New observations on the ecology and epidemiology of *Sporothrix schenckii* and sporotrichosis

2. Ecological niches of *S. schenckii* and zoonotic outbreaks

**Abstract**

Sporotrichosis is the most common subcutaneous mycosis in the Americas, Japan, India and South Africa. Classically, infection was associated with out-of-door occupations and the traumatic inoculation of soil, vegetables, thorns, straws and grasses, contaminated with *S. schenckii*. This paper presents the fungus ecology and disease risk-factors, according to recent epidemiologic and laboratory research work. From 2005 through 2008, 804 human new cases were diagnosed at the Evandro Chagas Clinical Research Institute (IPEC), most affected were women 40-49 years, engaged in domestic duties and from deprived social strata. Close contact with cats was reported in 91% of human cases, bites or scratches was recorded by 68% of patients. Also 64 dogs and 1,503 infected cats were confirmed at IPEC. Canine sporotrichosis presented as self limited mycosis. Feline disease varied from subclinical infection to systemic disease with hematogenous dissemination of *S. schenckii*. The cat’s zoonotic potential was demonstrated by isolation of *S. schenckii* from skin lesions biopsy, and the ma-

**Resumen**

La esporotricosis es la micosis subcutánea más prevalente en las Américas, Japón, India y Sudáfrica. Clásicamente, la infección se asociaba con la inoculación traumática del suelo, vegetales, espinas, paja o zacates contaminados con *S. schenckii*. Esta publicación presenta la ecología del microorganismo y los factores de riesgo de la enfermedad, según los trabajos de investigación más recientes, referentes a la epidemiología y laboratorio. Del 2005 al 2008, se diagnosticaron 804 casos nuevos en el Instituto de Investigación Evandro Chagas (IPEC) en Rio de Janeiro, Brasil. Las más atacadas fueron las mujeres de 40-49 años de edad, amas de casa procedentes de los estratos sociales más pobres. El contacto directo con los gatos se reportó en 91% de los casos humanos, el antecedente de mordeduras y arañazos fue recabado en 68% de los afectados. Además, en el IPEC se confirmaron más de 1,503 gatos y 64 perros infectados. La esporotricosis canina se comportó como enfermedad autolimitada. La enfermedad gatuna varió desde una infec-
Definition

Sporotrichosis is a chronic infection usually limited to cutaneous and subcutaneous tissues; it involves all layers of skin and the subcutaneous lymphatics. The disease is caused by different species of the dimorphic fungus Sporothrix schenckii. The fungus is present in the environment in soil, plants and animals, it invades through skin injury. Many times, injury is so minor that it goes unrecognized and neglected. The fungus produces indolent lesion which appears as small erythematous nodule or verrucous plaque, and may remain localized (fixed type), or spread centrally through the lymphatic draining the area, establishing a chain of granulomatous, ulcerating nodules (lymphocutaneous type). Sporotrichosis generally affects the exposed parts of the body, namely, the hands, arms, face and legs. Pulmonary sporotrichosis results from inhalation of the fungal spores, and occurs in humans with a variety of underlying conditions such as sarcoidosis, malignant neoplasm, diabetes mellitus, and chronic alcoholism. Infection may disseminate to become generalized involving skin, bones, joints, bursa and the central nervous system. Patients with acquired immunodeficiency syndrome (AIDS), or immunosuppressant, are at greater risk of multifocal, hematogenous spread and disseminated fatal infection.

Extracutaneous sporotrichosis shows marked male predominance 6:1 in widely scattered areas, most affected were males 24-50 years of age.

The new taxonomy and mycology

Sporothrix schenckii an eukaryotic organism, has chitin on its cell wall. For several year it was classified as Deuteromycotina, however, recent research imply it is an ascomycete (Ascomycota, class Pyrenomycetes, order Ophiotomatales, family Ophiostomataceae), since it has simple septum with Woronin bodies, and three chitin synthasa genes, the fungus produces dematiaceous (melanin +) conidia, and does not produce perithecium on malt, rice or potato media. The sexual form of S. schenckii is as yet unknown.

S. schenckii is one of several fungal pathogens that exhibit temperature dimorphism. In the environment or the laboratory, on standard culture media such as Mycosel-cycloheximide or Sabouraud-dextrose-agar at 25°C, after four days it forms moist, white to cream colored colonies, shiny at first and later become fuzzy. In time, the colony is membranous, brown to black and mycelial (M) (figure 1). Some primary isolates have the ability to form dark colonies from the beginning of growth.

At room temperature the fungus has thin septate and branching hyphae 1 to 2 μm in diameter. Microscopic observation of M-saprophytic stage shows thin walled hyaline conidia, however, the presence or dark brown thick walled solitary conidia, tear or globose shaped, arranged in sleeves around the main hyphal axis, and the oval borne sympodially on a lateral conidiophores (figure 3), their shape suggest a palm tree or flower head.
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Figure 1. Colonial Morphology. A: Two white colonies, initial isolate from a case of articular disease. B: Colonial side view of a “volcano on an island”. C: After two weeks, folded, black-brown with radial grooves (Courtesy Dr. J. W. Rippon).

Figure 2. A: Primary isolates from cavitary pulmonary disease, black and wrinkled folded colonies (25 °C). B: Transferred to BHI-agar at 37 °C, glistening, white, soft Yeast-colonies (Courtesy Dr. J. W. Rippon).

