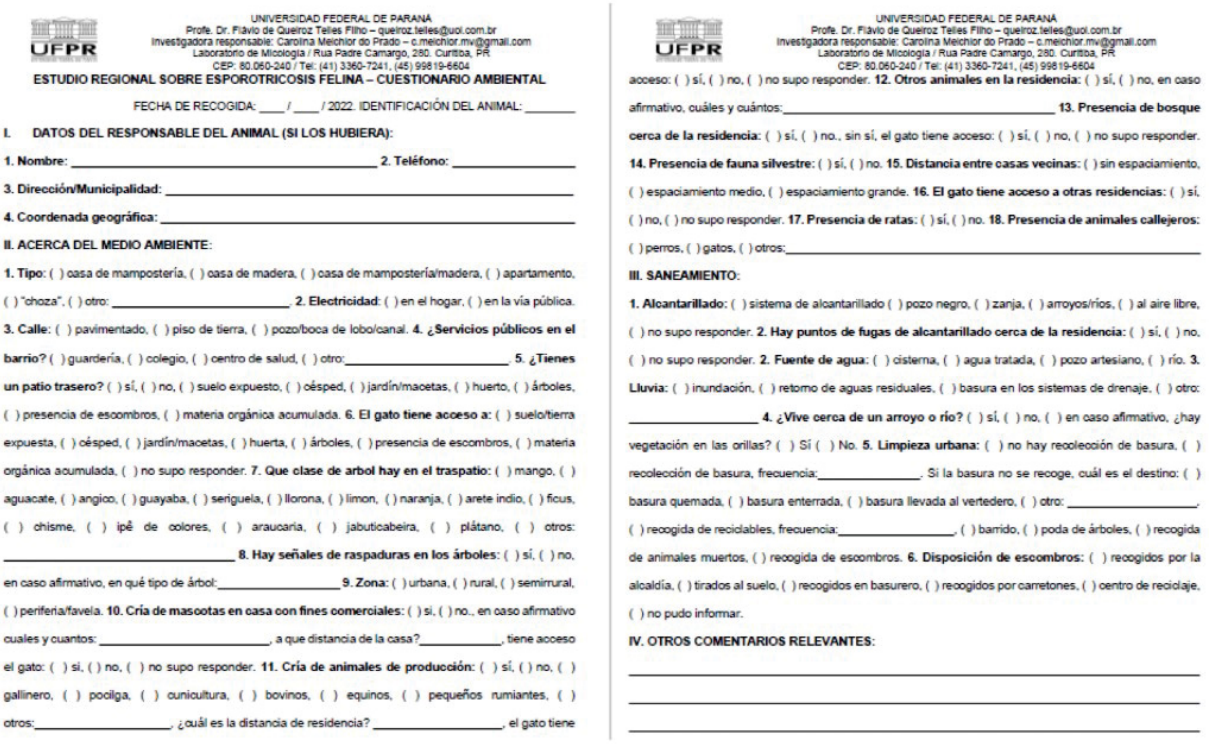
1. Tipo de muestra: _____

2. ¿Resultado de laboratorio? *Micológico directo:* _____

Cultura: _____

Secuenciación: _____

Figure A1. Clinical–epidemiological questionnaire applied to the owners.



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ESTUDIO REGIONAL SOBRE ESPOROTRICOSIS FELINA – CUESTIONARIO AMBIENTAL

FECHA DE RECOGIDA: ___ / ___ / 2022. IDENTIFICACIÓN DEL ANIMAL: _____

I. DATOS DEL RESPONSABLE DEL ANIMAL (SI LOS HUBIERA):

1. Nombre: _____ **2. Teléfono:** _____

3. Dirección/Municipalidad: _____

4. Coordenada geográfica: _____

II. ACERCA DEL MEDIO AMBIENTE:

1. Tipo: ( ) casa de mampostería, ( ) casa de madera, ( ) casa de mampostería/madera, ( ) apartamento, ( ) "choza", ( ) otro: _____. **2. Electricidad:** ( ) en el hogar, ( ) en la vía pública. **3. Calle:** ( ) pavimentado, ( ) piso de tierra, ( ) pozo/boca de lobo/canal. **4. ¿Servicios públicos en el barrio?** ( ) guardería, ( ) colegio, ( ) centro de salud, ( ) otro:_____. **5. ¿Tienes un patio trasero?** ( ) sí, ( ) no, ( ) suelo expuesto, ( ) césped, ( ) jardín/macetas, ( ) huerto, ( ) árboles, ( ) presencia de escombros, ( ) materia orgánica acumulada. **6. El gato tiene acceso a:** ( ) suelo/tierra expuesta, ( ) césped, ( ) jardín/macetas, ( ) huerta, ( ) árboles, ( ) presencia de escombros, ( ) materia orgánica acumulada, ( ) no supo responder. **7. Que clase de arbol hay en el traspatio:** ( ) mango, ( ) aguacate, ( ) angico, ( ) guayaba, ( ) seriguela, ( ) llorona, ( ) limon, ( ) naranja, ( ) arete indio, ( ) ficus, ( ) chisme, ( ) ipê de colores, ( ) araucaria, ( ) jabuticabeira, ( ) plátano, ( ) otros: _____ **8. Hay señales de raspaduras en los árboles:** ( ) sí, ( ) no, en caso afirmativo, en qué tipo de árbol:_____ **9. Zona:** ( ) urbana, ( ) rural, ( ) semirrural, ( ) periferia/favela. **10. Cría de masootas en casa con fines comerciales:** ( ) si, ( ) no., en caso afirmativo cuales y cuantos: _____, a que distancia de la casa?_____, tiene acceso el gato: ( ) si, ( ) no, ( ) no supo responder. **11. Cría de animales de producción:** ( ) sí, ( ) no, ( ) gallinero, ( ) pocilga, ( ) cunicultura, ( ) bovinos, ( ) equinos, ( ) pequeños rumiantes, ( ) otros:_____, ¿cuál es la distancia de residencia? _____, el gato tiene acceso: ( ) sí, ( ) no, ( ) no supo responder. **12. Otros animales en la residencia:** ( ) sí, ( ) no, en caso afirmativo, cuáles y cuántos:_____ **13. Presencia de bosque cerca de la residencia:** ( ) sí, ( ) no., sin sí, el gato tiene acceso: ( ) sí, ( ) no, ( ) no supo responder. **14. Presencia de fauna silvestre:** ( ) sí, ( ) no. **15. Distancia entre casas vecinas:** ( ) sin espaciamiento, ( ) espaciamiento medio, ( ) espaciamiento grande. **16. El gato tiene acceso a otras residencias:** ( ) sí, ( ) no, ( ) no supo responder. **17. Presencia de ratas:** ( ) sí, ( ) no. **18. Presencia de animales callejeros:** ( ) perros, ( ) gatos, ( ) otros:_____

III. SANEAMIENTO:

1. Alcantarillado: ( ) sistema de alcantarillado ( ) pozo negro, ( ) zanja, ( ) arroyos/ríos, ( ) al aire libre, ( ) no supo responder. **2. Hay puntos de fugas de alcantarillado cerca de la residencia:** ( ) sí, ( ) no, ( ) no supo responder. **2. Fuente de agua:** ( ) cisterna, ( ) agua tratada, ( ) pozo artesiano, ( ) río. **3. Lluvia:** ( ) inundación, ( ) retorno de aguas residuales, ( ) basura en los sistemas de drenaje, ( ) otro: _____ **4. ¿Vive cerca de un arroyo o río?** ( ) sí, ( ) no, ( ) en caso afirmativo, ¿hay vegetación en las orillas? ( ) Sí ( ) No. **5. Limpieza urbana:** ( ) no hay recolección de basura, ( ) recolección de basura, frecuencia:_____. Si la basura no se recoge, cuál es el destino: ( ) basura quemada, ( ) basura enterrada, ( ) basura llevada al vertedero, ( ) otro: _____, ( ) recogida de reciclables, frecuencia:_____, ( ) barrido, ( ) poda de árboles, ( ) recogida de animales muertos, ( ) recogida de escombros. **6. Disposición de escombros:** ( ) recogidos por la alcaldía, ( ) tirados al suelo, ( ) recogidos en basurero, ( ) recogidos por carretones, ( ) centro de reciclaje, ( ) no pudo informar.

IV. OTROS COMENTARIOS RELEVANTES:

_____

_____

_____

Figure A2. Clinical–epidemiological questionnaire applied to the owners.

Table A1. Reference strains used for CAL gene alignment.

Species	Isolate code	CBS code	Geographic origin	Source	GenBank	Reference
Sporothrix_ schenckii	Ss159	CBS_132976	Japan	Human	KF574464	[28]
Sporothrix_ schenckii	Ss141	CBS_132975	Brazil	Human	JQ041905	[29]
Sporothrix_ schenckii	-	CBS_359.36_(T)	NK	Human	AM117437	[30]
Sporothrix_ schenckii	-	CBS_211.61	South Africa	Not Know	KP101393	[31]
Sporothrix_ pallida	-	CBS_302.73_(T)	United Kingdom	Soil	AM398396	[32]
Sporothrix_ pallida	-	CBS_131.56(T)	Japan	Soil	KX590811	[33]
Sporothrix_ mexicana	Ss 132	CBS_132927	Brazil	Human	JF811340	[34]
Sporothrix_ mexicana	FMR 9108	CBS_120341_(T)	Mexico	Soil	AM398393	[32]
Sporothrix_ luriei	ATCC 18616	CBS_937.72_(T)	South Africa	Human	AM747302	[35]
Sporothrix_ humicola	-	CBS_118129	South Africa	Soil	KX590808	[33]
Sporothrix_ globosa	-	CBS_120340_(T)	Spain	Human	KP101459	[31]
Sporothrix_ chilensis	Ss469	CBS_139891_(T)	Chile	Human	KP711815	[36]
Sporothrix_ chilensis	Ss470	CBS_139890	Chile	Soil	KP711816	[36]
Sporothrix_ brunneoviolacea	-	CBS_101570	USA	Vegetal	KP017101	[31]
Sporothrix_ brasiliensis	Ss227	CBS_133004	Brazil	Canine	KC693876	[37]
Sporothrix_ brasiliensis	Ss226	CBS_133003	Brazil	Feline	KC693875	[37]
Sporothrix_ brasiliensis	Ss151	CBS_132994	Brazil	Canine	KC693864	[37]
Sporothrix	*0090013908*	-	Brazil	Human	*KJ769111*	[38]

brasiliensis						
Sporothrix brasiliensis	Ss05	*CBS 132985*	Brazil	Feline	*KC693830*	[37]
Sporothrix brasiliensis	HUPE117298	-	Brazil	Human	KC329494	[39]
Sporothrix brasiliensis	HUPE114500	-	Brazil	Human	KF048980	[40]
Sporothrix brasiliensis	IPEC 16490	CBS 120339 (T)	Brazil	Human	AM116899	[32]
Sporothrix brasiliensis	CMRP5787	-	Brazil	Feline	OR501574	This study
Sporothrix brasiliensis	CMPRP5786	-	Brazil	Feline	OR501573	This study

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2 CHAPTER 2 - The first autochthonous human case of sporotrichosis by *Sporothrix brasiliensis* in paraguay

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2.1 Abstract

Sporotrichosis *by Sporothrix brasiliensis* is increasingly reported in South America. Here, we present the first autochthonous human case in Paraguay, transmitted by a local infected cat. After 63 days of clinical signs onset, the patient was correctly diagnosed and antifungal treatment started, highlighting the need to increase awareness for this emerging disease.
Keywords: Sporotrichosis; *Sporothrix brasiliensis* infection; Diagnosis; Zoonotic Infectious diseases.

2.2 Introduction

Sporotrichosis is the most prevalent and globally distributed saprozoonotic implantation mycoses, previously referred to as a subcutaneous mycosis. Infections are caused by fungi of the genus *Sporothrix*. Generally, transmission occurs by traumatic inoculation in sapronotic, enzootic, or zoonotic routes. Since the 1990s sporotrichosis is hyperendemic in Brazil, with *S. brasiliensis* being the most common etiological agent in human and animal disease [1]. This major public health threat spreads by infected cats through exudates direct contact or respiratory droplets [2-4]. While the disease is common in Brazil, the prevalence in neighboring countries is steadily increasing, including Argentina and Chile [5,6].

From Paraguay, there has been one report from 2017 describing human sporotrichosis among relatives who moved from Brazil with an infected cat, although the species was not identified [7]. In 2022 the first feline *S. brasiliensis* infections in Paraguay were reported [8]. Here we report the first autochthonous human case of cat-transmitted sporotrichosis by *S. brasiliensis* in Paraguay.

2.3 Case report

This study was approved by the Committee for Ethics in Research of the Federal University of Parana (number—CAAE 52726021.8.0000.0102). Also, the

patient assigned a consent form to publication of information about her and the study adheres to the Declaration of Helsinki.

