Sporothrichosis

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Abstract

Sporothrix schenckii is currently recognized as a species complex consisting of *Sporothrix brasiliensis, Sporothrix schenckii* sensu stricto*, Sporothrix globosa,* and *Sporothrix luriei.* Due to divergent evolutionary process, each species possesses different virulence profiles, that allow it to thrive and persist in its niche. Currently the disease in cats is primarily caused by *S. brasiliensis, S. schenckii* sensu stricto and *S. globosa,* with cat fights and direct inoculation of the agent in the skin as the main mode of disease transmission. Expression of putative virulence factors, such as adhesins, ergosterol peroxide, melanin, proteases, extracellular vesicles and thermotolerance, determines the clinical manifestation in the feline patient, with thermotolerant *S. brasiliensis* exhibiting the highest pathogenicity, followed by *S. schenckii* sensu stricto*,* and *S. globosa*. Their ability to produce biofilm is documented, but their clinical significance remains to be elucidated. Despite comprehensive descriptions of the pathogenicity of the agent and of the disease, its prognosis remains guarded to poor, due to issues pertaining to cost, protracted treatment course, zoonotic potential and low susceptibility of some strains to antifungals.

Introduction

Sporothrix schenckii complex (also called *S. schenckii* sensu *lato*) causes a chronic, granulomatous, cutaneous or subcutaneous infection, mainly occurring in humans and cats. It has been recognised as an important cause of zoonotic subcutaneous mycosis since its description by Dr. Benjamin Schenk in 1896 [1].

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As a thermally dimorphic fungus, *Sporothrix schenckii* sensu *lato* exists as saprophyte in plant debris or decaying organic soil matter in its asexual filamentous form (25–30 °C). With favourable temperature and environment (35–37 °C), it phase transitions into its yeast form, and complete growth inhibition is achieved at 40 °C, with no sexual reproduction observed to date [2]. This characteristic underpins the epidemiology of clinical sporotrichosis where historically, the most common route of infection was reported to be the inoculation of conidia into broken skin via contaminated soil during horticultural activities. It is only in recent times that cats were perceived to be an important risk factor and disease propagators [3–7].

Etiologic Agent

Sporothrix schenckii is currently recognized as a species complex consisting of *Sporothrix brasiliensis*, *Sporothrix schenckii* sensu stricto*, Sporothrix globosa*, and *Sporothrix luriei* (Clinical clade) based on DNA sequencing, with each species having its own distinct virulence profiles and geographical distribution [8, 9]. *S. brasiliensis*, *S. s.* sensu stricto and S. *globosa*, in order of virulence, are the main species identified to cause pathology in cats [9]. *S. brasiliensis*, currently regionally restricted to Brazil, is characterised by its inherent thermotolerability which is responsible for causing systemic spread. This species was identified as the main cause of sporotrichosis epidemics in Rio de Janeiro and Sao Paolo, alongside *S. s.* sensu stricto *and S. globosa* [10–12]. *S. s.* sensu stricto is the second most pathogenic species with a worldwide distribution, especially in tropical or subtropical regions, with reports from the Americas, Africa, Australia and Asia. Zhou and colleagues demonstrated genetic diversity within this single species by subdividing *S. s.* sensu stricto into clinical clade C (most commonly isolated from Americas and Asia) and D (most commonly isolated from Americas and Africa), based on its internal transcribed spacer (ITS) [13]. The recent identification of a single clonal strain of *S. s.* sensu stricto clinical clade D from Malaysia (instead of the commonly isolated clinical clade C in Asia) suggests that this species is constantly evolving, with the ability to undergo a process of selection and subsequent population expansion, depending on local environmental or host selection pressure [14, 15]. *S. globosa* is commonly identified as the species responsible for sporotrichosis mainly in Asia and Europe, but is a rare cause in the Americas and Africa [11, 13, 16–20]. Exept *S. pallida*, Environmental clade associated sporothrix species such as *S.brunneoviolacea, S. lignivora, S. chilensis* and *S. mexicana* (*Sporothrix pallida* complex) have not been reported to cause disease in the feline patient at the time of writing [21]. These species are rare agents of sporotrichosis and normally causes low virulence, opportunistic infections from traumatic inoculation of fungus from soil into host tissue. This is in contrast to sporothrix species within the Clinical clade that is transmitted from animals.