(figure 4). M-stage grows well on pH around 3.0 to 11.5 and can withstand NaCl 7%.

The yeast (Y) stage can be produced in vitro by culturing mycelia or conidia in rich medium such as brain-heart-infusion agar (BHI) at 37 °C. The Y-colony is pasty, glabrous, dull white, or tan, soft and bacteria-like with a wrinkled surface. The yeast cells generated in vitro are oval, sub globose or cigar-shaped with one or several buds. In the hamster and human tissues, Y-cells are surrounded by radiated eosinophilic substance, with covering of approximately 10 mμ in thickness called asteroid bodies (figure 5).

The dimorphic M-Y transformation involves calcium/calmodulin-dependent protein-kinases and, signaling interaction between cytosolic phospholipase and protein G. Y-cells grow well within pH range 3.0-8.5, and NaCl 11%. When yeasts are cultured in albumin-containing medium, two extracellular proteinases are produced: 1) chymotrypsin-like, molecular weight (mw) of 36, 500, optimal pH 6.0, and 2) cathepsin-D-like, mw 39,000, optimal pH 3.5 these two enzymes may be related to the virulence of the fungus since they hydrolyse substrates such as stratum corneum, collagen I, and elastin. All strains are able to split urea, and can tolerate cycloheximide at 0.25%, thiamine is required for good fungal growth.

Laboratory animals such as mice, rats, guinea pigs and hamsters are susceptible to sporotrichosis. When a conidial suspension is injected intraperitoneally into male mice, orchitis is recorded within 10 days to two weeks. Testes with lower

Figure 3. Mycelial microscopic morphology. A: Globose dark conidia, on side of a hypha x 1,080. B: Elliptical to oblong hyaline and pigmented, dense conidia along the hypha axis x 1,417. C: Triangular deeply pigmented macro conidia. They appear to be more resistant to drying and UV-light, and are also more virulent for mice x 2,060 (Courtesy of Dr. F. Mariat).
isolates (37°C) multiply in the internal organs, but not in external organs. Higher thermo tolerance temperature, support growth of the fungus faster than internal organs. Intravenous injection (iv) of at least 10⁵ to 10⁶ cells of clinical isolate are necessary to produce disseminated and lethal disease. With a lower dose, osteoarticular infection of the paws and tail may be observed. Congenitally athymic (nu/nu) mice are found to be far more susceptible to iv-challenge than their phenotypically normal littermates, suggesting T-lymphocytes are critical to host resistance.⁷⁷

Molecular techniques have been used to assess genetic variability of the complex-species S. schenckii, however, only few were found to be useful in clinical research. Restriction-fragment-length-polymerorphism (RFLP) of mitochondrial-DNA (mt-DNA), and calmodulin gene sequencing are powerful tools for epidemiological analysis and intra-specific typing-clustering of new environmental and clinical isolates.¹³⁶

**Ecology of S. schenckii**

Open bogs are a feature of glaciated landscapes, they provide a vital wetland (92% of water), for Sphagnum moss (sm), a little plant that do not produce flowers or seeds, but can grow from fruiting bodies (capsules), full of spores. When the mosses dies, they do not rot away because the bog ground is acidic pH 3-4, and waterlogged with very little oxygen, the chemical composition is low in organic nutrients and higher amounts of ammonium 73ppm and nitrate 13.8ppm, therefore, bacteria and fungi that normally break down plant materials, cannot survive inside the anaerobic bog. Dead mosses...
remains pile up and get pressed together, so it can take from 7,000 to 10,000 years to produce giant bogs 7-10 meters ticks, such as those found at Wisconsin, USA and Southern Canada.

Sphagnum plants grow closely packed together with others of the same kind, providing support for each other’s tiny steams (figure 6). Sm acts like as sponge wet for long time, it can soak-up more than eight times its own weight in water. They also have high zinc content in the form of a naturally occurring antibiotic called tropolene. The anaerobic bacteria causing wood decay are nullified by antiseptic properties of Sphagnum. Hence, it usually serves as an ideal medium for re-invigorating the weak seedlings, and root-rot trees. Long fibered dried sm also has excellent water retention properties, and

Figure 6. A: Live Sphagnum moss on the surface of a bog, at Wisconsin, USA. B: Dried, long fibers of Sphagnum are used by its water-retention and antiseptic properties. C: A turtle-shaped topiary, supported on a metallic structure, and filled with sm.

Figure 7. A: Orchids, growing on wetted sm. B: Sphagnum moss mixed with soil are widely used by gardeners and C: Carnivorous plants and sm mixture.

Figure 8. A: A garden-basket was filled with wetted moss to grow ornamental plants. B: The moss can be changed by coconut husk fibers, to reduce the risk of sporotrichosis.
is able to keep an open, well aerated structured: perfect for growing bonsai, orchids and carnivorous plants (figure 7). A small amount of sm can be added to inorganic soil mixes, such as akadama and grit, to provide a stable organic ingredient and, to add color to the foliage of coniferous species such as Junipers and Pines. The lining of hanging garden-baskets are made up of sm, also used to make wreaths and to produce artistic topiaries. Thus, there is a definite potential for infection with S. schenckii in those who work with wetted sm, and do not wear protective globes. The fungus mass seems to increase in the moistness of most packing sheds. Population of S. schenckii increases on dead but not on living Sphagnum moss. Experimental and clinical epidemiologic observations support the contention that at some point during or after harvesting, the moss is contaminated with the pathogen, which then colonizes and reproduces on the dead plant, under favorable conditions (warm and moist). Nursery-workers have changed the traditional use of wetted moss, by the coconuts fibers (figure 8), to reduce risk of fungal infection.