On March 9, 2024 (T = 0d), a 39-year-old female resident of Presidente Franco, Paraguay, without known underlying diseases or immunosuppression, noticed a skin lesion on her right arm that initially looked like a mosquito bite, which progressed to an inflamed nodule. On April 2 (T = 24d), after consulting a medical center, she began treatment with cephalexin (oral 1 gram every 8 h for seven days) (Figure 1A, 1B). On April 9 (T = 31d), the skin lesion progressed, and reddish areas appeared that followed the lymphatic pathway, which was initially diagnosed as cellulitis, for which clindamycin was administered (oral 500 mg every 8 h for 10 days). On April 30 (T = 52d), trimethoprim-sulfamethoxazole (oral 160 mg/800 mg every 12 h for 10 days) was prescribed for insufficient clinical response to clindamycin. Finally, on May 3 (T = 55d) a cycle of vancomycin was started (1 gram every 24 h for 10 days). At this point, the skin lesions evolved to 13 swollen and painful nodules (Fig. 1C). All these treatments were accompanied by analgesics and anti-inflammatory drugs, except for no corticosteroids. Despite the use of antibiotics, no bacterial culture was performed.

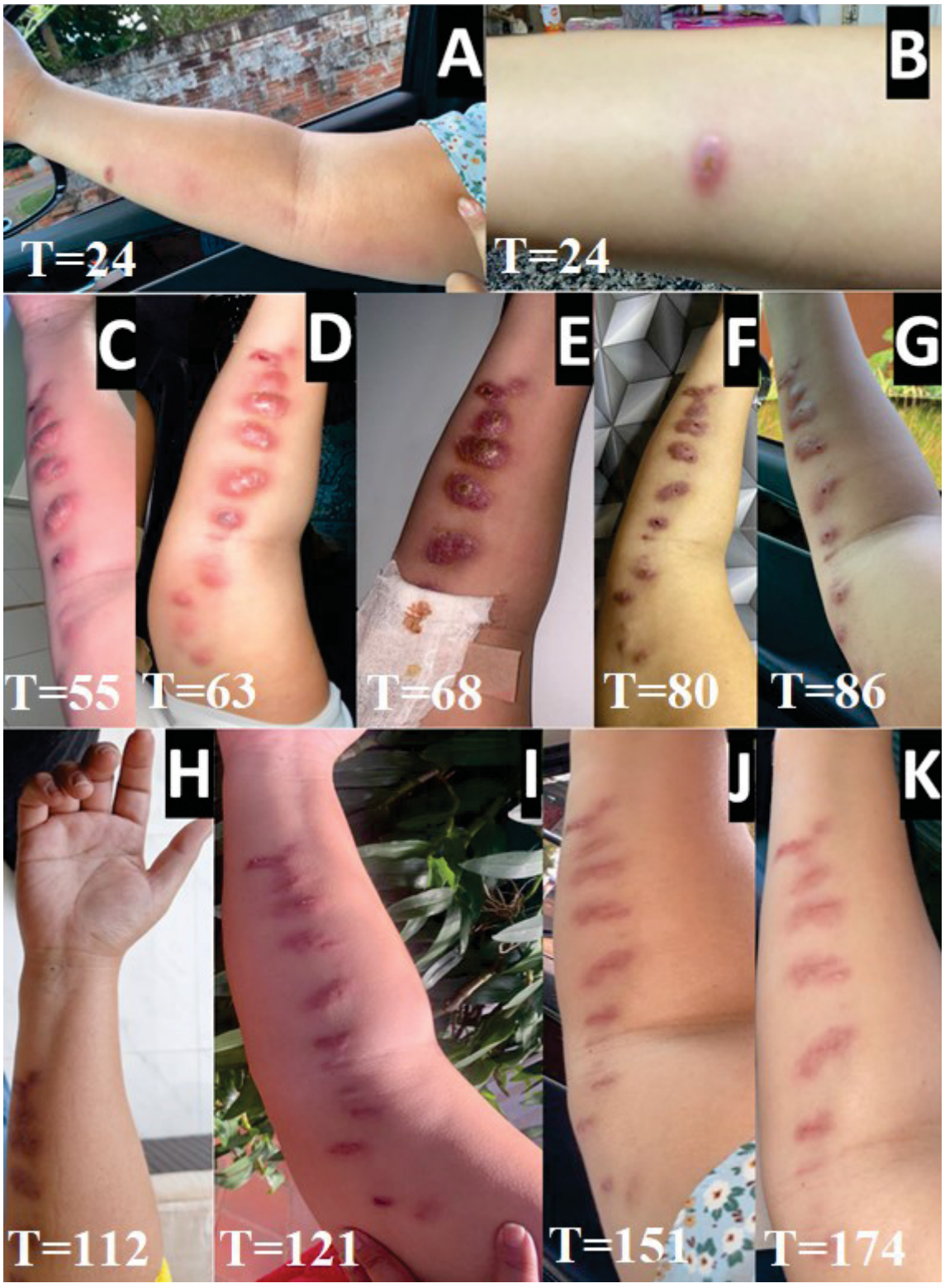

Figure 1. Clinical image across different time points. First consult on April 2 (Figure 1A, 1B), evolution to 13 swollen and painful nodules on May 3 (Figure 1C), consult with infectious disease specialist on May 11, (Figure 1D), itraconazole treatment for 6 days on May 16 (Figure 1E), for 18 days on May 28 (Figure 1F), for 24 days on June

3 (Figure 1G), for 50 days on June 29 (Figure 1H), for 59 days on July 8 (Figure 1I), for 89 days on August 7 (Figure 1J), and for 112 days on August 30 (Figure 1K).

On May 11 (T = 63), the patient consulted an infectious disease specialist that examined the skin lesions (Fig. 1D). During the consult, the patient reported to be scratched by her cat and after taking note of the clinical history of cat (lesion on head and cytopathological examination with observation of yeast-like structures, suggestive for *Sporothrix*, in the exudate of the cat's skin lesion), the specialist immediately requested a culture. Secretion from the skin lesions was collected using a swab and smeared onto Sabouraud Dextrose Agar medium. On the same day, treatment with oral itraconazole (200 mg daily) was started. In time the patient subsequently demonstrated clear clinical improvement (Fig. 1E-K) and continued with the treatment to date (January 8 [T = 305d], 2025).

In March 8, 2024, almost simultaneously with the patient clinical signs onset, her cat was also appeared with a lesion on its head. Antibiotic treatment was started. When no improvement was observed, the cat was admitted to a veterinary clinic on March 20 and continued with other antibiotics. At this point the cat was already weakened by the disease. Samples of the cat were collected for cytology and on April 17, the result revealed the presence of cigar-shaped yeast structures, suggestive for *Sporothrix*. No culture was performed, and despite starting itraconazole treatment (oral, 50 mg daily) at the same day (almost two months of the beginning of the lesion), few days later the cat was euthanized due to the declining medical condition.

The culture obtained on May 11 originated from a needle aspirate from the largest nodule containing a purulent secretion, which was sent to the microbiological laboratory. On direct examination using microscopy, an abundant inflammatory reaction was observed. Gram staining showed no bacterial or fungal structures. Culture was performed on SDA at 27 °C. After five days, the development of flat, white colonies with short aerial mycelia was observed that melanized over time. Under the microscope, hyphae with spherical conidia located in the apical part of the conidiophore were identified, in a characteristic teardrop or daisy flower arrangement, typical of the *Sporothrix* genus.

Calmodulin (CaM) sequencing was performed as described earlier [9] and the isolate was identified as *S. brasiliensis*. The sequence was deposited to the NCBI

Genbank database under accession number PQ550639. Using short tandem repeat (STR) genotyping as described previously [10,11], the genotype of the isolate appeared identical to previously genotyped isolates from the South of Brazil (Fig. 2).

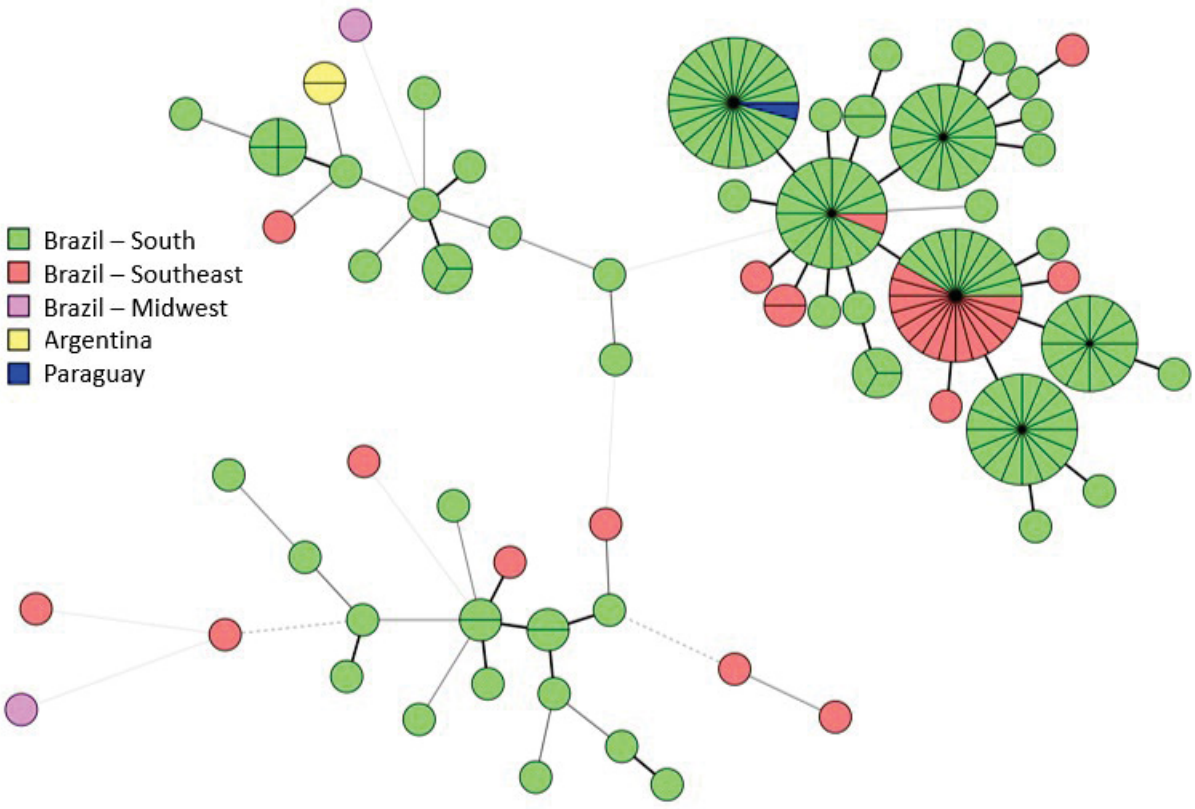

Figure 2. Minimum-spanning tree of 176 *Sporothrix brasiliensis* isolates, including one Paraguayan isolate from the current study. Branch lengths correlate with the similarity between isolates, which is further indicated by thick solid lines (variation in one allele), thin solid lines (variation in two alleles), thin dashed lines (variation in three alleles) and thin dotted lines (variation in four or more alleles). Isolates are colored according to different locations of origin.

Antifungal susceptibility testing was also performed by broth microdilution as outlined in the Clinical & Laboratory Standards Institute (CLSI) reference standard M38 for the mycelial form [12] and CLSI reference standard M27 for the yeasts form [13]. For the mycelial phase, isolate was cultured onto Potato Dextrose Agar (Difco Laboratories, Detroit, USA) plates at 30 °C for 7 days, which was confirmed microscopically. For the yeast phase, isolate was cultured onto Brain Heart Infusion (BHI) plates (Xebios Diagnostics GmbH, Düsseldorf, Germany), at 35 °C for 7 days,

followed by a second passage at BHI at 35 °C for 7 days, yeast presence was confirmed microscopically. *Aspergillus flavus* ATCC 204304 was used as quality control for the mycelial phase and *Pichia kudriavzevii* ATCC 6258 and *Candida parapsilosis* ATCC 22019 for the yeast phase. For both morphological phases, tentative epidemiological cut-off values (ECVs) for amphotericin B, itraconazole, voriconazole, posaconazole and terbinafine, were implemented [14]. All minimum inhibitory concentrations (MICs) were below the ECVs and for the other antifungals no elevated MIC values were found (Table 1).

Table 1. Minimum inhibitory concentrations (MICs) of the Paraguayan *Sporothrix brasiliensis* isolate in both yeast and mycelial phase according to clinical laboratory and standards institute (CLSI) guidelines. Concentrations in µg/mL.

Morphology	AMB	FLU	ITC	VOR	POS	ISA	TRB	AFG	MFG
Yeast	0.25	32	0.063	0.25	0.063	0.125	0.125	0.125	0.125
Mycelial	2	64	0.25	16	0.25	4	0.25	≤0.008*	≤0.008*

AMB, amphotericin B; FLU, fluconazole; ITC, itraconazole; VOR, voriconazole; POS, posaconazole; ISA, isavuconazole; TER, terbinafine; AFG, anidulafungin; MFG, micafungin.

