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New Insights on Transmission of *Sporothrix brasiliensis*

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Abstract

Background: Sporotrichosis is the most prevalent saprozoontic implantation mycosis worldwide.

Objectives: A prospective experimental study was performed to evaluate persistence on surfaces such as stainless steel medium-density fiberboard (MDF) wood, tile, and polyester fabric.

Methods: The different surfaces were homogeneously contaminated using the yeast phase of *Sporothrix brasiliensis*. Fungal culture was carried out in triplicate on Mycosel agar, at time 0 and then every 24 h, until 792 h (33 days).

Results: *Sporothrix brasiliensis* persists on MDF wood for up to 240 h (10 days), tile for 288 h (12 days), polyester fabric for 384 h (16 days), and stainless steel for up to 600 h (25 days).

Conclusions: Based on these findings, in concordance with published clinical reports suggesting fomite transmission, we propose an updated schematic of *S. brasiliensis* transmission. This is the

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Author Contributions

Regielly C. R. Cognialli: writing – original draft, investigation, conceptualization, methodology, visualization, writing – review and editing, supervision, formal analysis, data curation, software. **Flávio Queiroz-Telles:** supervision, writing – original draft, writing – review and editing, conceptualization. **Alyson M. Cavanaugh:** writing – original draft, writing – review and editing. **Bruno C. Redigueri:** writing – review and editing. **Giovanna C. D. Santos:** investigation. **Felipe Moreira Matias:** investigation. **Luciano Moreira Filho:** investigation. **Elias N. Monteiro Neto:** investigation. **Mônica Surek:** investigation. **Vânia A. Vicente:** writing – review and editing, resources. **Izabella C. R. Santos-Weiss:** supervision, conceptualization, methodology, investigation, writing – original draft, writing – review and editing, formal analysis, visualization, project administration, data curation, software.

Conflicts of Interest

The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control (CDC).

first in vitro study about the persistence of *S. brasiliensis* on different surfaces, which is essential to understand transmission routes for a One Health Approach.

Keywords

non-zoonotic fomites; persistence; sapronotic; sporotrichosis; transmission; zoonotic

1 | Introduction

Sporotrichosis, termed ‘Rose gardener’s disease’, has classically been associated with sapronotic transmission from traumatic implantation during gardening activities [1]. However, zoonotic transmission of sporotrichosis from infected cats was recognised in the 1990s in Brazil, and scientists identified an emerging species, *Sporothrix brasiliensis*, as the pathogen causing these sporotrichosis cases [2–6]. This species is more virulent than the other *Sporothrix* species due to its higher thermotolerance, biofilm synthesis, strong melanization capacity, and, particularly noteworthy, because this fungal pathogen is capable of being transmitted directly from the yeast phase [6–8]. Currently, cat-transmitted sporotrichosis (CTS) has emerged as an important epizoonosis in Brazil, affecting thousands of humans, and cats and hundreds of dogs [9–16]. Some regions of Brazil are hyperendemic, and the disease has rapidly spread geographically, establishing itself as a threat to other countries [2, 9, 17–22].

Most commonly, *S. brasiliensis* is transmitted from infected or apparently healthy cats to other cats, dogs, and humans by bites, scratches, or direct contact with exudate from ulcerative lesions [2, 5, 7, 23, 24]. Recent studies have shown that *S. brasiliensis* can be isolated from respiratory droplets during feline sneezing or cough episodes [23], and ocular infections have been attributed to respiratory droplet exposure [9, 25]. However, human cases of *S. brasiliensis* without direct contact with cats have been reported, suggesting additional pathways of transmission. For example, recently, 39 human cases of *S. brasiliensis* infection by non-zoonotic transmission were reported in a hyperendemic area of CTS, suggesting the potential for alternate transmission routes and highlighting the importance of natural source and environmental studies [26].

It is not uncommon for fungi to spread through fomite transmission, or transmission by inanimate objects that can carry the fungus. In fact, the persistence on surfaces for many yeasts and moulds is well described [27]. For example, *Candida auris*, a critical priority pathogen, can persist on surfaces for days, being a concerning source of transmission, as observed in Brazil, when an outbreak was likely caused by shared axillary digital thermometers [27–29]. For dermatophytes, many surfaces and objects have been attributed as sources of infection, including towels, hairbrushes, slippers, articles of clothing, and nail polish [30]. However, fomite transmission has not been thoroughly explored for *S. brasiliensis*, and, if occurring, represents a potential gap in prevention efforts. Current prevention methods for *S. brasiliensis* have largely focused on using physical barriers such as personal protective equipment (gowns, gloves, and eye protection) when handling infected cats to prevent human infection, as well as treating cats to reduce the risk of

transmission. A better understanding of the potential of fomite transmission is needed to inform prevention efforts for this rapidly spreading fungal pathogen.