The environmental niches in which the fungus grows most luxuriantly include: decaying vegetation, potting and garden soil, prairie hay and grasses. Persons exposed to these materials have a risk for developing sporotrichosis. Thus, most clinical cases occur in healthy young man, whose occupation or hobby takes him into the outdoors. Classically, farmers, nursery workers, gardeners, landscapers and laboratory workers develop cutaneous sporotrichosis because they are exposed to contaminated materials, and their daily activities frequently lead to nicks and cuts on their extremities and face (children), allowing the fungus easy access.

In children under 10 years insect stings, animal bites, falls and abrasions are mainly responsible; while in the group 11-20 years sporting activities such as foot-ball, rugby and cricket, contributed. Over the age 21, some occupational activities have been associated with sporotrichosis. Classically, rose gardeners and farmers, develops cutaneous sporotrichosis. Other specific occupations linked with sporotrichosis: forestry workers, packing and handling pine seedling in sm, artistic topiary production, masonry work, gold mining, hay baling, packing pottery with grass, and Christmas tree farming. Motor vehicle accident, hammer blows, injury by metal knifes or particles, handling of fish spines and other traumas may lead to inoculation of soil into skin tissues. Zoonotic transmission of S. schenckii is from bites or scratches of animals, most commonly reported are cats and armadillos, in the cases of South America armadillos, the mammal is not infected but, while trying to escape capture, inflict scratches and thereby inoculates S. schenckii. Cats develop disseminated skin ulcers, with often fatal infection. The fungus is spread from ulcer or from bites and scratches to cohabitant humans and dogs.

Several cases of sporotrichosis have been described among laboratory workers who handled animals infected with or cultures of S. schenckii. Generally, cutaneous inoculation has occurred, but ocular involvement subsequent to a splash of culture material into the eye has also been described. Primary infection potentiating mechanism include: higher number of virulent-pigmented fungal conidia inoculated in association with abrasives, so that minor skin trauma can easily occur, and the opportunity for sustained exposures. S. schenckii seems to thrive well in potting soils, Kenyon et al isolated the fungus from two of 12 samples, and the presence of melanin in the cell wall of the organism is thought to confer a selective advantage, allowing the fungus to survive desiccation and ultraviolet (UV)24 irradiation, and conidia may remain viable for several years.24 The fungus grows in soils plentiful of cellulose, pH range 3.5 to 9.4 and mean temperature 31 °C, but relative humidity cannot be below 92%. Sporothrix sp, has also been isolated from diverse sources: the floor of a swimming pool (Staib 1983), from desiccated
mushrooms (Kazanas, 1987), and from fleas, ants and horse hair. S. schenckii has the ability to grow in fabrics such as flannel, hessian, coarse felt and white canvas (Du Toit, 1942). Further research is needed to establish the possible role of diverse environmental sources in the spread of the disease.

Environmental sampling to isolate S. schenckii

The laboratory of Medical Microbiology receives samples such as packed Sphagnum moss, evergreen seedlings, soil and water. The purpose of environmental sampling is to determine the source of S. schenckii and to know the point at which the moss was contaminated with the fungus. A) Direct plating technique: the sample is first exposed to par dichlorobenzene crystals, in a closed clean container for 24 hr, to control mites. In the Mycology Laboratory it is usually processed by an aqueous extraction method. After placing 25 ml loose sample volume in flasks, adding penicillin and streptomycin in water to reduce bacterial contamination, preparing the suspension, and making dilutions for agar plating 0.2ml/plate on Mycosel-cycloheximide-agar, followed by daily examination of white, light brown or black colonies. Selected isolates are deposited in a sterile water culture collection and, stored at room temperature. Macroscopic colonial morphology was evaluated after seven days, on potato dextrose-agar (PDA), cornmeal-agar supplemented with tween-80 at 1%, and Saboureaud-dextrose-agar (SDA) incubated at 27 °C. Microscopic morphology was evaluated with PDA-slide-culture, incubated at 30 °C for 14 days. Isolates producing one celled conidia sympodially on lateral, erected conidiophores were given preliminary identification of Sporothrix sp., with emphasis on the presence of oval, melanin (+) conidia, arranged in cylindrical sleeves around the hyphal axis.

B) Virulence has been defined as the relative ability of a microbe to cause damage in the host or to produce disseminated life-threatening infections, following iv-injection of >10⁶ conidia/mouse. A laboratory model has been proposed: Male mice were inoculated iv by injection of 5x10⁶ to 2x10⁸ conidia /mouse. Virulent (vi) fungal strains, grew exponentially in livers and testes throughout a 14 days period, with a 100% mortality between 12 to 24 days, after injection. These vi isolates grew at 37 °C, and were melanized in PDA-media. Some few dematiaceous strains grew at 35 °C, but not a 37 °C, and were mice-avirulent. Remaining strains were not melanized and avirulent, finally identified as Ophistoma stenoceras and Sporothrix sp.