2.4 Discussion

While the first human sporotrichosis cases in Paraguay occurred in 2017, involving an imported cat, this case describes the first autochthonous case. Recently, feline cases in the same region were reported, known for their propensity to cause outbreaks [8]. Most sporotrichosis human cases present cutaneous or lymphocutaneous clinical forms, affecting lower and upper members and face [15]. Usually, a papular lesion starts on an inoculating site after some days or weeks, and may progress to nodular or ulcerative form. Lesions are characterized by swelling and itching erythematous halos [11]. In the lymphocutaneous form, lesions progress in a sporotrichoid pattern, following lymphatic vessels and reaching lymph nodes. The clinical signs of the current case are in line with this.

Despite noticing the clinical signs early and having an affected cat, it took a prolonged time to accurately diagnose human sporotrichosis and start antifungal treatment, while even antibiotics were administered initially. This highlights the need

to raise awareness among clinicians and veterinarians for this disease, which is characterized by its typical sporotrichoid spread, and underscores the importance of a One Health approach and rational antifungal usage. Especially in the early stage of the infection, when itraconazole is administered accordingly, the treatment time is considerably lower than with more severe cases, as was shown here. Nonetheless, the isolate was found to have in vitro wildtype MICs to itraconazole, which was in line with the favorable clinical response to systemic itraconazole treatment.

STR genotyping demonstrated that this isolate is genetically closely related to isolates from Southern Brazil, suggesting an introduction by sick cats from there, although whole genome sequencing would be needed for definitive confirmation.

Our findings once more support the necessity of implementing an One Health approach to improve surveillance, diagnosis, and treatment of sporotrichosis, considering the importance of early diagnosis and treatment for an optimal prognosis.

Declaration of competing interest

EFJM received research grants from Mundipharma and Scynexis, is in the scientific advisory board for Pfizer and has received speaker fees from Gilead Sciences. All other authors declare no conflict of interest.

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3 CHAPTER 3 - Emergence of feline sporotrichosis in Puerto Iguazú (Argentina) near the Argentina-Brazil Border

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3.1 Abstract

We describe a large urban outbreak of feline sporotrichosis caused by *Sporothrix brasiliensis* in Argentina. Over a 7-month period in Puerto Iguazú, which borders Brazil, culture-proven sporotrichosis was identified in 9 cases across 7 households. Notably, 2 of these cases presented without cutaneous lesions, and 2 households experienced multiple cases, indicating potential intradomestic transmission.

3.2 Text

Sporotrichosis is an implantation mycosis caused by thermal-dimorphic fungi belonging to the *Sporothrix schenckii* complex (1). Among the pathogenic species, *Sporothrix brasiliensis* stands out for its high virulence, epidemic potential, and a zoonotic/enzootic transmission which occurring through bites, scratches, or contact with exudates from infected animals, particularly domestic cats (2-4).

In South America, *S. brasiliensis* was first identified in Brazil, and has since been reported in other Latin American countries (1-8). Over recent decades, sporotrichosis in Brazil has seen a significant epidemiological shift, marked by intense, widespread urban zoonotic outbreaks, initially concentrated in Rio de Janeiro, affecting cats, dogs, and humans (2, 9). These outbreaks have spread to several cities in the southern and southeastern states, including Foz do Iguaçu, located on the triple border between Argentina and Paraguay (2, 3, 5, 7, 10), and recently, a cat infection by *S. brasiliensis* was reported in Ciudad del Este, the Paraguayan side of the triple border (8). In Argentina, the first human isolation of *S. brasiliensis* was documented in 1986 in the south of province of Misiones, with no identified source of infection (4). Since then, zoonotic sporotrichosis cases have increased, with most occurrences reported in the central and southern regions of the country (1, 4).

This study reports the emergence of urban transmission of feline sporotrichosis in Puerto Iguazú, Misiones, Argentina (25°36′39″S, 54°34′49″W). Located in the extreme northeast of Argentina, on the border of the Triple Frontier, the city has a population of approximately 54,675 and is 1,278 km from Buenos Aires City. Puerto Iguazú is a major tourist destination due to the Iguazu Falls, and is

characterized by significant cross-border dynamics, including high population and commercial movement.

Between August 2023 and February 2024, we conducted an intensified passive surveillance on 21 domestic cats (*Felis catus*) from 12 households in Puerto Iguazú's urban area (Figure 1).

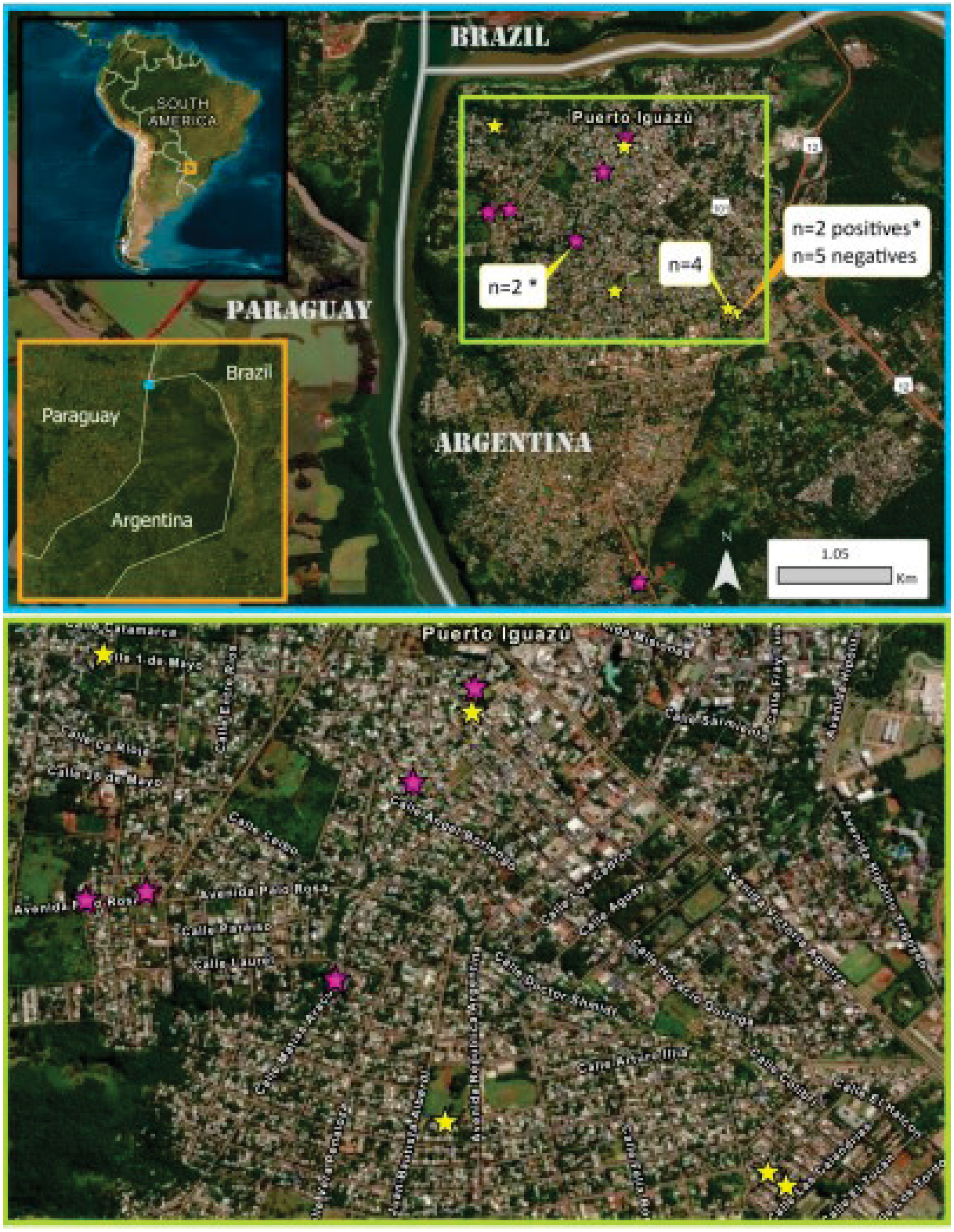

Figure 1. Map of Puerto Iguazú, Misiones, Argentina, located at the tri-border with Brazil and Paraguay, showing the spatial distribution of positive cases of feline sporotrichosis confirmed (n=9; pink) and ruled out cases (n=12; yellow) in the different neighborhoods studied. For those areas where multiple cases (positive and negative) overlap in location, the number of individual cases is indicated. The cases

of where intra-household transmission occurred between two cats (FSCMi24 - FSCMi25 and FSCMi41 – FSCMi43) are indicated by an *. In the spatial analysis, the distance between households with feline sporotrichosis cases was measured using nearest-neighbor calculation in ArcGIS Pro, which calculates the shortest distance between two points.

Cats with lesions consistent with feline sporotrichosis (ulcers, scabs, soft nodules, and ulcerated subcutaneous nodules with exudate) were included, as well as those without lesions but in contact with affected cats or living in areas with documented cases. None had received treatment before sample collection. Pet owners provided written consent, and clinical, epidemiological, and demographic data were recorded. All suspected cases and their contact were evaluated by veterinarians, with ongoing prevention and awareness campaigns directed at owners and the community.

Nasal and lesion swabs were collected from cats with skin lesions, and nasal swabs from cats without lesions. Diagnosis and species identification of *Sporothrix* were conducted using phenotypic (Giemsa stain and culture) and genotypic methods (sequencing ITS region and partial sequencing of the calmodulin gene) (1, 4, 10).

Among the 21 cases studied, 9 (42.9%) were suspected cases, while 12 (57.1%) were contacts. Feline sporotrichosis was confirmed by culture in 77.8% (7/9) of the suspected cases and 16.7% (2/12) of the contacts (Appendix Figure 1); of them, molecular confirmation of *S. brasiliensis* was achieved in 77.8% (7/9) and two samples were inconclusive due to mold and bacterial contamination, which hindered identification of *Sporothrix* species (Table 1).

Table 1. Features of domestic cats (*Felis catus*) suspected of feline sporotrichosis and their contacts, from 12 households in various neighborhoods of Puerto Iguazú's urban area, Misiones, Argentina; August 2023 to February 2024.

Household no. /Cat ID	Date	Sex/Age	Neutered	Inhabit¥	Type/Location of lesion	Collected material	DE/Culture	Identification / DMic no.
1/FSCMi-011¶	1-Aug-23	♂/Adult	No	Feral	Multiple dog bites	NS/LS/C	N/N	
2/FSCMi-017	13-Nov-23	♂/Adult	yes	Outdoor	NI	NS/C	N/N	
2/FSCMi-024	24-Nov-23	♂/Adult	Yes	Outdoor	NI	NS	ND/*Sporothrix* sp.	*S. brasiliensis*/247479
2/FSCMi-025*	13-Nov-23	♂/Adult	Yes	Outdoor	NI	NS	N/*Sporothrix* sp.	*S. brasiliensis*/247478
2/FSCMi-019	13-Nov-23	♀/Adult	Yes	Outdoor	NI	NS	N/N	
3/FSCMi-021	16-Nov-23	♀/Adult	No	Indoor	Localized/Head	NS/LS	yeast/*Sporothrix* sp.	*NC*
4/FSCMi-023	24-Nov-23	♂/Adult	Yes	Outdoor	Multiple/Head, dorsum, and extremities	NS/LS	ND/*Sporothrix sp.*	*S. brasiliensis*/247481
5/FSCMi-026	24-Nov-23	♂/Adult	Yes	Feral	Localized/Head	NS/LS	ND/*Sporothrix* sp.	*S. brasiliensis*/247480
6/FSCMi-031	20-Dec-23	♂/Kitten	No	Outdoor	NI	NS	N/N	
6/FSCMi-032	20-Dec-23	♀/Juvenile	No	Outdoor	NI	NS	N/N	
6/FSCMi-033	20-Dec-23	♂/Kitten	No	Outdoor	NI	NS	N/N	
7/FSCMi-034	20-Dec-23	♂/Adult	Yes	Outdoor	NI	NS	N/N	
7/FSCMi-035	20-Dec-23	♂/Adult	Yes	Outdoor	NI	NS	N/N	
7/FSCMi-036	20-Dec-23	♂/Adult	Yes	Outdoor	NI	NS	N/N	
7/FSCMi-037	20-Dec-23	♀/Adult	Yes	Outdoor	NI	NS	N/N	
8/FSCMi-038	20-Dec-23	♀/Juvenile	Yes	Outdoor	NI	NS	N/N	
9/FSCMi-040	10-Jan-24	♀/Juvenile	No	Feral	Multiple/Head and extremities	NS/LS	yeast/*Sporothrix* sp.	NC
10/FSCMi-041	15-Jan-24	♂/Adult	Yes	Outdoor	Multiple/Head, dorsum, and extremities	NS/LS	yeast/*Sporothrix* sp.	*S. brasiliensis*/247599
10/FSCMi-043	22-Jan-24	♂/Adult	Yes	Outdoor	Multiple/Head and extremities	NS/LS	yeast/*Sporothrix* sp.	*S. brasiliensis*/247600
11/FSCMi-042¶	15-Jan-24	♀/Juvenile	No	Outdoor	Alopecia	NS/LS	ND/*Microsporum canis*	
12/FSCMi-049	28-Feb-24	♂/Senior	Yes	Outdoor	Multiple/Head, dorsum, and extremities	NS/LS	yeast/*Sporothrix* sp.	*S. brasiliensis*/247735

* The cat had frequent sneezing. ♂ Male, ♀ Female. ¥ Inhabit; Indoor: Cats kept exclusively indoors or in enclosed spaces, without outdoor access; Outdoor: Cats with free access to roam outdoors, including streets, neighboring properties, or vacant lots; Feral: Cats living in the wild with minimal or no human interaction. NI: No injuries. NS: Nasal Swab. LS: Lesion Swab. C: Claw. N: Negative. ND: Not Determined. DE: Direct examination of the sample, with Giemsa staining. NC: Inconclusive due to mold and bacterial contamination. ¶Dog bites and diagnosis of dermatophytosis.