Pathogenesis

Upon inoculation, the expression of putative virulence factors, such as adhesins, ergosterol peroxide, melanin, proteases, extracellular vesicles (EV) and thermotolerance, determines the pathogenicity and clinical presentation of sporotrichosis in the feline patient [22, 23]. The expression of adhesins and a 70 kDa glycoprotein (Gp70) on the cell wall mediates adhesion of the fungus to fibronectin, type II collagen and laminin in the host [24]. Upon invasion, the fungal cell wall composed of glucans, galactomannans, rhamnomannans, chitin, glycoprotein, glycolipids and melanin provides the ability to survive within host tissues and aids evasion from host innate immune response [25–27]. Melanin production in both mycelial and yeast form shields against a broad range of toxic insults. Melanin reduces susceptibility to antifungals and enzymatic degradation, and confers protection against oxygen nitrogen free radicals, macrophagic and neutrophilic phagocytosis [28]. The fungus readily produces ergosterol peroxide and proteinases (Proteinase 1 and 2), which allow it to evade phagocytosis and host immune response [29, 30]. EV (exosomes, microvesicles and apoptotic bodies) are membranous compartments composed of lipid bilayers, released by all living cells to the extracellular medium, that contain cargos of lipids (neutral glycolipids, sterols and phospholipids), polysaccharides (glucuronoxylomannan, alpha-galactosyl epitopes), proteins (lipases, proteases, urease, phosphatase) and nucleic acids (RNA) [31]. These cargos represent virulence factors that contribute to drug resistance, facilitate cell invasion and are eventually recognized by the innate immune system. EV contribution to fungal virulence was described in *Cryptococcus neoformans, Histoplasma capsulatum, Paracoccidioides brasiliensis, Malassezia sympodialis, Candida albicans* and, recently, also in *Sporothrix brasiliensis* [32–39]. Specifically, the EV cargos of *Sporothrix brasiliensis,* such as cell wall glucanase and heat shock proteins, were shown to increase phagocytosis but not pathogen elimination, stimulate cytokine production (IL-12p40 and TNFα) and favour the establishment of the fungus in the skin [38, 40, 41]. Current proteomic analyses revealed that 27% of EV proteins in *S. brasiliensis* and 35% in *S. schenckii* remain to be characterized, including the identification of their assigned biological process [38].

Thermotolerance, the ability of a fungus to grow or not at 37 °C, is another important virulence factor that has been identified in *Sporothrix* spp. Isolates that are able to grow at 35 °C but not at 37 °C in humans cause fixed cutaneous lesions, but those that grow at 37 °C (a close approximation to human and animal core body temperature) produce disseminated and extracutaneous lesions. Pathogenic thermotolerant species, such as *S. brasiliensis* have the ability to produce disseminated disease, compared to non-thermotolerant, less pathogenic species such as *S. globosa*. *S. s.* sensu stricto displays variable thermotolerability [14].

The ability of *Sporothrix schenckii* complex to produce biofilm has recently been documented, and an early report suggests that biofilm production alters the fungus sensitivity to antifungals, however, the full extent of its clinical significance has yet to be elucidated [42].

Both innate and adaptive immune responses play important roles in the prevention of disease progression. The first contact between fungal pathogen associated molecular pattern (PAMPs) and host pattern recognition receptors (PPRs) is mediated by toll-like-receptors (TLR)-4 and TLR-2 [43, 44]. During the initiation of infection, these receptors recognize lipid extracts from yeast cells that lead to an increased production of tumour necrosis factor alpha (TNF-alpha), interleukin (IL)-10 and nitric oxide (NO). While NO demonstrates antifungal activity in vitro, in vivo it is associated with immunosuppression during the initial and the terminal stages of the infection, due to its ability to increase apoptosis of immune cells [45]. The role of NO in the infection was also documented in histoplasmosis by *Histoplasma capsulatum* and paracoccidioidomycosis by *Paracoccidioides brasiliensis* [46, 47].