A complete understanding of an infectious disease requires knowledge of the geographic distribution of the causal fungus, in diverse environments. Specific research of the natural sources of the organism is important in establishing the mode of infection, and demographic characteristics such as age, sex, occupation, socioeconomic status and to measure the occurrence of disease by time, place and persons, to determine whether there has been an increase or decrease of sporotrichosis new cases over the years; whether one geographical area has a higher frequency of sporotrichosis than another; and whether the characteristics of infected persons distinguish them from those without it. Numerous interesting reports are available on the ecology-epidemiology of this pathogenic fungus (Findlay, Vismer et al.); (Howard and Orr et al.); (Mackinnon, Conti-Diaz, Gezuele E et al.); Shippee JK; Fukushiro R; (Gosh, Chakrabarti et al.); (Dixon, Salkin, Cooper, Bruce-Coles).

Rio de Janeiro, outbreak of epidemic zoonotic sporotrichosis

In Latin-America, sporotrichosis is the most common subcutaneous mycoses. Classic descriptions were associated with traumatic inoculation of soil, splinters and organic matter contaminated with S. schenckii. An increasing number of human sporotrichosis cases have been reported in Rio de
Janeiro, Brazil, associated with contacts of cats, affected by the same fungal disease. From 1998-2004, there were 759 humans and 1,503 cats, diagnosed by culture-isolation of S. schenckii, in the laboratory specimens submitted at the Evandro Chagas Clinical Research Institute, Oswaldo Cruz Foundation (IPEC/Fiocruz). This represents an epidemic increment vis-à-vis previous recorded case, because during the previous 12 years, there were only 13 cases diagnosed at the IPEC/Fiocruz. From 2005-2008, 804 human patients with sporotrichosis were recorded, an annual increase of 85% (figure 9). The clinical-type diagnosis was: lymphocutaneous form 66%, fixed cutaneous 25% and disseminated 9%, (multiple cutaneous with or without extracutaneous lesions). The most attacked population segment were housewives taking care of cats, aged 40-49 years, who came from deprived and poorer social-strata. As a rule, feline disease preceded human and canine disease, domiciliary or professional contact with sick or asymptomatic cat was reported in 91% of human cases. Bites and or/scratches were reported by 68% of these patients, suggesting such lesions as the putative mean of fungus-transmission (figure 10). Repeated isolation of S. schenckii from nails and oral cavities of cats reinforces evidence indicating traumatic-skin primary infection, whereas isolation from nasal fossae and ulcerated cutaneous lesions, together with the plethora of yeast-like cells observed in histological sections of skin biopsies, demonstrates the factibility of contamination through secretions. The results of molecular typing of S. schenckii isolated from humans and animals support this hypothesis.

The treatment of choice was oral itraconazole (64%), and terbinafina (23%). Amphotericin-B was used only in six patients. Almost 2% of clinically cured patients had relapses, whereas 90 (11%) did not needed therapy, because of spontaneous cure. Post treatment follow-up was from 3-6 months: Nine patients were lost, and six were hospitalized, with two deaths. Irrespective of the drug regimen, the cure-rate was 89%.

**Domestic felines langer epizootic in Brazil**

Medical records of 374 infected cats with S. schenckii infection were evaluated. Of these, 151 (43.5%) were from Rio de Janeiro and 196 (56.5%) from neighboring municipalities. Cats came from 225 different houses, the number of infected cats per house ranged from 1 (n 173=76.9%) to 22 (n 1=0.4%). Of male sex were 228 (65.7%) and sexually intact 186 (53.6%). The cats habits of digging holes and covering their excrements with soil and sand, and sharpening its nails on trees, woods or trunks, are probably responsible for the carriage of the fungus on its nails and claws, even as a healthy carriers (figure 11). Intact male cats frequently have fights and territorial disputes with other cats and dogs (figure 12). Souza and Meirelles were able to isolate S. schenckii from the claws of 16.5% of examined cats, in Rio Grande do Sul, Brazil (Souza LL, Meirelles MCA. (Sporothrix Schenckii: estudio epidemiológico em populações de gatos, Universidade de São Paulo, Brasil, 2001; p 146). The large number of dogs infected with sporotrichosis in Rio de Janeiro, may be attributed to cats bites, scratches and purulent secretions as the main sources of fungal infections.
The known common route of infection was: fights with other cats 127 (71.0%); contact with sick cats 40 (22.3%); iatrogenic 10 (5.6%), others 2 (1.1%). The mean duration of skin lesions varied from one to 128 weeks (median, eight weeks). Lymphangitis and regional lymphadenitis were observed in 92 (26.5%), however, the most frequently recorded lesions were extensive and exudative, multiple ulcers, with zones of necrosis (figure 13). Most cutaneous lesions were localized

Figure 10. Domestic cats infected by *S. brasiliensis* and *S. schenckii* generated the simultaneous epidemic epizootic increases: Feline disease was transmitted through the bites, scratches or purulent secretions, as a means of fungus contamination to humans and dogs.

Figure 11. A: Domestic cats roaming out-of-doors may be infected by repeated contacts with soils, wood, rodents and grasses of the environment. B: Intact young male cats have frequent fights and territorial disputes with other cats; these provide new chances of epizootic fungal transmission of virulent *S. brasiliensis* strains, confirmed by isolations from cat’s nails and oral cavity.

Figure 12. Domestic cats are the main carriers of virulent *S. brasiliensis* strains, feline epizootic disease preceded canine-sporotrichosis cases, and domiciliary contact with sick cats was recorded in 84% of the infected dogs.