Among the two confirmed cases in contacts, sneezing was identified as the sole symptom in one case, and two suspected cases were ruled out due to differential diagnosis (dog bite and dermatophytosis) (Table 1). All cases have been reported to health authorities.

Feline sporotrichosis cases were identified in 58,3% (7/12) of the households. In 2 households, multiple cases were detected, suggesting intradomestic transmission. However, in 5 households, only one cat with sporotrichosis was found. Most of the cats had free access to streets, neighboring properties, and vacant lots. The average distance between the nearest households with feline sporotrichosis cases was 1.84 km ± 1.22 km (range: 0–4.54 km) (Table 1, Figure 1).

Our results describe the largest outbreak the *S. brasiliensis* in Argentina to date. We

identified 9 proven feline sporotrichosis in 7 months in Puerto Iguazú, province of Misiones, being higher than that reported for the whole country (1). The outbreak reflects transmission dynamics similar to the Brazilian epidemic, with evidence of multiple foci of transmission and asymptomatic carriers that facilitate the spread of the fungus (2, 5, 7, 10). In addition, the nearest epidemic focus is on the Brazilian side of the triple border (7), and to our knowledge, the latest cases of feline sporotrichosis in Argentina have been reported in Buenos Aires and Santa Cruz (1).

Asymptomatic carriers hinder control efforts by delaying diagnosis and treatment. Screening all contacts of confirmed cases is essential to minimize the risk of transmission (1, 10). Addressing these challenges requires mandatory case reporting and public health measures. The expansion of this emerging disease outside Brazil in contiguous countries calls for coordinated cross-border One Health actions and context-specific interventions, which will be crucial to safeguard local communities and tourists (1,5). Further studies are needed to understand its transmission dynamics.

Ethical Considerations

This study adhered to established ethical standards for veterinary research, ensuring the welfare and humane treatment of all animals involved. Sample collection was performed by qualified veterinarians using minimally invasive methods to reduce discomfort and stress. Procedures followed veterinary best practices and complied with international guidelines. Informed consent was obtained from all pet owners, who were briefed on the study objectives, methods, and potential benefits. The study protocol was reviewed and approved by the Institutional Ethics Committee of the Faculty of Agricultural and Veterinary Sciences, Universidad del Salvador (CICUAE - FCAyV, USAL-06-2021), with permits for the collection of natural resources and/or genetic material granted by the Instituto Misionero de Biodiversidad (IMiBio) and the Ministerio de Ecología y Recursos Naturales (Expte. 9950- 70-2023-1). The strains were deposited in the culture collection of the Mycology Department at INEI-ANLIS (DMic), Buenos Aires, and in the Biobank of the Instituto Misionero de Biodiversidad, Puerto Iguazú, Misiones.

Acknowledgment

The data presented are part of a broader ongoing project, Proyecto SIGEVA-USAL 2022-2025, entitled Estudio eco-epidemiológico y sanitario de *Sporothrix brasiliensis* en localidades correntinas y misioneras del Corredor Jesuítico Guaraní Argentino. We extend our gratitude for the support and collaboration of the Ministerio de Salud Pública (Misiones) and IMiBio, as well as trust and cooperation of the Consejo de Veterinarios de Misiones, Zoonosis Iguazú, private veterinary clinics in Puerto Iguazú, the Sociedad Rural de Ganado Menor (Misiones), and all pet owners involved in the study.

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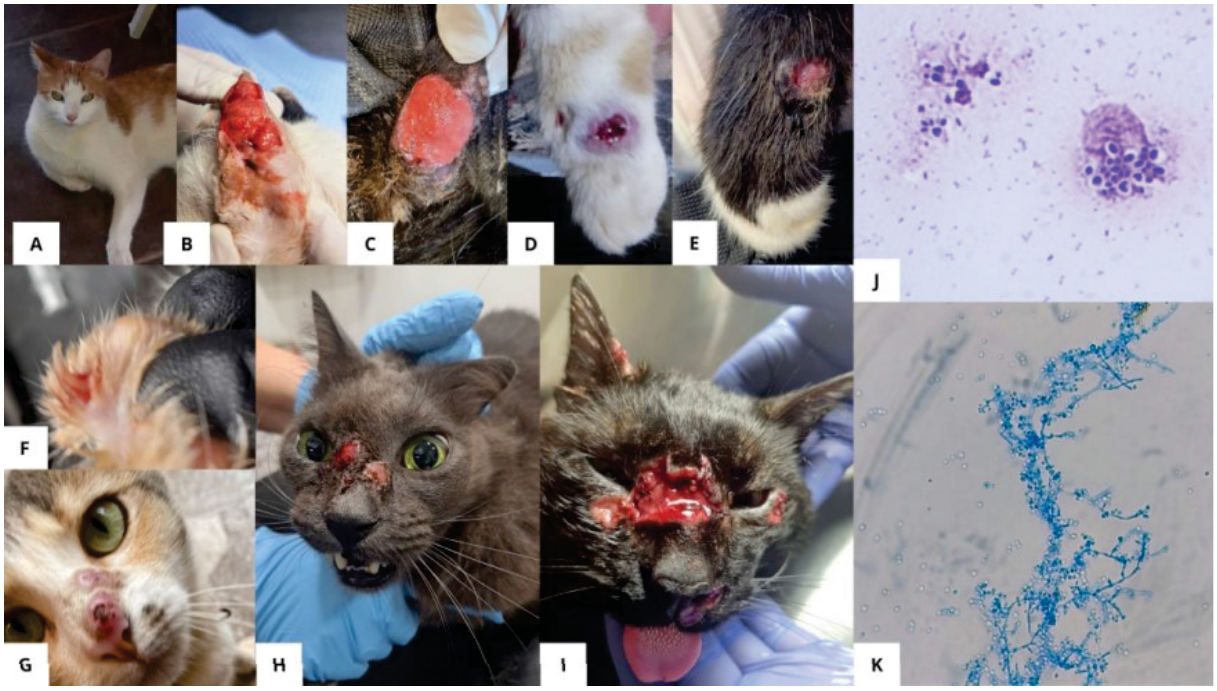
Appendix



Appendix Figure 1. Feline sporotrichosis and phenotypic studies. A. Cat without lesions, with frequent sneezing (FSCMi25). B - C. Multiple ulcerative lesions in the ear (FSCMi021 - FSCMi026). D - E. Ulcerative lesion on forelimbs (FSCMi043 -

FSCMi049). F. Ulcerative lesion around the claw (FSCMi040). G. Crusty ulcerative lesions on the nose (FSCMi043). H - I. Multiple crusty ulcerative lesions on the head (FSCMi041 - FSCMi026). J. Cigar-shaped oval capsulated yeasts (Giemsa stain. 1000x). K. Micromorphology of *Sporothrix* sp. Colonies (lactophenol cotton blue. 400x).

4 CHAPTER 4 - Absence of initial elevated itraconazole minimum inhibitory concentrations in *Sporothrix brasiliensis* from felines with treatment failure

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4.1 Abstract

Cat-transmitted sporotrichosis caused by *Sporothrix brasiliensis* is an emerging zoonosis in Latin America. As treatment of feline sporotrichosis is often not effective, we determined whether this is caused by infection of *S. brasiliensis* strains with pre-existing elevated itraconazole MICs, which is the drug of choice. At the triple border region of Brazil, Paraguay and Argentina from 2021 to 2023, 108 *S. brasiliensis* strains were isolated from felines before antifungal treatment. Antifungal susceptibility testing (AFST) to nine antifungal compounds was evaluated for the mycelial and

yeast phase. The main clinical presentation was cutaneous disseminated sporotrichosis (61%) and was the only form showing sporotrichosis-induced mortality (61%). AFST demonstrated only non-wild type MICs for terbinafine (n = 9). While MIC levels were low for most antifungals, they were higher in the mycelial phase. We conclude that the varying clinical presentations with large differences in mortality were not caused by pre-existing elevated itraconazole MICs.

4.2 Text

Sporotrichosis is a globally neglected epizoonotic and sapronotic disease, mostly involving infection of the skin and subcutaneous tissues, caused by fungi of the *Sporothrix* genus and the most prevalent implantation mycosis (1). *Sporothrix* species are thermally dimorphic fungi from the order Ophiostomatales, showing filamentous forms at 25-30°C and yeast-like forms at temperatures of 35-37°C as in mammals (2). The main clinical pathogenic species include *S. brasiliensis*, *S. schenckii*, *S. globosa* and *S. luriei*. *S. schenckii* and *S. globosa* are usually transmitted via the sapronotic route, involving traumatic implantation with plant/soil debris (3). Remarkably, in the last 30 years transmission of *S. brasiliensis* to humans, other felines and canines through infected cats by the zoonotic route has led to various outbreaks in Brazil and other Latin American countries (4–7). Currently cat-transmitted sporotrichosis (CTS) by *S. brasiliensis* is a major public health concern in Latin America, since infections are rapidly spreading from Brazil to other countries and cases were described in Brazil (8,9), Argentina (5,10), Paraguay (4), and Chile (6). In addition, imported cases in United Kingdom (11) and United States (12) were recently reported. Transmission by infected cats, via yeast form (13), occurs through bites, scratches, direct contact with exudate from skin lesions and probably through respiratory droplets, via cat sneezing (2,14). Cats are the primary animal hosts and main source of infection for other cats, dogs and humans (15).

Outbreaks often involve clonal zoonotic transmission (8,16). To curb these outbreaks, one of the necessary measures is antifungal treatment of cats (15). The drug of choice is itraconazole, although various refractory cases have been reported (17,18). Whether treatment failure is due to high antifungal MICs is unknown as this is poorly investigated (19). Recently high antifungal MICs against itraconazole were reported in isolates obtained from both cats and humans (19–21). It is not clear

whether strains with reduced susceptibility are also transmitted or whether this only develops during treatment. Antifungal susceptibility testing, applicable to both the yeast and mycelial form, has not been standardized leading to different protocols. As a consequence, published MICs are currently difficult to compare. In the present study, we investigate the spread of feline sporotrichosis in the triple border region between Brazil, Paraguay and Argentina by molecular genotyping. Additionally, MICs of common antifungals using microbroth dilution methods of both the yeast and mycelial phase, were obtained, to determine whether cats with sporotrichosis had *S. brasiliensis* with elevated itraconazole MICs at the onset of treatment.

4.3 Materials and methods

4.3.1 Isolate and data collection

Between July 2021 to October 2023, swab samples were collected from 108 symptomatic cats presenting with lesions compatible with sporotrichosis. Cats were selected through notification of the owners, health agents of the Zoonosis Control Center of Foz do Iguaçu (FIG) (CCZ – FIG, Brazil), veterinarians from private clinics and hospitals, and the receipt of suspected animals by CCZ. In FIG, the service was provided at the homes of the cats or at the place of indication by the citizen of stray cats. In Paraguay the service was provided at private veterinary clinics. All cats lived in the triple border region between Brazil, Paraguay and Argentina. Clinical and environmental data were obtained with questionnaires send to the owners of each cat. All cats were evaluated and classified according to the types of the lesions and divided in 3 groups: 1) cutaneous disseminated; 2) fixed cutaneous and 3) extracutaneous. Cartographic bases from the Brazilian Institute of Geography and Statistics (IBGE) were used for georeferencing the coordinates. Quantus Gis software (QGIS) was used to assemble the maps. To determine the clinical outcome, felines were followed for the complete duration of treatment, or until they passed away or were lost to follow-up.

4.3.2 Diagnosis

Sporotrichosis was diagnosed via fungal culture as previous described (4). Swabs collected from the wounds were sent for fungal culture. Specimens were cultivated on Sabouraud dextrose agar (SDA, KASVI, Pinhais, Brazil) containing chloramphenicol and incubated at 25-27°C for up to 10 days. Micromorphology of colonies was performed and *Sporothrix* growth was confirmed. Calmodulin (CaM) sequencing was performed for species identification as previous described (4). As control isolates, *S. brasiliensis* CBS 133017 (KP101458.1), *S. schenckii* CBS 117440 (KP101386.1), *S. globosa* CBS 129721 (KP101478.1), *S. luriei* ATCC 18616 (KT427639.1), *S. mexicana* Ss133 (JF811341.1), *S. chilensis* Ss470 (KP711816.1), *S. humicola* CBS 118129 (KX590808.1) and *S. phasma* CBS 119721 (KX590795.1) were included. Sequences generated in this study were deposited to NCBI Genbank (accession numbers OR501574, OR501573 and PQ741608-PQ741713) (Table S1).