Yeast cells are also able to activate the antibody-dependent classical and alternative complement pathways [48, 49]. The main antigen recognized by antibodies is a 70 kDa cell wall glycoprotein, named Gp70 [50]. This protein plays a crucial role in fungal opsonisation, allowing macrophages to phagocytose and the production of pro-inflammatory cytokines [51]. Nevertheless, the cornerstone for an effective fungal eradication is based on an effectively coordinated innate and adaptive immune response (humoral and cell mediated) [52]. Recently, the nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3 (NLRP3) inflammasome was shown to be critical to link the innate immune response to the adaptive arm, contributing to effective protection against this infection by promoting the production of pro-IL1β [53]. Fungal interaction with dendritic cells drives a mixed Th1/Th17 immune response that activates macrophages, neutrophils and CD4+ T cells, that release IFN-gamma, IL-12 and TNF-alpha that ultimately culminates in the reduction of pathogen burden [54, 55].

Clinical Signs

Feline sporotrichosis occurs most commonly in young adult, free roaming intact male cats and is associated with fighting, with no known breed predisposition [4]. In the human patient, clinical signs of sporotrichosis may be classified into 3 forms: fixed cutaneous, lympho-cutaneous and disseminated forms, depending on the pathogenicity of the fungal species and the status of host immunity (Fig. 1). Such clear and distinct categorisation of clinical forms does not apply to cats and thus is seldom used.

In cats, chronic non-healing lesions such as nodules, ulcers and crusts are commonly found on the head, especially at the bridge of the nose (Fig. 2), on the distal limbs and tail base region (Fig. 3) and on the pinnae (Fig. 4). The majority of lesions occur in cooler regions of the host body such as at the nasal passages and ear tips.

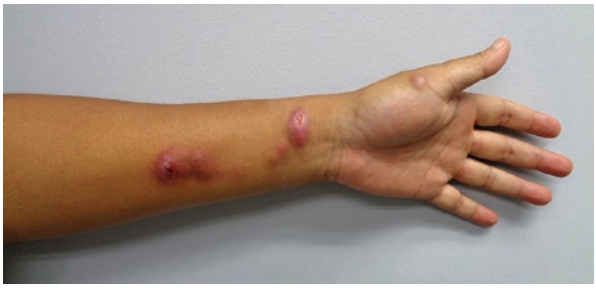

Fig. 1 A human patient manifesting lymphocutaneous sporotrichosis after being bitten by a cat with sporotrichosis (nodule at base of thumb). Due to the lack of thermotolerability of the infectious agent, the lesion did not progress beyond the arm

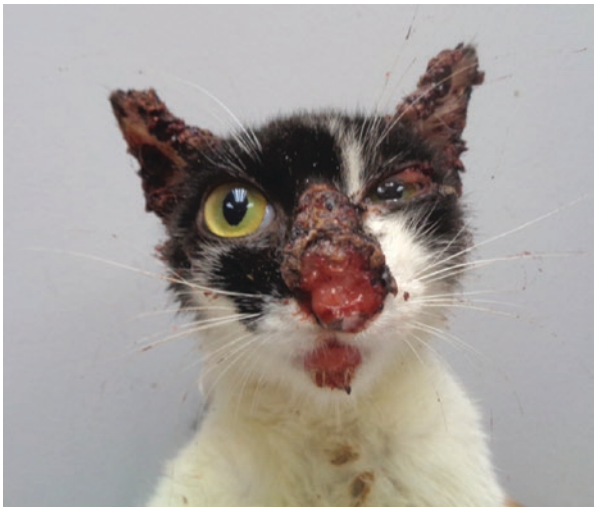

Fig. 2 Classical presentation of feline sporotrichosis: chronic non-healing wounds affecting the bridge of the nose