Figure 13. Sick domestic cats with sporotrichosis, frequently had multiple lesions. A: On the dorsum of nose, B: and C: Extensive, widespread, exudative ulcers with necrosis, on the head, cheeks, nose, ears and hind limbs, with a high burden of infectious yeast-cells.
on the head (56.8%), especially the nose (27.9%), ears (21.6%), and hind-limbs (13.8%). Nasal, oral or genital mucosal involvement was recorded in 34.9%. According to number of lesions (L): Absence of skin lesion L/0=10 (2.9%); skin lesion on one anatomic site L/1=114= 32.8%; on two anatomic sites 86 (24.8%); and >L/3=137 (39.5%). The statistical difference between >L/3 and other groups was significant (P<0.001). L3 was associated with lesions on the nose and nasal mucosa (P<0.001).

General bad condition of the cat was observed in 108 (31.1%), and respiratory signs in 154 (44.4%). Histopathology examination of 90 cat’s biopsies showed a widely distributed dermal inflammatory infiltrate of mononuclear cells and polymorph nuclear neutrophils. Granuloma formation was confirmed only in 11 (12.2%) biopsy specimens, extracellular yeast-like elements in 56 (62.2%), but not «asteroid-bodies» were seen. There was inverse correlation between presence of granuloma and histopathology finding of S. schenckii-yeasts. Anemia, leukocytosis with neutrophilia, hypoalbuminemia and hyperglobulinemia were the main hematologic abnormalities, but anemia (P<0.07), neutrophilia (P<0.01) and hipoalbuminaemia (P<0.001), predominated in group L/3, as compared to the L/1 an L/2 groups. There were no significant clinical or laboratory differences in the cats with or without feline immunodeficiency virus or feline leukemia virus, tested in 142 animals. During the study 118 (34.0%) cats were lost and 124 (35.7%) died. Only 266 were treated and 68 (25.6%) were cured, complete healing was reported regardless the presence of respiratory signs, general condition status or drug treatment schedule applied.42 Human epidemic disease raised-up in parallel with cats and dogs epizootic sporotrichosis (figure 14).

Canine sporotrichosis in Rio de Janeiro, Brazil

Sporotrichosis was considered to be rare in dogs, usually acquired after hunting activities, with possible traumatic inoculation of the fungus through thorns and wood splinters. Canine disease was characterized by fixed skin lesions on the head, ears and thorax, very few cases of disseminated and
ostearticular form have been published, the largest series of canine cases consisted of only 12 dogs (Freitas D et al. Rev Fac Med Vet Sao Paulo. 1965; 7: 381-387), and (Londero AT et al. Sabouraudia 1964; 3: 273-274).

In a five years period from July-1998 to October 2003, sporotrichosis was diagnosed in 44 dogs at the Zoonotic Service of IPEC/Fiocruz; 23 (52.3%) were males and 21 (47.7%) females, including one pregnant. Age varied from six months to 12 years (median = 4 years); 23 (52.3%) were mongrels, 14 (31.8%), Toy miniature-breeds, seven (15.9%) other breeds. Thirty-seven (84.1%) had contact with sporotrichosis confirmed cats, that inhabited the same household environment. Previous unrelated traumatic injury only two (4.5%).

Skin ulcers, fixed plaques or nodules were namely observed (figures 15 and 16). The known duration of dermatosis ranged 2 to 48 weeks, median = 6 wks. Subcutaneous nodules softened slowly, draining sera purulent material and progressing to an exudative ulcer, with elevated borders and granulomatous funds. Accordingly to number of lesions (L): Solitary L/1=18 (40.9%); L/2-4=17 (38.6%); L/5 or more=9 (20.5%). L-Topography: on nose 25 (56.8%); forelimbs 13 (29.5%), nasal mucosa involvement 9 (20.5%), and with lymphangitis-lymphadenitis were 22 (50%).

Mycological diagnosis was confirmed as follows: a) Direct microscopy of secretions 6 (13.6%); b) S. schenckii was isolated by culture of exudates in 25 out of 33 specimens (75.8%). Higher number of isolates were recorded by culture of tissue-fragment 41/42=97.6% of samples. Swabs from nasal lesions 7/12 (58.3%). Histopathology examination of skin biopsy tissue showed: an inflammatory chronic process and granuloma formation in 19 (45.2%), yeast-like cells were visualized 7 (16.7%), and no asteroid bodies were observed.

Most common hematologic abnormalities were anemia, eosinophilia, neutrophilia and lymphopenia. In 34 dogs, biochemical serum-examination was performed: High concentration of serum-proteins in 11 (32.4%) within ranges 5.4-7.1 g/dl; hipoalbuminemia <2.6 g/dl in 21 (61.8%) and hyperglobulinemia >4.4 g/dl in 18 (52.9%).