Fomite transmission, commonly seen with other fungal pathogens, has yet to be evaluated as a transmission route for *S. brasiliensis*, thus representing a gap in knowledge necessary to guide prevention efforts. Therefore, the aim of this study was to evaluate in vitro the viability of *S. brasiliensis* on different environmental surfaces to establish the possibility of transmission through fomites.

2 | Materials and Methods

A prospective experimental study was performed. Common inanimate surfaces were selected to simulate home, laboratory, and veterinary clinic environments. Therefore, stainless steel surfaces, medium density fibreboard (MDF) wood, tile, and polyester fabric were selected for the study. Each surface with a total area of 450 cm² was sterilised and divided into 102 quadrants with equal areas. The metal surface (stainless steel) was cleaned and sterilised in an autoclave. The wood and tile were cleaned with formaldehyde and left under ultraviolet light (UV) for 20 min. The polyester fabric was new and left under UV for 20 min.

One isolate of *S. brasiliensis* (CMRP6191) characterised by Calmodulin gene sequencing was selected from Microbiological Collections of the Paraná Network—Taxonline (CMRP). We elected to use *S. brasiliensis* in the yeast phase to simulate contamination of the surface from an infected cat. Reversion from mycelial to yeast phase was performed using brain heart infusion (BHI) agar at 35°C–37°C for 7 days; then the fungus was transferred to other BHI at the same temperature for a further 7 days for full reversion. Suspension containing the yeast phase of *S. brasiliensis* was prepared in sterile saline, and turbidity was measured using a nephelometer (BD PhoenixSpec).

The different surfaces were homogeneously contaminated using 600 mL of suspension with a final turbidity of 1.42. For the polyester fabric, we dipped it into a container with the fungal suspension and left it for 5 min. All surfaces were kept at room temperature.

Fungal culture was carried out in triplicate from the previously delimited quadrants on all surfaces, at time 0 and then every 24 h, until 792 h (33 days). Collections were performed using a sterile swab moistened and seeded on Mycosel agar plates. The cultures were incubated at 28°C–30°C and monitored daily through macro and microscopic analysis of the colonies. It was considered growth if there was at least one plate from the triplicate collection with *Sporothrix*, independently of the number of colonies.

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the research in this article related to micro-organisms.

3 | Results

Persistence of *S. brasiliensis* was different on each surface studied. After surface contamination, *Sporothrix* persisted on MDF wood for up to 240 h (10 days), tiles for 288 h (12 days), polyester fabric for 384 h (16 days), and stainless steel for up to 600 h (25 days) (Figure 1A–D). After 25 days, only the development of contaminating environmental fungi was observed.

4 | Discussion

Sporotrichosis caused by *S. brasiliensis* has become an important public health issue due to the increased number of cases and geographic expansion [9, 15]. Critical aspects of the disease need to be understood to effectively implement public health prevention strategies through a One Health approach, a transdisciplinary collaborative approach that recognises the relatedness of humans, animals, and the environment and addresses critical elements through multisector actions [7]. Human cases of *S. brasiliensis* by non-zoonotic transmission have been reported, and it is fundamental to elucidate all transmission routes [26].

Sapronotic transmission is well documented for other *Sporothrix* species; however, for *S. brasiliensis*, it was recently described [1]. Previously, scientists have tried to isolate *S. brasiliensis* by culture from soil samples in endemic regions. Despite no success, possibly due to limitations with culture methods and contamination with saprobic microorganisms, which can grow faster than *Sporothrix*, molecular methods have shown the presence of *S. brasiliensis* DNA [31, 32]. There are few studies that have successfully isolated the fungus from environmental sources. A study conducted in São Paulo, Brazil, evaluated 24 environmental samples, and the authors were able to isolate *Sporothrix* from only one bark sample [33]. Another study analysed 24 strains of *Sporothrix* isolated between 1984 and 2017 in Argentina [34]. In this study, *S. brasiliensis* was identified using molecular methods in two environmental samples of soil collected from nine-banded armadillo caves in the Chaco Province in 2003 [34]. Another environmental study collected demolition wood in a region with feline and human sporotrichosis cases [35]. In this study, fungal culture was performed using serial dilutions, and the authors reported 100% similarity between a *Sporothrix* isolate from a wood sample and an isolate from a local cat who had sporotrichosis 3 years prior [35]. This data supports the microbiological evidence of sapronotic transmission and prolonged persistence of *S. brasiliensis* on organic matter.