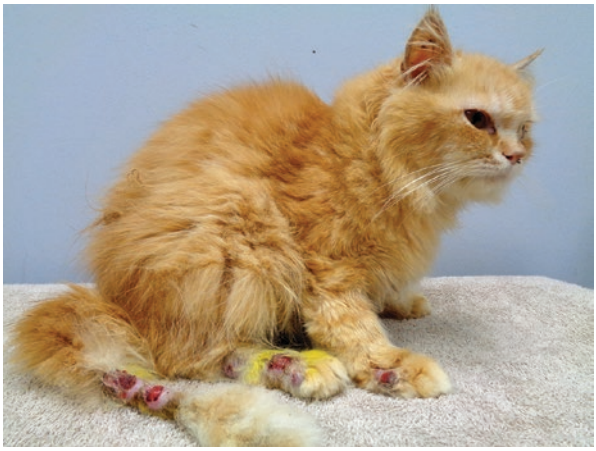

Fig. 3 Chronic non-healing wounds affecting the paws and the tail

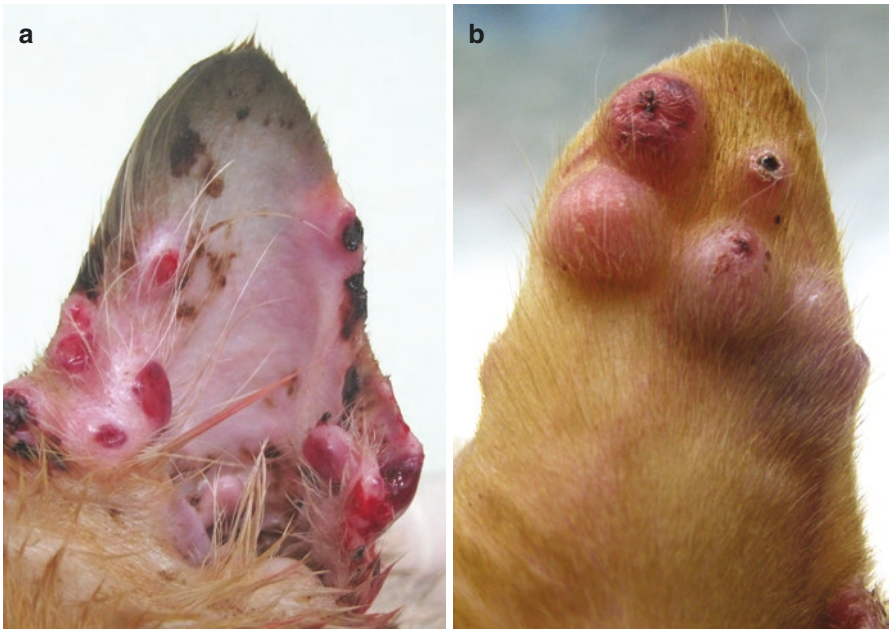

Fig. 4 (**a** and **b**) Concave and convex aspects, respectively, of the pinna of a cat with sporotrichosis presenting numerous ulcerated nodules

If nasal passages are affected, extracutaneous signs such as sneezing, dyspnoea and respiratory distress are commonly reported in tandem with cutaneous manifestations [5]. Cutaneous screwworm myiasis as secondary infestation was recently reported [56]. The fatal disseminated form of the disease is associated with *S. brasiliensis* infection. Co-infection with either feline immunodeficiency virus (FIV) or feline leukaemia virus (FeLV) has no significant effect on the clinical manifestations or on the prognosis of the disease [57].

Diagnosis

A definitive diagnosis of feline sporotrichosis requires the isolation and identification of the agent in culture. The species identification can be obtained by morphologic studies and physiologic phenotyping, as well as by polymerase chain reaction targeting the calmodulin gene [5]. At 25–30°, the fungus exists in its mycelial form and is seen as small and white or pale orange to orange-grey colonies with no cottony aerial hyphae. Later, the colony becomes black, moist, wrinkled, leathery or velvety with narrow white borders (Fig. 5). Some colonies are however black from the onset. At 35–37°, yeast colonies are cream or tan, smooth and yeast-like [2].