During the study period-time eight dogs were lost to follow-up, three were euthanized upon owner´s request, five (15.2%) had spontaneous regression of lesions, and 28 (84.8%) were cured after treatment with itraconazole-ketoconazole, duration of chemotherapy ranged from 2 to 5 months (median=2.5 months). Adverse drug effects were recorded in 3 animals: anorexia, vomiting, diarrhea and elevation of the hepatic enzymes levels. Dogs with culture confirmed sporotrichosis, responded well to chemotherapy and skin lesions frequently were self-limited.43

Sporotrichosis in HIV-infected patients, Rio de Janeiro, Brazil

In the medical literature only 34 isolated cases of sporotrichosis associated with human immune deficiency virus (HIV) infection, have been described since beginning of AIDS-epidemic through 2009, namely, and with disseminated cutaneous, osteoarticular, central nervous system (CNS), pulmonary and ocular lesions (figure 17). (Gutierrez-Galhardo MC et al, 2010). In Brazil, in the period 1980-2008, a total of 506, 499 AIDS-cases were diagnosed, 60% from the Southeast Region, and Rio de Janeiro is the Brazilian State with second largest number of AIDS-cases (Ministry of Health, Epidemiologic Bulletin, May 19th, 2010).

From February 1999 to December 2009, the Dermatology and Infectious Disease Team at IPEC/Fiocruz, reported 21 clinical cases of sporotrichosis in HIV-infected patients, an increase of 126%: this sample represents 1.2% of human sporotrichosis seen at IPEC from 1999-2009. 16 (76.2%) were men and five (23.8%) women, the mean CD4+ count was 346.4 cells/ul, and most cases gave an epidemiologic history of transmission from cats 66.7%. Mean duration of symptoms before clinical care was 5.75 months (range 0.5-6 months) how-
Figure 15. Dog’s skin on the muzzle and nasal mucosa were confirmed as infected in 57% of the animals with sporotrichosis.

Figure 16. Dogs with fixed cutaneous sporotrichosis. A: Alopecic plaque with two subcutaneous ulcerated nodules of the cervical-dorsal area. B: Two open granulomatous ulcers of the left foreleg (Courtesy Dr. K Dantas-Filgueira, RF University of Mossoro, RN, Brazil).

Figure 17. Cutaneous distribution of lesions in HIV-sporotrichosis disseminated cases, with multiple facial ulcers, also on the limbs, and lower abdomen.

Figure 18. A 24 years old gardener from Curitiba, Brazil. He presented with multiple ulcerated painful lesions, raised indurate margins and fetid secretions, distributed over the face, thorax, arms an legs. The CD4+ cells count 62/ul (Courtesy of Hospital of Clinics, UF Paraná, Curitiba, Brazil).

However, the HIV-positive patients without clinically defined-AIDS and no signs of immune deficiency, manifested as lymphocutaneous and fixed forms. By contrast, disseminated-widespread cutaneous lesions were observed in those with AIDS and CD4+ count <200 cells/μl, associated mucosal lesions and hematogenous propagation to bones and CNS. The skin was commonly affected 95.2%, and a rare case of destructive auricular chondritis, nodular cystic lesions and cervical lymphadenopathy. Cutaneous lesions manifested as ulcerated nodules 11 (52.4%), ulcers in eight (38.1%) and cystic masses one (4.8%). Ten (47.6%) patients reported prolonged fever for more than 1 month and seven (33.3%) with weight loss greater than 10 percent of body weight.
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Numbers and types of lesions (L) recorded: Twelve (57.1%) had up to ten-L; six (28.6%) had 11-20-L; one (4.8%) with 51-L, and one 130-L. Extensive ulcerative L>4 cm, were observed in eight (38.1%). Localized L were distributed on the upper limbs eight (38.1%) and one on abdomen (4.8%), the others had widespread disseminated skin-L (figure 18). Three patients had nasal inflammatory-L, including two with nasal septum destruction, and two with hypertrophic infiltration of the gingival with involvement of the palate and lips is Ls of the oral mucosa (figure 19).

Osteomyelitis was diagnosed only in two patients, a) localized on the ankle and knee, b) left index finger and tenosynovitis of left forearm. There were two cases with CNS-involvement. Four patients developed immune-reconstitution-syndrome (IRIS), 2-5 weeks after initiating antiretroviral therapy, with a significant increase of the CD4+ count and simultaneous viral load reduction-suppression. Two cases (9.5%) were observed with erythema multiform (a hypersensitivity reaction) which improved after combined treatment of prednisone-itraconazole. A rare case of granulomatous conjunctivitis was also described (figure 20), and a patient with bilateral auricular chondritis and lymphatic cervical cystic lesions (figure 21).

Mycologic diagnosis of sporotrichosis was based on microscopic observation of S. schenckii in the skin of 20 (95.2%), and the nasal mucosa in 2 (9.5%), cerebral-spinal fluid 2 (9.5%), and conjunctiva mucosa, urine blood, and sputum collected one of each sample from four separate patients. Histopathology examination of 12 skin biopsy specimens showed: diffuse inflammatory dermal response, with mononuclear cells, neutrophils and some giant cells. Disseminated cases were characterized by the presence of numerous round, elongated, or cigar-shaped, elliptical yeast-cells, best visualized under PAS and silver staining-GG-methods (figure 22).