Regarding our experiment, surprisingly, *in vitro*, the fungus persisted for at least 10 days on the tested inanimate surfaces, indicating an important source of environmental contamination and suggesting the possibility of infection through fomites. These data reinforce the importance of keeping infected animals in isolation to avoid additional spread and transmission. It has been well documented that *S. brasiliensis* transmission can occur through bites, scratches, contact with exudate, and respiratory droplets from infected cats; additionally, we observed that the fungus can be viable on inanimate surfaces for many days, enabling transmission by fomites (Figure 2A) [2, 23]. Transmission of *S. brasiliensis* to humans and dogs is also possible through contact with soil or plants (sapronotic route), as was reported in 19 human cases in Rio de Janeiro and one canine case in

Santiago, Chile [26, 36]. Although four case reports have suggested human sporotrichosis transmitted by dogs, robust microbiological evidence was lacking in the published reports [37–40]. Moreover, fungal burden is low in humans (immunocompetent) and in canines with sporotrichosis; therefore, transmission from humans or dogs to others is unlikely [7, 36, 41]. Based on previously published evidence of epidemiologically relevant sources and new evidence gained from this study, we propose an update of *S. brasiliensis* transmission, emphasising the role of fomites as a potential source of transmission (Figure 2B).

Our finding of the extended persistence of *S. brasiliensis* on indoor surfaces suggests the potential for fomite transmission, and this is supported by some published reports suggesting the possibility of fomite transmission causing human infection. In a case series of 39 non-zoonotic sporotrichosis cases, all confirmed by culture to be caused by *S. brasiliensis*, 19 patients reported sapronotic exposures, 10 reported unusual potential sources of inoculation exposures (insect bites, trauma with iron gate and fishbone, human aggression and human bite), and 10 reported no known potential exposure source [26]. There is also a published report of two cases of sporotrichosis, confirmed by culture and sequencing of the calmodulin gene, that occurred after tattooing in a hyperendemic area at studios that failed to comply with appropriate hygiene standards; both patients denied any contact with cats [42]. Another case report of sporotrichosis describes a female patient with a lesion in her earlobe for 6 months; a biopsy showed growth of *Sporothrix*. She denied contact with animals or soil/plants, and the suspected source of infection was the handmade wooden earrings bought in the street market in Rio de Janeiro [43]. Unfortunately, molecular assays were not performed in this case for species identification; however, *S. brasiliensis* must be considered as the patient lived and bought the earrings at a street market in a hyperendemic area. These cases report the transmission route as uncertain, but surfaces were suspected as potential sources of infection. Unusual and interesting sources of *Sporothrix* inoculation have been described for other species. For example, it was reported in Mexico that four members of two families developed sporotrichosis after sleeping in an old, rust-stained camping tent [44]. Another case involved a 36-year-old Samoan man who developed sporotrichosis following a traditional body tattooing procedure [45]. Twelve weeks after the procedure, he presented with nodules on his thigh and knee, which were diagnosed as sporotrichosis [45]. In this case, the source of infection was likely associated with the non-sterile material used during the tattooing process, such as boar's tusk or bone and the components used for pigment [45].

Fomite transmission may be a possible transmission route, as suggested by extended persistence on surfaces and previously published case reports. However, this is not one of the most common transmission routes, and disruption of the skin barrier appears to be necessary for human infection. This study was qualitative in methodology, using a high fungal burden to evaluate the hypothesis; additional information with more isolates about the quantitative fungal burden that persists on surfaces and investigation into the possibility of reversion to a mycelial phase (a less infective form) may be helpful to support the hypothesis of fomite transmission.

The possibility of fomite transmission in indoor settings, including veterinary settings, should be considered and would have substantial public health ramifications. In this study,