4.3.3 Short tandem repeat genotyping

Genotyping of isolates was performed using short tandem repeats (STRs) as described before (16). Briefly, nine markers were amplified with three multiplex PCRs and subsequently analyzed with a 3500 XNL genetic analyzer (Applied Biosystems, Foster City, CA, USA). Copy numbers were determined using GeneMapper 5 (Applied Biosystems) and the genetic relatedness between the current and previously genotyped isolates was assessed with BioNumerics v7.6.1 (Applied Maths NV, Sint-Martems-Latem, Belgium) (16).

4.3.4 Antifungal susceptibility testing (AFST)

AFST was performed for the mycelial and yeast phase of all isolates. For the mycelial phase, isolates were cultured on Potato Dextrose Agar (Difco Laboratories, Detroit, USA) plates at 30°C for 7 days, and the absence of yeast cells was confirmed microscopically. AFST for the mycelial phase was performed by broth microdilution as outlined in the Clinical & Laboratory Standards Institute (CLSI) reference standard M38 for filamentous fungi (22). *Aspergillus flavus* ATCC 204304 was used as quality control. Amphotericin B (Bristol Myers Squib, Woerden, The Netherlands), fluconazole (Merck, Darmstadt, Germany), itraconazole (Janssen Cilag, Breda, The Netherlands), voriconazole (Pfizer Central Research, New York, USA), posaconazole

(Merck), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), terbinafine (VWR, Leicestershire, UK), anidulafungin (Merck) and micafungin (Astellas Pharma, Tokyo, Japan) were tested. The minimum inhibitory concentrations (MICs) were read visually after 72 hours of incubation at 30°C. For amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole, the MIC endpoint was the lowest concentration that produced complete inhibition of growth. For echinocandins, it was the lowest concentration producing a visual change in the appearance of the growth, defined as the minimum effective concentration (MEC). For terbinafine, the MIC was defined as the lowest concentration at which there was ≤80% growth reduction when compared to the growth control. For mycelial phase, tentative epidemiological cut-off values (ECVs) according to Espinel-Ingroff were implemented for amphotericin B, itraconazole, voriconazole, posaconazole and terbinafine (23).

For the yeast phase, isolates were cultured onto Brain Heart Infusion (BHI) plates (Xebios Diagnostics GmbH, Düsseldorf, Germany), at 35°C for 7 days, followed by a second passage at BHI at 35°C for 7 days again, and the absence of filamentous fungi was confirmed microscopically. AFST was performed by broth microdilution according to the CLSI reference standard M27 for yeasts (24). *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality controls. The same antifungals as for the mycelial phase were tested. The MICs were read visually after 120 hours of incubation at 35°C. For amphotericin B and terbinafine, the MIC endpoint was identical as used for mycelial phase and for fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, anidulafungin and micafungin, the MIC was defined as the lowest concentration at which there was a ≤50% growth reduction.

4.3.5 Ethics

This study was approved by the Committee for Ethics in Research of the Federal University of Paraná (number—CAAE 52726021.8.0000.0102) and by the Animal Use Ethics Committee of the Federal University of Paraná, Curitiba, Brazil.

4.4 Results

4.4.1 Clinical epidemiology

Samples of symptomatic cats from the triple border region were collected between 2021 and 2023 and put on culture, obtaining 108 isolates of *Sporothrix* spp. originating from 88 households (Table S1). Of all animals, 100 were from FIG (Brazil), four from Ciudad del Este (CDE) (Paraguay) and four from Hernandarias (Paraguay). None of the animals had a previous travel history to other regions. Sporotrichosis cases were initially only found in neighborhoods in the eastern region of FIG (Figure 1A), while in following years cases were spreading to other regions, close to the country border, especially around the international bridges (Figure 1B and C). The prevalence of sporotrichosis cases was highest in the northern, southern and eastern districts (Table 1), regions with the highest density of humans (Figure S1). Furthermore, most cases were found at or close to favelas and poor urban communities (Figure S2), which are low-income dense housing settlements, characterized by poor socio-economic status, precarious conditions and lack of essential services, mostly found at the eastern region of FIG (Figure S2). Notably, all cats had easy access to the street, other homes, backyards and vacant lots. Feline sporotrichosis was more frequently in males (ration 2.8:1), adults, uncastrated cats, those not vaccinated for any disease, and those with little or no access to veterinary services, while most of them had an owner (Table 2). Characteristics of the cats with negative cultures can be seen in Table S2.

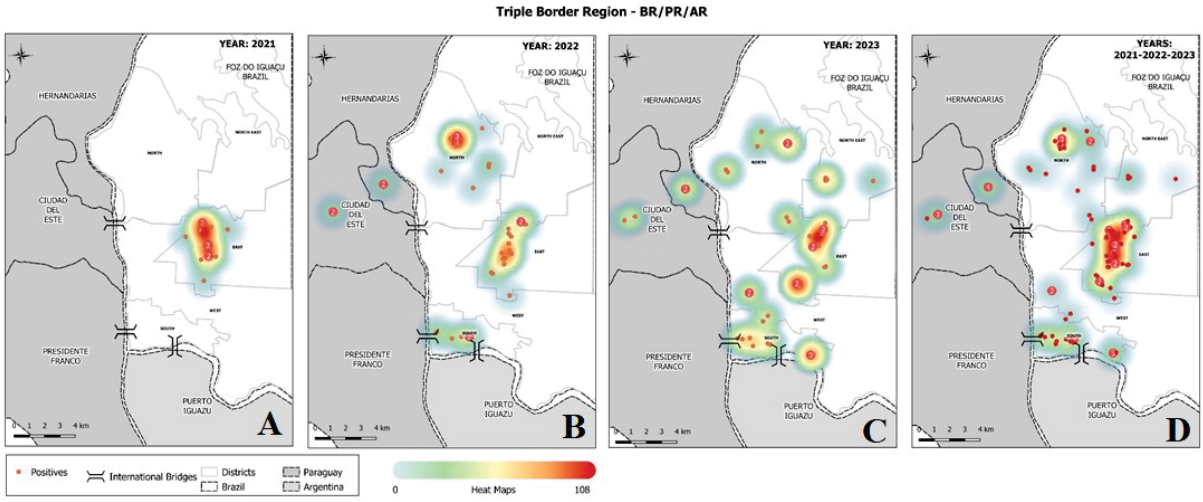

Figure 1. Heat map showing the spatial distribution of the animals with proven sporotrichosis in the triple border region (Brazil – white map; Paraguay – dark grey

map; Argentina – light grey map) between 2021 and 2023. Feline sporotrichosis cases and localization (red dots) in 2021 (A), 2022 (B), 2023 (C); and accumulated (D). Red circles with “2” and “3” refer to the number of positive cats in the same house.

Table 1. Prevalence of sporotrichosis per district

District	**Nºof residences**	**Nº of positive animals**	**Prevalence in 1000 residences**
North	32610	24	0.73
West	32705	3	0.09
South	16175	17	1.05
North East	18804	4	0.21
East	36315	52	1.43

Table 2. Characteristics of 108 cats with sporotrichosis

Characteristic	Category	Number of animals (%)
Sex	Male	80 (74%)
	Female	28 (26%)
Age	≤ 12 months	13 (12%)
	≥ 12 months	95 (88%)
Castration status	Uncastrated	81 (75%)
	Castrated	23 (21%)
	Unknown	4 (4%)
Vaccination status	Never vaccinated	64 (59%)
	Full vaccination scheme	11 (10%)
	Only primary vaccination	12 (11%)
	Occasional vaccination	4 (4%)
	Unknown	17 (16%)
Access to veterinary	No access	76 (70%)
	With access	32 (30%)
Tutored	With an owner	95 (88%)
	Stray cat	13 (12%)

The main clinical presentation consisted of cutaneous disseminated sporotrichosis (61%), followed by fixed cutaneous (34%) and extracutaneous (5%) sporotrichosis (Table 3). The mortality rate was 61% in those with the disseminated form, while cats with the fixed cutaneous or extracutaneous form did not die due to sporotrichosis. Ten animals were euthanized, as they lived on the street without owner, hindering any possibility of treatment. Other outcomes were lost to follow up (7%).

Table 3. Outcome of 108 cats with sporotrichosis based on clinical form

Clinical form	Outcome	Number of animals
Cutaneous disseminated	Death	40 (61%)
	Clinical cure	16 (24%)
	Lost to follow up	3 (4%)
	Euthanized	7 (11%)
Fixed cutaneous	Clinical cure	29 (78%)
	Lost to follow up	5 (14%)
	Euthanized	3 (8%)
Extracutaneous	Clinical cure	5 (100%)

All cats received itraconazole 25-100 mg daily, while those with the disseminated and extracutaneous form also received potassium iodide (2.5-20 mg/kg/24h, depending on severity of symptoms). For the clinically cured cats treatment duration ranged between two and 15 months with no correlation to the clinical form (Table 4). For the dead cats, 12 never received treatment and for the rest treatment duration ranged between 1 week and 7 months (Table 5).

Table 4. Treatment regime and duration of 50 clinical cured cats with sporotrichosis based on clinical form

Clinical form	Treatment regime and duration	Number of animals
Cutaneous disseminated	ITRA + PI 3 – 6 months	3
	ITRA + PI 8 – 12 months	11
	ITRA + PI 13 – 15 months	2
Fixed cutaneous	ITRA 2 – 6 months	12

	ITRA 7 – 12 months	17
Extracutaneous	ITRA + PI 2 – 6 months	5

ITRA, itraconazole; PI, potassium iodide.

Table 5. Treatment regime and duration of 40 dead cats with sporotrichosis

Clinical form	Treatment regime and duration	Number of animals
Cutaneous disseminated	No treatment applied	12
	ITRA + PI 1 – 4 weeks	12
	ITRA + PI 2 – 4 months	6
	ITRA + PI 5 – 7 months	10

4.4.2 Phylogenetic analysis

With CaM sequencing, all 104 isolates were identified as *S. brasiliensis*, displaying low genetic variation within this gene (Figure S3). By performing STR genotyping, 20 genotypes were found, of which six were found earlier (16) (Figure 2). All isolates from the triple border region were highly related and all grouped within the Rio de Janeiro (RdJ) clade, a previously described dominant group of genotypes originating from RdJ (16). In this study they often clustered with isolates from other regions of Paraná, RdJ and several other states.

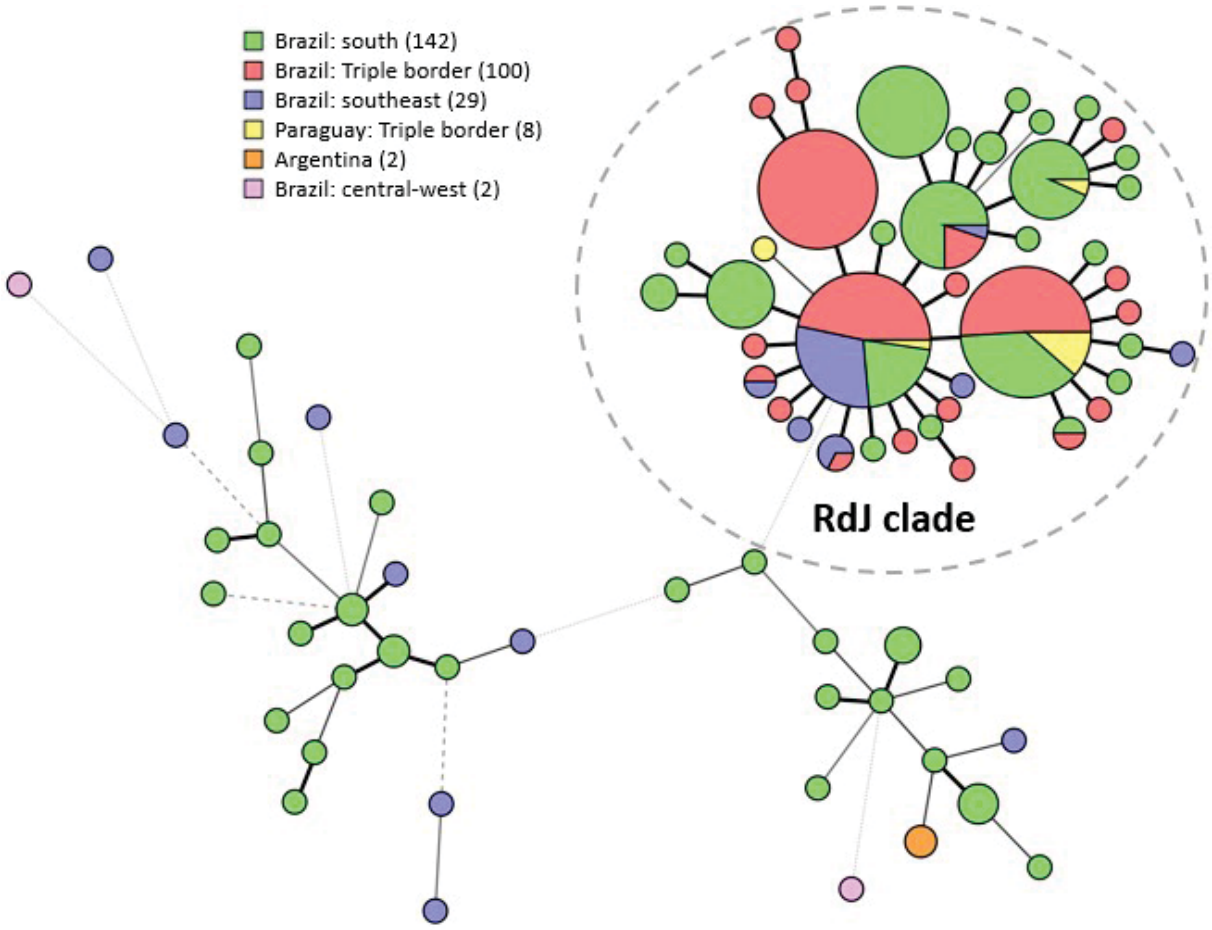

Figure 2. Minimum-spanning tree of 283 *Sporothrix brasiliensis* isolates, including 108 isolates from the current study. Isolates are colored according to different locations of origin and numbers in the color key represent the number of isolates. South: Paraná, Rio Grande do Sul; Southeast: Rio de Janeiro, Minas Gerais, São Paulo, Espírito Santo; Central-West: Federal District.