Eleven (52.4%) patients were treated with itraconazole, and eight (38.1%) also received iv-amphotericin-B, with an overall cure-rate 81%. Spontaneous cure was recorded in one (4.8%) patient. Sporotrichosis as opportunistic infection, must be considered in all countries where sporotrichosis and AIDS are both endemic.45,46

Laboratory research was a powerful tool to establish the origin of the Rio de Janeiro zoonotic epidemic: S. schenckii was isolated from 100% of cat´s cutaneous lesions; 66.2% of nasal cavities, 41.8% from the oral cavity and 39.5% of the nails. In addition, the fungus was cultured from the oral cavity of three clinically healthy cats. The presence of large numbers of intra- and extracellular yeast cells, and the absence of organized granulomas is characteristic of feline sporotrichosis.47

Santos-Reis et al., used molecular-DNA-techniques, by typing 19 human and 25 animals S. schenckii isolates from the epidemic. By random amplified polymorphic DNA (RAPD), 5-10 genotypes were clustered, but the profiles of the
Brazilian isolates could be distinguished from the control, United-States isolates. DNA-fingerprints of S. schenckii isolated from the nails (42.8%) and oral cavities (66%) of infected cats were identical to the human clinical human isolates, suggesting a common source epidemic source. Clearly, cats acted as link for the widespread dissemination of S. schenckii.

Phenotypic-molecular identification of 246 fungal isolates of patients attending IPEC/Fiocruz between 1998-2008 was as follows: 206 (83.4%) were characterized as virulent S. brasiliensis; 15 (6%) S. schenckii sensu stricto and 1 (0.5%) as S. mexicana, and one single atypical strain of S. globose was identified (Mycopathologia 2011; 172: p 257).

Discussion

Several factors may explain the endemicity of sporotrichosis in developing countries: adults and children spend many hours of activities in the out-of-doors, being frequently exposed to punctures from thorns, splinters, and cuts from sedge barbs, handling of reed, Sphagnum moss, hay, and grasses. S. schenckii has been isolated from soil, humus, fertilizer, vegetables debris, moist wood, cat’s nails and oral cavity and refrigerated meat. It has also been isolated from many types of plants, such as: horsetails, rose bushes, cacti, salt meadow hay, residual packing straw, carnations, wood splinters, gold mine timbers and most commonly from wetted Sphagnum moss. A practical preventive implication is: Sphagnum moss, hay and grasses, should be dried properly before packing, protected from wetting during storage, and not wetted any sooner than necessary prior to use.
Epidemiologic research is a powerful tool to evaluate the risk of infection. In spring 1994, an outbreak of lymphocutaneous sporotrichosis (LC) occurred at a tree nursery in Florida, USA, 65 workers were involved in production of sphagnum artistic topiaries (pto) which consist of shaping plants into ornamental forms, pto also involved handling of metal frames with chicken wires, and filling the artistic form with sphagnum, and then planting small ivy plants into the wetted sphagnum.

A case of sporotrichosis was defined as either a skin biopsy specimen or swab of a skin lesion, yielding \textit{S. schenckii}. To determine risk factors, a questionnaire was administered to all nursery employees in pto, to collect demographic characteristics, various gardening activities and experience, local skin trauma and specific exposure to sphagnum. Odd ratios (OR) and 95% confidence interval (CI) for univariate analysis were calculated. All variables statistically significant (P<0.05), were included for multivariate logistic regression (SAS-software, version 6).

Among 65 workers nine cases of LC-sporotrichosis were identified, therefore: 9/65x100 = 14% attack rate, median age was 39 years (range 27-59), seven (78%) were male. Median time from observation of skin lesions until diagnosis was established was 17 days (range 1-38). Risk factors associated with sporotrichosis-disease were: A) spending >20 h/week working with sphagnum (P<0.01) B) spending more time filling topiaries with sphagnum, (P=.05); C) spending <10 h in gardening work, per week (P=.05), which reflected less gardening experience. Wearing cotton globes (P<.05) or rubber globes (P<.005), were factors clearly protective. The nurseries purchased sphagnum from local supplier in Florida, who received moss shipped from Wisconsin in plastic bales. The bale-moss was broken manually and soaked in water overnight. Wet moss was tightly packed inside chicken wires frames, the unprotected manipulation of metallic wires, resulted in minor injuries on hands and arms.59,60

A high index of early clinical suspicion and hospital quality surveillance, and prescription of appropriate therapy, remain most important for prevention. Primary health physicians should suspect sporotrichosis in any patient who presents ulcerative or verrucous plaque skin lesion, especially if there is evidence of lymphatic spread.60,61 Sporotrichosis is not a reportable disease in most countries, there is little accurate information on case-incidence, and known data are those generated by publications.1,3 Isolation of \textit{S. schenckii} from skin lesions is the gold-standard of diagnosis.1,5

Although information about the incidence of LC-sporotrichosis in the United States is limited, a few studies have estimated the annual incidence to be 0.2-2.4 cases per 100,000 persons, however; the large number of good publications reflects the research development in that country. Newton et al. after reporting a patient with LC-sporotrichosis from Laos,62 highlighted the difficulties in laboratory diagnosis, the lack of infrastructure and scarce research work on fungal infections in Southeast Asian countries, resulting in shortage of publications. In regions as the Highlands of China, Laos, Vietnam, Cambodia, Burma, Iran, Bangla-Desh, Indonesia, which have favorable conditions for \textit{S. schenckii} endemic growth, the prevalence of cases must be higher than estimated by published literature.62 In India, the number of cases published between 1932,63 to 2012 increased,64 partly due to higher number of medical and laboratory personnel who have the expertise to make diagnosis.65 In the Middle East few cases have been reported from Israel,66 Turkey,67 two cases of equine sporotrichosis were diagnosed in the Kingdom of Saudi Arabia,68 and one human case from Thailand.69 In Malaysia, a small outbreak of cat’s transmitted sporotrichosis were reported. Five adult male cats were presented with fight wounds on the forelimbs, cheeks and nose. The wounds did not heal, but instead several ulcerated nodules developed above the eyes, on the nose and behind both ears. All cats were confirmed to have sporotrichosis by culture.
Four veterinary studies who handled these cats were scratched or bitten, and three weeks later developed suppurative skin ulcers. *S. schenckii* was observed in biopsy samples taken from skin or lymph node of two students. The owner of a surviving cat, also presented similar symptoms.