stainless steel and polyester fabric were the main surfaces on which *S. brasiliensis* remained viable for a long time. Stainless steel was tested to simulate a procedure table of veterinary clinics. Polyester fabric was chosen to simulate coats used by healthcare workers, including veterinarians. *S. brasiliensis* could be viable for up to 25 days and 16 days, respectively, on these surfaces. This long period may be associated with biofilm formation, which is an important factor for persistence on surfaces to avoid desiccation and has been previously described for *Sporothrix* species [8, 27, 46]. The persistence on surfaces could be even higher with low temperatures and high humidity [28]. The veterinary clinic environment may act as a possible source of infection for both cats and humans, facilitating the transmission of sporotrichosis; however, it is worth noting that it would be difficult to epidemiologically link the veterinary clinic to new feline sporotrichosis cases, given the potential long latency from exposure to illness in cats. As an example, the first human cases of *S. brasiliensis* in the United Kingdom were attributed to scratches from an indoor pet cat; the cat was brought to the United Kingdom from Brazil more than 3 years previously and was in a healthy condition at that time [22]. Given the findings from this study, veterinary clinics, if not properly disinfected, would theoretically have the potential for widespread transmission. Additional environmental sampling studies and epidemiologic studies would be helpful to better understand transmission risk within veterinary clinics. Additionally, disinfectant studies that could guide infection prevention and control efforts in veterinary clinics and other indoor settings are needed.

Sporotrichosis by *S. brasiliensis* is a challenging disease, marked by a higher prevalence of atypical clinical manifestations, a higher proportion of cases refractory to conventional treatment, and, notably, a different primary transmission route than those of *Sporothrix* species [9, 23, 26, 47, 48]. Many aspects of this fungus are not completely understood, limiting the ability to apply effective control