Cytologically, yeasts are found in abundance from cutaneous impression smears. They are located intra- and extracellularly, in pleomorphic shapes, ranging from

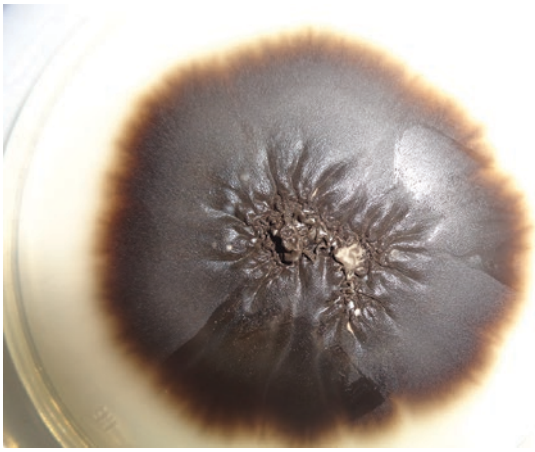

Fig. 5 In its mature mycelial form the fungi becomes black, moist, wrinkled, leathery or velvety with narrow white borders

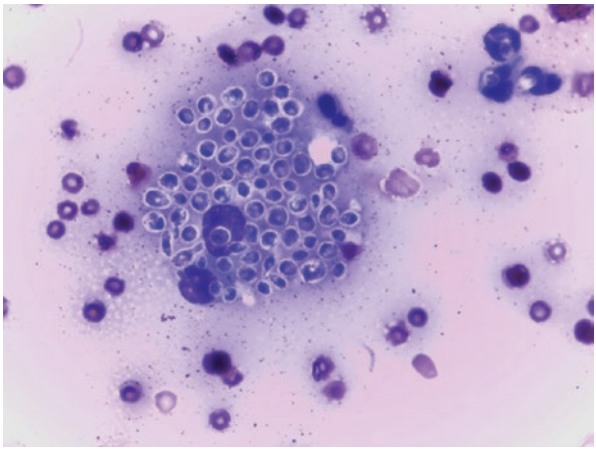

Fig. 6 Cytologically, the yeasts are found in abundance intra- and extracellularly in pleomorphic shapes, ranging from the classical cigar-shaped to round or oval, measuring 3–5 μm in diameter with a thin, clear halo around a pale blue cytoplasm (Diff Quick, 1000×)

the classical cigar-shaped to round or oval bodies, measuring 3–5 μm in diameter with a thin, clear halo around a pale-blue cytoplasm (Fig. 6) [58]. The sensitivity of cytology to detect *Sporothrix* yeasts in the feline patient is estimated to range from 79% to 84.9% [59, 60].

On histology, a diffuse pyogranulomatous inflammation with large foci of necrosis is seen throughout the superficial and deep dermis, sometimes extending to the subcutis. There are abundant round to cigar-shaped organisms, 3–10 μm in length and 1–2 μm in diameter, seen both free and within macrophages. Commonly, organisms in cytoplasm of macrophages create large clear pockets full of yeast due to poorly visualized yeast cell wall (Fig. 7) [61]. Periodic acid of Schiff (PAS) stain may also be utilized to visualize yeasts as magenta stained organism on histological preparation. Other diagnostic techniques such as serology (enzyme-linked immunosorbent assay, ELISA) and polymerase chain reaction (PCR) may also be used for the diagnosis [62, 63].

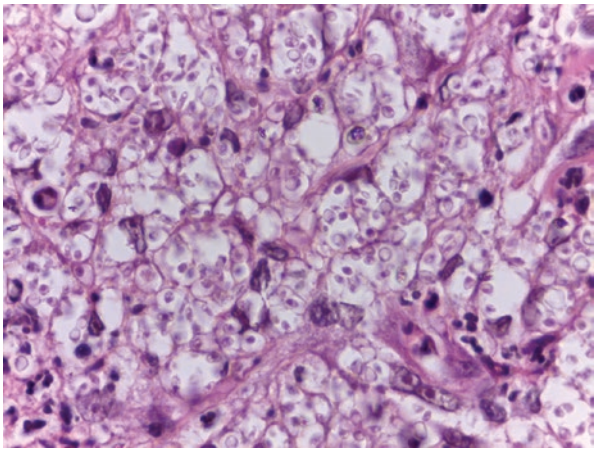

Fig. 7 On histology there are abundant round to cigar-shaped organisms, 3–10 μm in length to 1–2 μm in diameter seen both free and within macrophages. Organisms in cytoplasm of macrophages create large clear pockets full of yeasts due to poorly visualized yeast cell wall

Treatment

Treatment of feline sporotrichosis requires several months and must be continued for at least 1 month beyond clinical cure. Luckily, despite a protracted treatment course, it is current understanding that the fungus does not develop resistance during treatment [14].