4.4.3 Minimum inhibitory concentration investigation

AFST of all isolates was performed for the mycelial and yeast phase. Based on the study of Espinel-Ingroff which proposed ECVs based on the M38 CLSI protocol, nine isolates were classified as non-wild-type for terbinafine (Table 6). For the other tested antifungals with available ECVs all isolates were classified as wild-type. For the mycelial phase, itraconazole and posaconazole had the highest in vitro activity, followed by amphotericin B. In contrast, fluconazole, voriconazole and isavuconazole, had low activity with high MICs. For the yeast phase, itraconazole, posaconazole and isavuconazole showed the highest activity followed by

voriconazole and amphotericin B. Comparing susceptibility levels between both phases, isolates at the mycelial phase had higher MICs for all azoles and amphotericin B, while for terbinafine and echinocandins higher MICS were found at the yeast phase. Finally, the MIC50 of strains isolated from cats with disseminated disease that were cured were identical to those from cats that died (Table S3).

Table 6. Distribution of minimum inhibitory concentration (MIC) values against 108 clinical isolates of *Sporothrix brasiliensis* according to CLSI M38 and M27 guidelines. M lines refer to mycelial phase and Y lines refer to yeast phase. MICs in µg/mL

Antifungal		≤0.063	0.125	0.25	0.5	1	2	4	8	16	32	≥64	MIC_{50}*
FLU	M											108	≥64
	Y					1	1	1	13	49	30	13	16

		≤0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	≥16	
AMB	M						1	38	57	12			2
	Y				2	9	27	66	4				1
ITC	M				3	42	62	1					0.5
	Y	14	35	37	18	4							0.063
VOR	M										7	101	≥16
	Y		2	8	18	26	19	21	13	1			0.25
POS	M				2	31	65	10					0.5
	Y	11	29	36	28	4							0.063
ISA	M									31	51	26	8
	Y	20	18	29	25	10	6						0.063

		≤0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	≥8	
TRB	M				53	44	9						0.063
	Y			2	14	65	29						0.125
AFG	M	101	6	1									0.008
	Y	7	9	20	34	32	6						0.063
MFG	M	101	2	5									0.008
	Y	4	21	14	40	24	5						0.063

AMB, amphotericin B; FLU, fluconazole; ITC, itraconazole; VOR, voriconazole; POS, posaconazole; ISA, isavuconazole; TRB, terbinafine; AFG, anidulafungin; MFG, micafungin; M, mycelial phase; Y, yeast phase. Red dotted line indicates division of WT vs non-WT isolates based on ECV values when available. The ECV value for

voriconazole is 32 µg/mL. MIC50, the MIC value that inhibited 50% of isolates. *, for AFG and MFG the MEC50 (filamentous phase) was determined.

4.5 Discussion

This study is the first to report cases of CTS in triple border region of Brazil, Paraguay and Argentina. The epidemiological data shows that within three years *S. brasiliensis*-induced sporotrichosis spread across this region. The rapidly increasing number of feline cases highlights the severity of sporotrichosis as a public health problem and the potential for outbreaks (25). The data also suggests that CTS mainly affects an urban population with a high concentration of humans and likely also cats as compared to areas with a low density of humans (8,26). Notably, all cats had easy access to the street, other homes, backyards and vacant lots. Besides, cats can freely across national borders in this region and we observed introduction of sporotrichosis in Paraguay near to international bridges. Furthermore, cases correlate with poor socioeconomic status as the eastern region of FIG has the lowest standard of living. As a consequence, most cats had owners but had no access to the veterinarian (27). In most cases owners were financially not able to provide basic resources for their cats health, and in cases of sporotrichosis not capable to provide diagnosis and treatment. Therefore, public policies that provide these tools free of charge are crucial.

Although all reported sporotrichosis cases in both the Brazilian and Paraguayan side of the border were included, most isolates originated from Brazil, while the population number in the Brazilian and Paraguayan cities at the triple border region is similar (2024 population FIG: 295.000 (28), CDE: 339.000 (29)). The number of feline population is not known for both cities. The differences in sporotrichosis cases are partly due to the spread originating from the east of Brazil, which is halted by the river between both countries. In addition, differences between the healthcare systems of these two countries might play a role. In Brazil, the Health Unic System (SUS) is a decentralized system, meaning that the city decides how the resources from the state and the federal government will be used (30). In Paraguay there is a centralized health system, with most of the actions concentrated in the capital Asunción, which is 324 km from the border (30). Although the Epidemiological Laboratory in CDE, Paraguay is capable of to track cases and diagnosing

sporotrichosis in cats and humans for free, there are no enough clinicians and veterinarians available.

In this study, uncastrated and unvaccinated males represented the majority of patients with feline sporotrichosis, as previously described (31). Unvaccinated cats may have comorbidities such as FeLV, Calicivirus, Herpes, and panleukopenia, leading to immunosuppression and rapid evolution to the disseminated form of sporotrichosis (32). Castration reduces the behavior of territory disputes and sexual intercourse between males and females, which in cats usually involves fights with injuries, decreasing the chance of transmission to females and newborn kittens (33). Besides, production of testosterone can affect male's behavior. As the majority of cats in this study were over 12 months old, public interventions (vaccination and neutering programs) before this age would likely reduce the risk of transmission of sporotrichosis. Therefore, these measures are important to control and prevent sporotrichosis based on a One Health Approach, and reinforces the importance of public health education, especially about responsible feline ownership. Furthermore, the lack of awareness about this disease in health professionals is the main difficulty in identifying sporotrichosis in humans and animals, making searching for cases in these locations even harder (12). Thus, public health education on responsible feline ownership and increasing disease awareness in health professionals are the first step towards the prevention of sporotrichosis outbreaks and effective treatment of patients (34).

To show the genetic relatedness between the *S. brasiliensis* isolates, STR analysis was performed (35). All isolates clustered in the RdJ clade and were closely related to Brazilian isolates from the south and southeastern parts of the country. Curitiba and other cities in the Brazilian state of Paraná steadily report *S. brasiliensis* cases since 2011, and these isolates display identical or highly related genotypes (16). The introduction of *S. brasiliensis* in regions may happen by the movement of sick or colonized cats (11). Also, our isolates were closely related to those from Curitiba, which like FIG, belongs to Paraná state, although these regions are more than 600 kilometers apart. All our isolates clustered with the RdJ clade (16), but since different genotypes were identified, multiple introductions cannot be excluded. WGS of these isolates is needed to elucidate the origin of *S. brasiliensis* in this region.

Next, we determined MIC values for nine different antifungals against *S. brasiliensis* isolates with both pure yeast and mycelial phase, which was

microscopically confirmed. According to the interpretation of an international multicenter study for definition of tentative ECVs for mycelial *S. brasiliensis* (23), we found, with exception of nine non-WT isolates for terbinafine, only wild-type isolates for amphotericin B, itraconazole, voriconazole and posaconazole (36). This multicenter study used standard incubation of two to three days at 35°C according to the CLSI reference standard M38 for filamentous fungi. We found in this study that incubation at 35°C induces transition to the yeast phase taking up to two weeks for full transition. As microscopy was not performed in the multicenter study it cannot be excluded that the ECVs were established on a mixture of filamentous and yeasts phases. Moreover, many centers in this multicenter study were excluded due to insufficient or unsuitable data, suggesting suboptimal methodology or implementation thereof. Thus, additional studies are required to establish the ECVs, although the normal distribution of our MIC values indeed suggests an absence of non-WT isolates. Notably, mycelial phase MICs were overall higher than in the yeast phase, especially for the azoles fluconazole, voriconazole and isavuconazole. One explanation for this difference is the higher concentration of melanin in the cell wall in the filamentous phase. Melanin is associated with a reduced susceptibility to antifungal drugs. It is important to note however that MICs in the mycelial phase were read at 100% inhibition compared to growth control, while at the yeast phase this was 50%. Direct comparisons of the MICs between both phases should therefore be made with caution.

AFST results may not reflect in vivo treatment in the absence of clinical breakpoints (34). Nonetheless for itraconazole, which is the first-choice drug for feline treatment (15,34), MICs of all isolates in both phases were below the tentative ECV and similar results were reported earlier (17,37). In contrast, others recently found MICs of itraconazole and other azoles above the tentative ECV (19,38). Notably, the reported bimodal distribution with low and elevated MICs for itraconazole and the identification of cyp51 mutants (19) suggests that these MICs would also be well above the tentative ECV in conditions of pure mycelial and yeast phase (23). This discrepancy with our study might be due to the inclusion of different strains. Our collection consisted of one genotype only and strains were isolated before start of treatment. Smaller MIC differences could also be explained by differences in AFST protocols, including the mixed presence of filamentous and yeast phases as discussed earlier. In addition, other factors including panel preparation,

media/reagents, and inoculum preparation might influence AFST results. Finally, since the mycelial form is the one with the least variation in our genotypically similar isolates, is easiest to employ, and mycelial AFST data seemingly corresponds to in vivo failure of voriconazole (39), this phase might be most suitable for AFST. A multicenter evaluation comparing robust AFST methodologies in a genotypically variable cohort would be needed to establish the best method to determine antifungal susceptibility for *S. brasiliensis*.

We observed a high mortality in cats with the disseminated form and no sporothrichosis-related deaths in cats with the fixed cutaneous form. Importantly, all isolates were genetically similar and displayed initially low MICs of itraconazole, used for treatment in all cats. MIC50 levels of strains isolated from cats with the disseminated form that were cured were also not different from those that were not cured. Thus, the mortality in cats with the disseminated form is not due to initial elevated MICs, although we cannot exclude the development of reduced susceptibility in time, as we did not collect isolates after itraconazole treatment. The observation that transmission only involved itraconazole susceptible isolates, as we did not find an isolate with high MIC in any cat, suggests that an increase in MICs was probably uncommon, if present at all. Moreover, some cats from the same household became infected months after each other. Also in these households no elevated MICs were found. It is more likely that other factors, like disease progression, treatment variations and host factors, were involved in treatment failure in this cohort. Besides clinical outcomes, treatment duration was different in both clinical forms. Cats with the fixed cutaneous form were treated for fewer months. While disease progression is also likely to play an important role here, erratic itraconazole pharmacokinetics might be involved too. For feline sporotrichosis, the proposed drug dose by the Guideline for the management of feline sporotrichosis caused by *S. brasiliensis* is 100 mg/24h for cats over 3 kg. A robust dose-response study evaluating the efficacy of this dose, is lacking to our knowledge. When administering the medication, it is recommended to open the capsules over a small amount of wet food, however, there are currently no studies that verify the absorption degree of itraconazole administered in this way. In disseminated cases it is also unknown whether itraconazole can reach the mucous membranes at an adequate level for cure. However, suboptimal itraconazole blood levels prolong treatment and the risk for resistance development in other diseases (40,41). Optimal dosing to

reach effective serum itraconazole concentrations in severe disease would allow best standard of care but is currently unavailable for cats. In conclusion, early diagnosis and effective treatment seem crucial to prevent disease progression, mortality and transmission to other humans and animals, while reduced antifungal susceptibility has not been detected in the triple border region as of yet.

Conflict of interest

EFJM received research grants from Mundipharma and Scynexis, is in the scientific advisory board for Pfizer and has received speaker fees from Gilead Sciences. All other authors declare no conflict of interest.