through a One Health approach. Patients with sporotrichosis due to *S. brasiliensis* but without evidence of contact with healthy or sick cats should be thoroughly evaluated for possible sapronotic or fomite transmission. More studies are needed to understand the potential of fomite transmission, with active searches of surfaces when fomite transmission is suspected. Additionally, studies are needed to test different disinfectant agents that can be used on surfaces to prevent contamination. Comparing the persistence of *S. brasiliensis* with other pathogenic *Sporothrix* species could also provide information to guide infection prevention and control efforts. This is the first in vitro study on the viability of *S. brasiliensis* on different surfaces and fills some gaps about the viability of the fungus and the possibility of transmission by fomites. This study contributes to a better understanding of possible transmission routes and an improved capacity for a One Health approach to tackling this emerging fungal threat.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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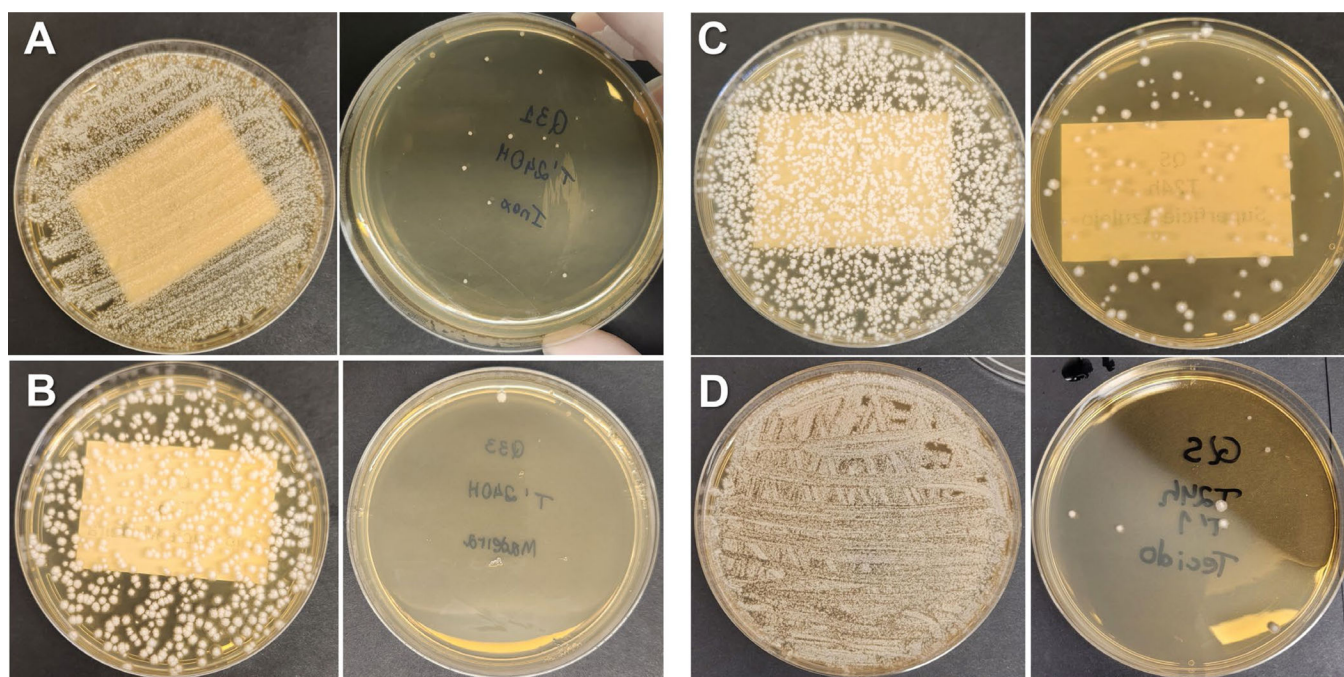
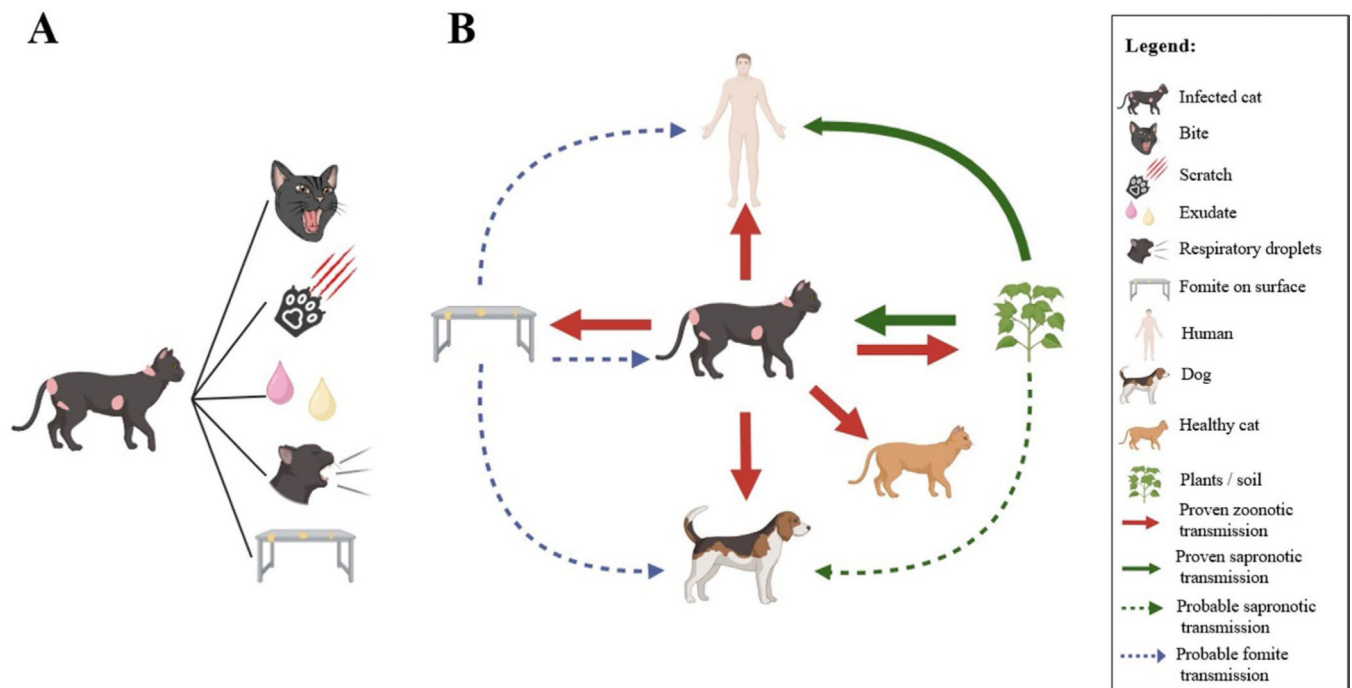


FIGURE 1.

The figure shows the growth of *S. brasiliensis* in culture: Stainless steel (A) 24 and 240 h, on MDF wood in 24 and 240 h (B), in tiles in 24 and 240 h (C) and in polyester fabric in 24 and 240 h (D).

**FIGURE 2.**

(A) Cat-transmitted sporotrichosis by *S. brasiliensis* can occur by Bites, scratches, direct contact with exudates, respiratory droplets, and contact with several kinds of fomites, including non-disinfected inanimate surfaces; (B) *S. brasiliensis* transmission routes. Red arrow—zoonotic transmission: Infected cats can transmit the disease to humans, other cats, dogs and can contaminate the environment (organic and inorganic material). Green arrow—sapronotic transmission: Organic matter as plant/soil contaminated with *S. brasiliensis* can transmit to cats, humans, and dogs. Blue spotted arrow—fomite transmission: Contact with inanimate surfaces contaminated with *S. brasiliensis* can likely transmit to humans, cats, and dogs.