Due to the high cost of treatment, high risk of therapeutic side effects and of zoonosis and existence of low susceptibility strains, feline sporotrichosis carries a guarded to poor prognosis. Currently, potassium iodide, azolic antifungals (ketoconazole, itraconazole), amphotericin B, terbinafine, local heat therapy, cryosurgery and surgical resection have all been documented as treatment options in the feline patient. Potassium iodide has traditionally been the treatment of choice, either in its saturated form (saturated salt of potassium iodide, SSKI) or in its powder form re-packaged into capsules. Dosages range from 10 to 20 mg/kg every 24 hours [64, 65]. The powder form re-packaged into capsules is favoured over SSKI for the feline patient, due to the latter's tendency to cause hypersalivation. From a report of 48 cats receiving potassium iodide, 23 (47.9%) patients achieved clinical cure with treatment failure in 18 cats (37.5%), two reported deaths (4.2%) and treatment period averaging from 4 to 5 months. The most commonly observed side effects were hyporexia, lethargy, weight loss, vomiting, diarrhoea plus an increase in the liver enzyme alanine transaminase. No signs of iodism (lacrimation, salivation, coughing, facial swelling, tachycardia) nor thyroid hormone abnormalities were observed in this study [64]. Due to its low cost, potassium iodide is still often used either singularly or in conjunction with azole antifungals to treat feline sporotrichosis [65].

Imidazoles such as ketoconazole and itraconazole currently represent the cornerstone therapy for feline sporotrichosis. Itraconazole is favoured over ketoconazole as the latter is commonly associated with a higher rate of side effects, such a vomiting, hepatic dysfunction and altered cortisol metabolism. Itraconazole at 5–10 mg/kg has been used successfully to treat feline sporotrichosis, with a maximum plasma concentration of 0.7 ± 0.14 mg/L achieved after a 5 mg/kg oral dosing [66]. Based on the updated Clinical and Laboratory Standards Institute (CLSI) reference method for broth dilution antifungal susceptibility testing of filamentous fungi (document M38-A2), the minimum inhibitory concentration (MIC) of antifungals against *S. brasiliensis, S. s* sensu stricto and *S. globosa* is presented in Table 1 [14, 19, 20, 67, 68]. Itraconazole may be the treatment of choice but there are isolates with MIC above 4 mg/L, the putative breakpoint for this antifungal agent. This variability in MIC values may reflect the extensive divergent evolutionary process within the *Sporotrix* complex, where each species developed its own repertoire of virulence factors allowing thriving and persisting in its niche. Clinically, this is reflected by the fact that some cases of feline sporotrichosis are refractory to treatment and thus protocols based on higher dosages of itraconazole and/or its combination with other antifungals have been explored to treat these refractory cases [65, 69]. *Sporothrix schenckii* sensu *lato* generally displays low susceptibility towards fluconazole and exhibits species-dependent susceptibility towards terbinafine and amphotericin B (Table 1). Despite reports of successful treatment of human sporotrichosis with terbinafine, results are still inconclusive for the feline patient [70, 71]. The recent description of the protective effects of pyomelanin and eumelanin, synthesized by *S. brasiliensis* and *S. s.* sensu stricto, against the antifungal terbinafine may partially explain why in vitro results do not always correlate with in vivo responses when patients are treated with this drug [72]. The administration of amphotericin B is associated with toxicity, high cost and side effects, such as localized sterile abscess formation from intralesional injections [5]. It is interesting to note that *Sporothrix* spp. displays variable susceptibility towards antifungals rarely used in veterinary medicine such as micafungin, 5-flucytosine and even posaconazole, highlighting the importance of susceptibility testing [14, 20, 68]. Resolving granulomas are visually and tactile-wise indistinguishable from normal adjacent healthy skin under normal room lighting, and may be better visualized when held against a bright light source (Fig. 8). Treatment should be continued for 1 month beyond the resolution of all granulomas. Localized heat therapy is based on the fact that the fungus does not grow at temperatures above 40 °C. This treatment modality, however, is associated with issues of practicality and perhaps welfare concerns in its application on animals and has not been pursued as a feasible treatment option in the feline patient. Cryosurgery, used in conjunction with itraconazole has been used successfully to treat and cure 11 of 13 cats with sporotrichosis, with treatment lasting 3–16 months and a median of 8 months [73]. Surgical resection is possible for localized singular lesions but unpractical for generalized, disseminated forms.