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Appendices

Table S1 Accession numbers (NCBI), date of sampling and geographic data of the animals with proven sporotrichosis in the triple border region.

CMRP	ACCESSION NUMBER	DATE	NEIGHBORHOOD	CITY	COUNTRY
CMRP6060	PQ741666	07/07/2021	Jardim Alice I	Foz do Iguaçu	Brazil
CMRP5585	PQ741608	09/07/2021	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6061	PQ741667	09/07/2021	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6062	PQ741668	09/07/2021	Jardim Copacabana	Foz do Iguaçu	Brazil
CMRP5586	PQ741609	30/07/2021	Parque Residencial Morumbi	Foz do Iguaçu	Brazil
CMRP6063	PQ741669	30/07/2021	Parque Residencial Morumbi	Foz do Iguaçu	Brazil
CMRP6064	PQ741670	09/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6065	PQ741671	09/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6066	PQ741672	16/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6067	PQ741673	16/08/2021	Portal da Foz	Foz do Iguaçu	Brazil

CMRP6068	PQ741674	16/08/2021	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6069	PQ741675	16/09/2021	Parque Residencial Santa Rita	Foz do Iguaçu	Brazil
CMRP5587	PQ741610	16/09/2021	Jardim Alice I	Foz do Iguaçu	Brazil
CMRP6070	PQ741676	24/09/2021	Jardim Europa	Foz do Iguaçu	Brazil
CMRP6071	PQ741677	24/09/2021	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6072	PQ741678	24/09/2021	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6073	PQ741679	24/09/2021	Parque Residencial Morumbi	Foz do Iguaçu	Brazil
CMRP5588	PQ741611	29/12/2021	Beverli Falls Park	Foz do Iguaçu	Brazil
CMRP5589	PQ741612	03/01/2022	Jardim das Flores	Foz do Iguaçu	Brazil
CMRP5590	PQ741613	18/01/2022	Jardim das Flores	Foz do Iguaçu	Brazil
CMRP5591	PQ741614	18/01/2022	Jardim das Flores	Foz do Iguaçu	Brazil
CMRP5592	PQ741615	01/02/2022	Jardim Curitibano	Foz do Iguaçu	Brazil
CMRP5593	PQ741616	04/02/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP5594	PQ741617	04/02/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6074	PQ741680	10/03/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6075	PQ741681	16/03/2022	Jardim Nacional	Foz do Iguaçu	Brazil
CMRP6077	PQ741682	05/04/2022	Cidade Nova II	Foz do Iguaçu	Brazil
CMRP6078	PQ741683	19/04/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6079	PQ741684	19/04/2022	Cidade Nova II	Foz do Iguaçu	Brazil
CMRP6080	PQ741685	27/04/2022	Parque Residencial Santa Rita	Foz do Iguaçu	Brazil
CMRP6082	PQ741686	02/06/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6083	PQ741687	02/06/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6084	PQ741688	07/06/2022	Residencial Morumbi III	Foz do Iguaçu	Brazil
CMRP5785	PQ741618	07/06/2022	Parque Residencial Italia	Foz do Iguaçu	Brazil
CMRP6085	PQ741689	08/06/2022	Vila São Sebastião	Foz do Iguaçu	Brazil
CMRP6086	PQ741690	14/06/2022	Vila Borges	Foz do Iguaçu	Brazil
CMRP6087	PQ741691	14/06/2022	Vila Borges	Foz do Iguaçu	Brazil
CMRP6088	PQ741692	20/06/2022	Jardim São Paulo II	Foz do Iguaçu	Brazil
CMRP6089	PQ741693	21/06/2022	Jardim São Paulo II	Foz do Iguaçu	Brazil
CMRP6090	PQ741694	21/06/2022	Porto Belo	Foz do Iguaçu	Brazil
CMRP5786	**OR501573**	22/06/2022	-	Ciudad del Este	Paraguay
CMRP6091	PQ741695	07/07/2022	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6092	PQ741696	07/07/2022	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6093	PQ741697	14/07/2022	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6094	PQ741698	14/07/2022	Portal da Foz	Foz do Iguaçu	Brazil
CMRP6095	PQ741699	28/07/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6096	PQ741700	11/08/2022	Vila Borges	Foz do Iguaçu	Brazil
CMRP6097	PQ741701	11/08/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6098	PQ741702	19/08/2022	Vila São Sebastião	Foz do Iguaçu	Brazil
CMRP5787	OR501574	19/08/2022	-	Ciudad del Este	Paraguay
CMRP6099	PQ741703	24/08/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6100	PQ741704	26/08/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6101	PQ741705	26/08/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6102	PQ741706	26/08/2022	Conjunto C	Foz do Iguaçu	Brazil
CMRP6103	PQ741707	06/09/2022	Jardim Residencial São Roque	Foz do Iguaçu	Brazil

CMRP6104	PQ741708	09/09/2022	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP6105	PQ741709	20/09/2022	Profilurb I	Foz do Iguaçu	Brazil
CMRP6106	PQ741710	05/10/2022	Jardim Bela Vista de Itaipu III	Foz do Iguaçu	Brazil
CMRP6107	PQ741711	05/10/2022	Jardim Oriente	Foz do Iguaçu	Brazil
CMRP6108	PQ741712	05/10/2022	Profilurb I	Foz do Iguaçu	Brazil
CMRP5791	PQ741619	30/11/2022	-	Hernandarias	Paraguay
CMRP5792	PQ741620	10/12/2022	-	Hernandarias	Paraguay
CMRP5915	PQ741621	19/01/2023	-	Hernandarias	Paraguay
CMRP5916	PQ741622	19/01/2023	-	Hernandarias	Paraguay
CMRP5917	PQ741623	12/05/2023	Portal da Foz	Foz do Iguaçu	Brazil
CMRP5918	PQ741624	12/05/2023	Portal da Foz	Foz do Iguaçu	Brazil
CMRP5919	PQ741625	25/05/2023	Portal da Foz	Foz do Iguaçu	Brazil
CMRP5940	PQ741640	25/05/2023	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP5941	PQ741641	25/05/2023	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP5921	PQ741626	01/06/2023	Vila Guarani	Foz do Iguaçu	Brazil
CMRP5922	PQ741627	08/06/2023	-	Ciudad del Este	Paraguay
CMRP5923	PQ741628	12/06/2023	Jardim Novo Horizonte	Foz do Iguaçu	Brazil
CMRP5924	PQ741629	12/06/2023	Jardim Novo Horizonte	Foz do Iguaçu	Brazil
CMRP5925	PQ741630	22/06/2023	Centro	Foz do Iguaçu	Brazil
CMRP5942	PQ741642	22/06/2023	Profilurb I	Foz do Iguaçu	Brazil
CMRP5926	PQ741631	28/06/2023	Jardim São Luiz	Foz do Iguaçu	Brazil
CMRP5927	PQ741632	28/06/2023	Jardim São Luiz	Foz do Iguaçu	Brazil
CMRP5943	PQ741643	30/06/2023	Vila A	Foz do Iguaçu	Brazil
CMRP5944	PQ741644	30/06/2023	Jd. Califórnia II	Foz do Iguaçu	Brazil
CMRP5928	PQ741633	06/07/2023	Jardim Guaira	Foz do Iguaçu	Brazil
CMRP5945	PQ741645	06/07/2023	Bubas	Foz do Iguaçu	Brazil
CMRP5946	PQ741646	11/07/2023	Profilurb I	Foz do Iguaçu	Brazil
CMRP5929	PQ741634	11/07/2023	Cohapar III	Foz do Iguaçu	Brazil
CMRP5947	PQ741647	13/07/2023	Conjunto C	Foz do Iguaçu	Brazil
CMRP5948	PQ741648	13/07/2023	Loteamento Bela Vista de Itaipu II	Foz do Iguaçu	Brazil
CMRP5930	PQ741635	13/07/2023	Jardim Cedro	Foz do Iguaçu	Brazil
CMRP5931	PQ741636	20/07/2023	Conjunto C	Foz do Iguaçu	Brazil
CMRP5949	PQ741649	20/07/2023	Portal da Foz	Foz do Iguaçu	Brazil
CMRP5932	PQ741637	26/07/2023	Jardim Duarte	Foz do Iguaçu	Brazil
CMRP5950	PQ741650	28/07/2023	Jardim Novo Horizonte	Foz do Iguaçu	Brazil
CMRP5933	PQ741638	28/07/2023	Jardim Novo Horizonte	Foz do Iguaçu	Brazil
CMRP5935	PQ741639	28/07/2023	Jardim Eliza II	Foz do Iguaçu	Brazil
CMRP5952	PQ741651	16/08/2023	Jardim California	Foz do Iguaçu	Brazil
CMRP5953	PQ741652	16/08/2023	Centro	Foz do Iguaçu	Brazil
CMRP5955	PQ741653	19/08/2023	-	Ciudad del Este	Paraguay
CMRP5956	PQ741654	24/08/2023	Jardim das Flores	Foz do Iguaçu	Brazil
CMRP5957	PQ741655	24/08/2023	Parque Residencial Morumbi II	Foz do Iguaçu	Brazil
CMRP5958	PQ741656	04/09/2023	Jardim São Paulo II	Foz do Iguaçu	Brazil
CMRP5959	PQ741657	04/09/2023	Jardim São Paulo II	Foz do Iguaçu	Brazil
CMRP5960	PQ741658	04/09/2023	Conjunto Habitacional Fernanda	Foz do Iguaçu	Brazil

CMRP5961	PQ741659	06/09/2023	Jardim Tropical	Foz do Iguaçu	Brazil
CMRP5962	PQ741660	06/09/2023	Parque Ouro Verde	Foz do Iguaçu	Brazil
CMRP5963	PQ741661	12/09/2023	Vila Guarani	Foz do Iguaçu	Brazil
CMRP5964	PQ741662	12/09/2023	Parque Residencial Morumbi III	Foz do Iguaçu	Brazil
CMRP5965	PQ741663	13/09/2023	Cohapar III	Foz do Iguaçu	Brazil
CMRP5966	PQ741664	15/09/2023	Loteamento Bela Vista de Itaipu II	Foz do Iguaçu	Brazil
CMRP5967	PQ741665	15/09/2023	Loteamento Bela Vista de Itaipu II	Foz do Iguaçu	Brazil
CMRP6111	PQ741713	25/10/2023	Jardim São Luiz	Foz do Iguaçu	Brazil

Table S2. Characteristics of 25 cats without sporotrichosis.

Characteristic	Category	Number of animals (%)
Sex	Male	17 (68%)
	Female	8 (32%)
Age	≤ 12 months	3 (12%)
	≥ 12 months	22 (88%)
Castration status	Uncastrated	12 (48%)
	Castrated	12 (48%)
	Unknown	1 (4%)
Vaccination status	Never vaccinated	14 (56%)
	Full vaccination scheme	4 (16%)
	Only primary vaccination	3 (12%)
	Occasional vaccination	3 (12%)
	Unknown	1 (4%)
Access to veterinary	No access	22 (88%)
	With access	3 (12%)
Tutored	With an owner	24 (%)
	Stray cat	1 (%)

Table S3. Comparison between clinical outcome with MIC50.