Table 1 All results are expressed in mg/L and based on the Clinical and Laboratory Standards Institute (CLSI) reference method for broth dilution antifungal susceptibility testing of filamentous fungi document M38-A2 (2008) in mycelial phase

	Origin	*n*	Itraconazole	Fluconazole	Amphotericin B	Terbinafine	References
S. globosa	Japan subgroup I	29	0.5–4	>128	1–4	Not tested	[20]
	Japan subgroup II	9	0.25–2	>128	2–4	Not tested	[20]
	Brazil	4	0.83 (0.06–16)	53.8 (16–128)	1 (0.2–4)	0.03 (0.01–0.06)	[67]
	Iran	4	8 (1 -> 16)	>64 32 -> 64	5.66 (4–8)	1.68 (1–2)	[19]
S. s. sensu stricto	Malaysia	40	1.3 (0.5–4)	>256	Not tested	2.85 (1–8)	[14]
	Japan	9	0.5–1	>128	2	Not tested	[20]
	Brazil	61	0.42 (0.03–16)	57.7 (8–128)	1.06 (0.03–2)	0.05 (0.01–0.50)	[67]
	Iran	5	0.76 (0.25–2)	>64	3.03 (1–8)	0.38 (0.13–1)	[19]
S. brasiliensis	Brazil	32	2	Not tested	1.2	0.1	[68]
	Brazil	23	0.36 (0.06–2)	56.7 (16–128)	1.03 (0.2–4)	0.06 (0.01–0.50)	[67]

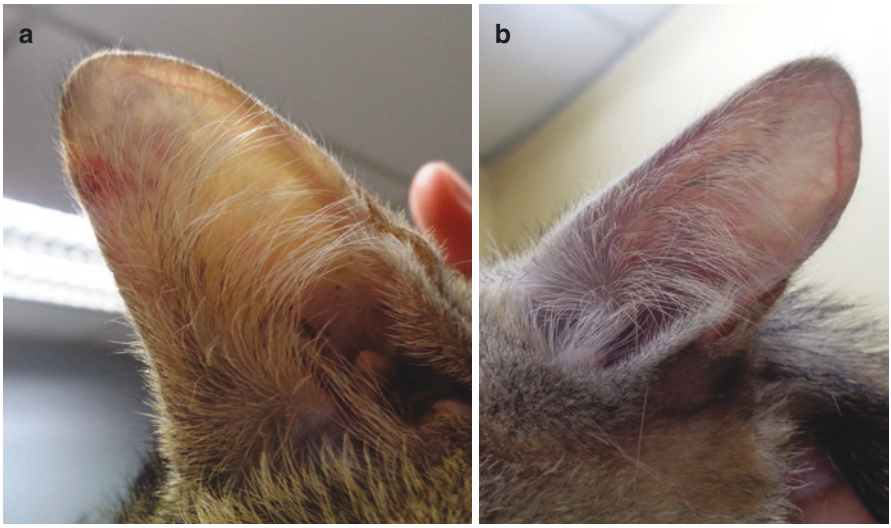

Fig. 8 The author utilizes a bright light source to evaluate and ascertain cure. (**a**) A resolving granulomatous reaction at the left ear tip, tactile and visually indistinguishable from adjacent normal tissue but is visualized with a bright light source. (**b**) Same patient after cure with complete resolution of granuloma

Conclusion

The prognosis of feline sporotrichosis remains guarded to poor due to cost, protracted treatment course, risk of zoonosis and low susceptibility of some strains. Despite the fact that antifungal susceptibility testing provides essential guidance for the treatment, its lack of commercial availability and validated breakpoints remains a stumbling block in the treatment of this disease. Unfortunately, the current repertoire of veterinary antifungals classes are inadequate to address the issue of fungal low fungal susceptibility.

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