Clinical form	**Outcome**	**MIC50**																	
		AMB		FLU		ITC		VOR		POS		ISA		TRB		AFG		MCF	
		M	Y	M	Y	M	Y	M	Y	M	Y	M	Y	M	Y	M	Y	M	Y
Cutaneous disseminated	Death	2	1	64	16	0.5	0.063	16	0.375	0.5	0.063	8	0.063	0.063	0.125	0.008	0.063	0.008	0.063

	Clinical cure	2	1	64	16	0.5	0.047	16	0.5	0.5	0.063	8	0.063	0.063	0.125	0.008	0.125	0.008	0.063
	Lost to follow up	2	1	64	16	0.5	0.125	16	1	0.5	0.125	8	0.25	0.063	0.063	0.008	0.063	0.008	0.063
	Euthanized	1	0.5	64	32	0.25	0.031	16	1	0.25	0.063	8	0.063	0.063	0.125	0.008	0.031	0.008	0.063
Fixed cutaneous	Clinical cure	2	1	64	16	0.5	0.063	16	0.25	0.5	0.063	8	0.063	0.063	0.125	0.008	0.063	0.008	0.063
	Lost to follow up	1	0.5	64	32	0.25	0.063	16	0.25	0.25	0.063	8	0.063	0.125	0.125	0.008	0.125	0.008	0.063
	Euthanized	2	1	64	32	0.5	0.031	16	0.25	0.5	0.031	8	0.016	0.125	0.125	0.008	0.031	0.008	0.016
Extracutaneous	Clinical cure	2	1	64	16	0.5	0.063	16	0.5	0.5	0.063	16	0.063	0.125	0.25	0.008	0.016	0.008	0.063

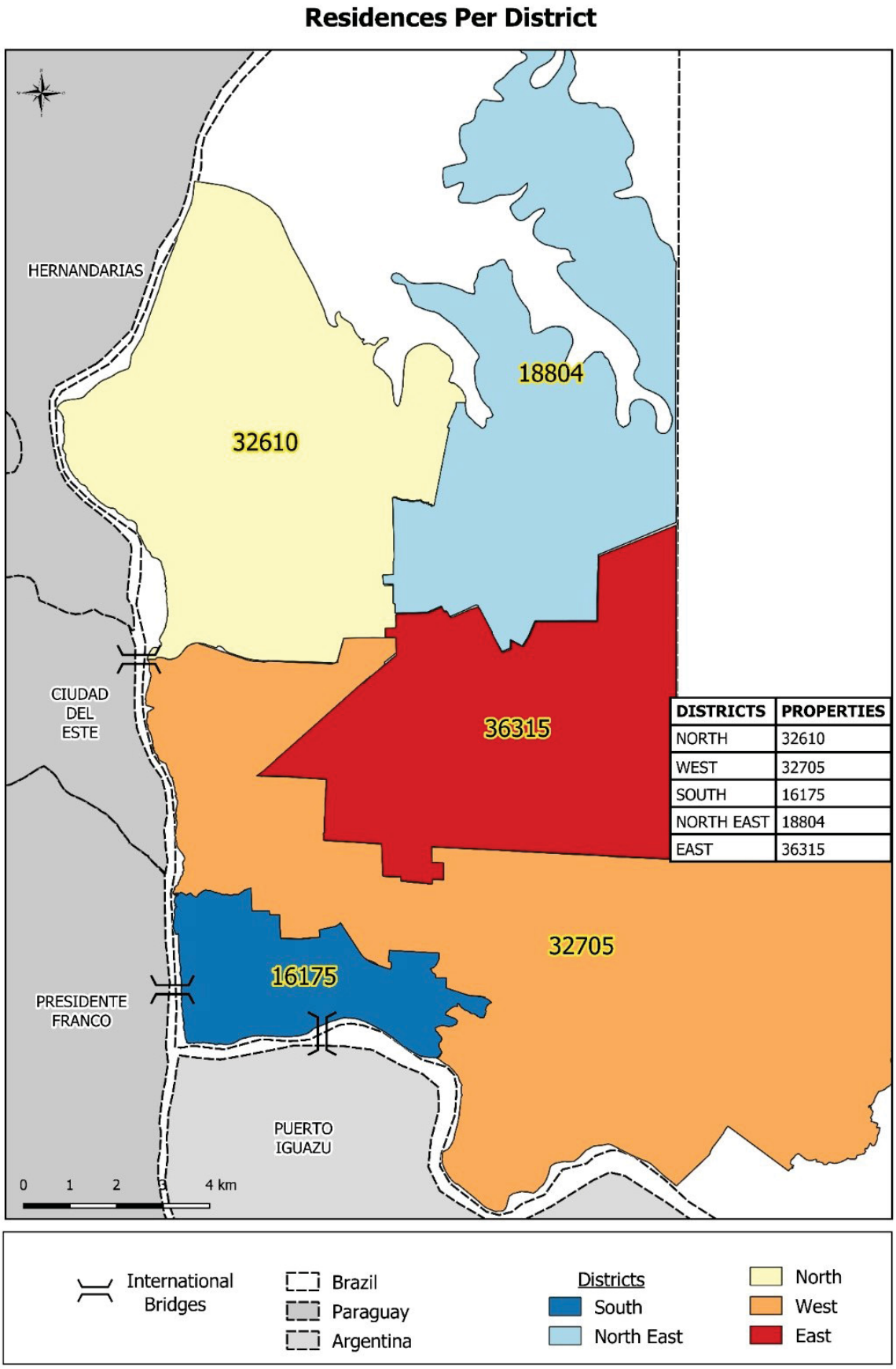

Figure S1. Number of residences per district of Foz do Iguaçu, Brazil.

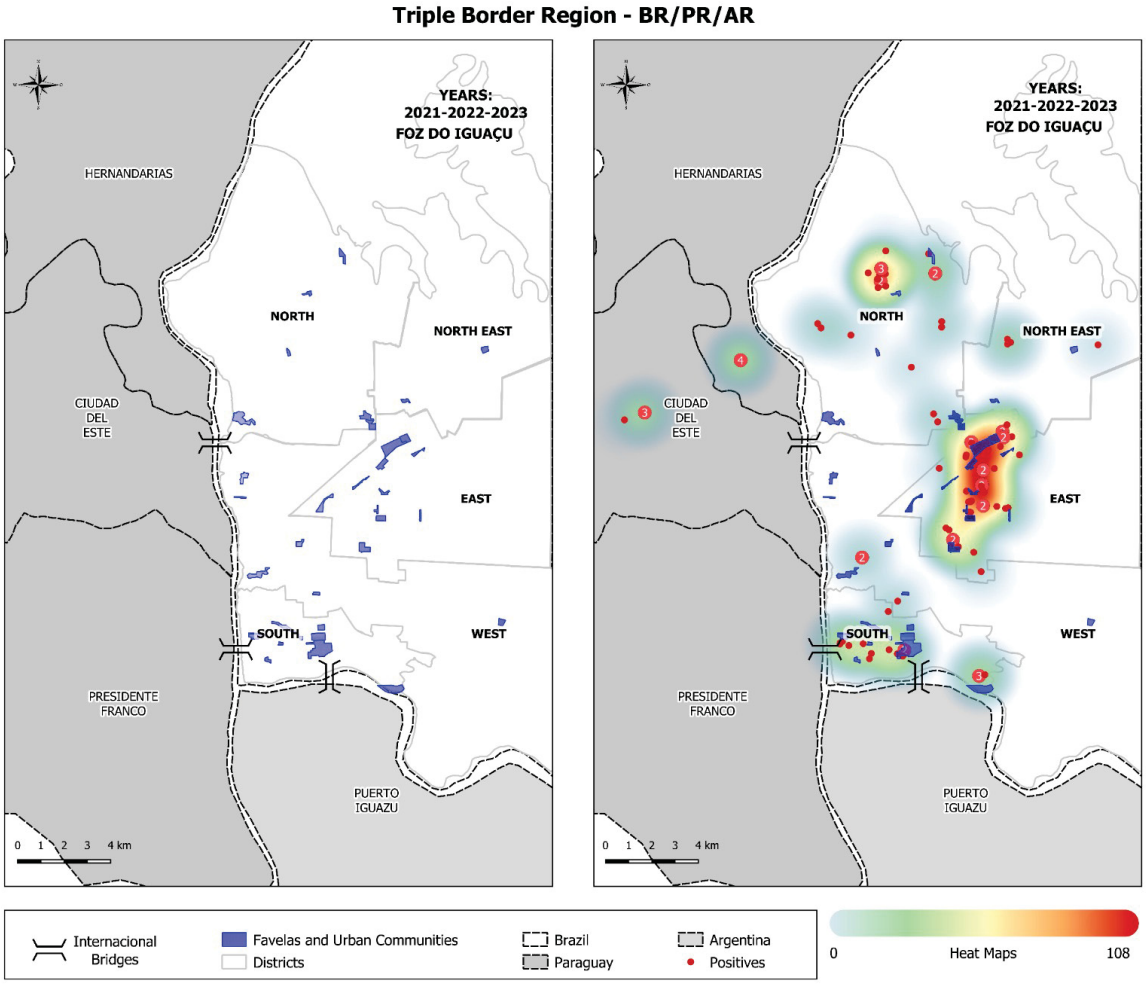

Figure S2. On the left side is the location of the favelas and poor urban communities (FPUC) in Foz do Iguaçu, Brazil. On the right side is the Map with FPUC + all sporotrichosis cases 2021-2023.

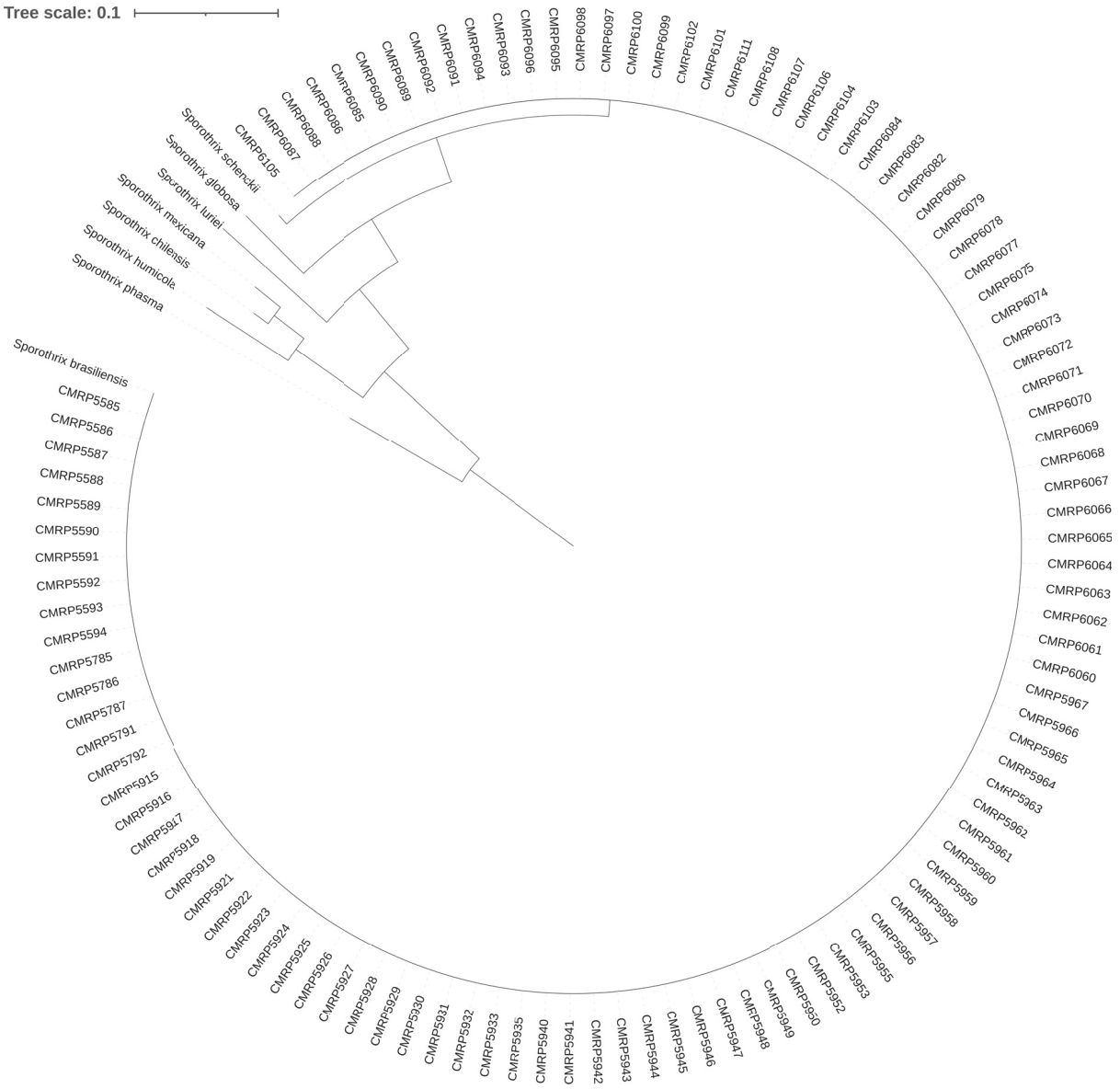

Figure S3. Phylogenetic tree of *Sporothrix brasiliensis* based on multiple sequence alignment of calmodulin sequences. The tree was rooted to *Sporothrix phasma*.

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CONCLUSION

This research identified the first autochthonous cases of feline and human sporotrichosis caused by *S. brasiliensis* in the border region between Brazil and Paraguay, as well as an emerging outbreak in Argentina. It was also revealed that treatment failures in cats are not due to pre-existing itraconazole resistance but may be related to other factors, such as pharmacokinetic/pharmacodynamic issues, disease progression, and host conditions.

These findings highlight the urgent need to strengthen disease surveillance, increase public awareness, such as educating veterinarians, health professionals, and the public about early detection and preventive measures, and adopt a coordinated One Health approach to address this growing public health threat in Latin America.

The increase in cases in Latin America, especially in high transboundary mobility regions, like the one we studied, presents significant challenges for public health authorities. Climate change, urbanization, and high densities of cats with access to the street exacerbate the spread of sporotrichosis.

The study emphasizes the importance of early diagnosis and effective treatment to prevent disease progression and transmission. Public health measures, such as vaccination and sterilization programs for cats, are critical for controlling sporotrichosis. Additionally, raising awareness among health professionals and the public is essential for timely interventions.

Molecular diagnostics such as Short Tandem Repeat Genotyping are useful to monitor the spread of *S. brasiliensis* and its introduction to new areas. Future studies are warranted to assess whether this method for AFST is better reproducible between laboratories and whether the high mortality can be attributed to mere late clinical presentation and/or pharmacokinetics and pharmacodynamics (PK/PD) issues in cats.

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