In Rio de Janeiro and nearby municipalities of Brazil, highest incidence of feline disease was observed, namely in males cats of reproductive age. The presence of *Sporothrix schenckii* in the skin, oral cavity, nasal fossae and nails of cats, together with the habits of leaving home to roam; the frequent scratching of soil, woods and the disputes with other cats, surely favored the epizootic spread of the fungus, into the feline, canine and human populations. In epidemic transmission areas, many subclinical infections and spontaneous cure may have gone unnoticed. However, the lack of research studies on the feline immune response against the fungus leaves many questions unanswered.

Probably, the widespread cutaneous lesions recorded in cats, have occurred because of self-trauma, grooming, bites from other cats and hematogenous dissemination, such diagnosis is considered likely if the cat presents with a history of lethargy, depression, anorexia and fever. The repeated isolation of *Sporothrix schenckii* from sick cat’s blood and testis, suggested that acute dissemination may be more frequent, than previously reported in animal models. Also because of high burden of yeast-cells in feline lesions, the laboratory diagnosis of sporotrichosis is often possible by cytological examination (Clinkenbeard KD. Diagnostic cytology: Sporotrichosis. The Compendium. Small Animal. 1999; 13: (No 2) 207-212). It is important for veterinarians to be aware that in some cats and dogs, the organism may be difficult to see in smears or histological sections, and highlights the importance of submitting a fresh tissue sample for fungal culture, when sporotrichosis-leishmaniasis or cryptococcosis are considered in the differential diagnosis. Recently, a technique involving DNA-extraction and polymerase-chain-reaction amplification from a tissue biopsy specimen was used in diagnosing sporotrichosis in cats. Currently, the test is not commercially available. Cost is an important consideration when choosing treatments. In Latin Americans fluconazol, itraconazole and terbinafine in generic formulations are available, the cure-rate may be good in fixed and cutaneous lymphatic forms, but the prognosis is bad in disseminated cases and malnourished animals.

Most general physicians and dermatologists do not know or don’t give proper attention, to cat’s role as important source of sporotrichosis-transmission. Frequently, they attribute human infection to contacts with organic soils, plants and mosses, and forged completely the possibility of zoonotic infections from scratches-bites or skin’s secretions of diseased cats. Veterinarians also ignore a basic rule of felines dermatology: "Ulcerated skin lesions observed in cats, may potentially be produced by neoplasia, cryptococcosis or sporotrichosis" (Larsen CE. University of Sao Paulo, Brazil, 2001). In tropical countries, mucocutaneous leishmaniasis, paracoccidiomycosis, tuberculosis, coccidioidomycosis and blastomycosis should be considered in the differential diagnosis of HIV-sporotrichosis patients, with disseminated cutaneous lesions.

In dogs, the fixed cutaneous form was most commonly reported. Multiple and firm nodules, ulcerated plaques with raised borders or, annular crusted and alopecic areas have been observed on the head, pinnae or trunk. In the Brazilian canine epizootic, the nose was affected in 25 (56.8%), nasal mucosal involvement 9 (20.5%), and three cases with isolated mucosal lesions, however, the presence of sick cats in the homes of humans and dogs with sporotrichosis was reported in 83%. The high incidence of muzzle lesions in dogs, was explained by the habit to sniff their environment, and the injuries caused by cats. Dogs usually have a scarcity of yeast-fungal cells seen in histological sections, but well organized granulomes formation was reported in 45.2%. Canine disease is easily treated and has a good prognosis, in contrast, cats
frequently had a severe disease, often systemic and difficult to treat. So far, there is no reports of human cases associated with contamination from dogs. All infected animals should be handled with care, gloves and protective clothing should be worn when samples are taken of exudates or tissues and, these should be carefully removed and disposed. The arms, wrists and hands should be washed in chlorhexidine gluconate or povidone-iodine.75

The large number of sporotrichosis-HIV-infected patients, was probably due to the overlapping epidemics in the State of Rio de Janeiro, and the poverty, low-income of the affected population, with precarious health service. In the period 2005-2008, there was an epidemic increase of AIDS-sporotrichosis, all patients except one came from areas with zoonotic-epidemic spread, and two thirds of patients gave a history of transmissions from cats with sporotrichosis. Men were most affected by HIV, because in Brazil the sex-rate male/female, recorded in the ongoing AIDS epidemic was 1.5. Severe, disseminated forms were observed in HIV-infected patients when CD4+ counts were of <200 cells/ul, frequently they also showed mucosal, bones and CNS attacks, and conjuntival mucosa involvement, but nasal mucosa was the most common site of extracutaneous lesions 14.3% in the series.76-87

In terms of clinical evolution and treatment response, 81% were cured regardless the immune status or clinical-type, most patients received 100 mg oral itraconazole/daily and associated highly active antiretroviral therapy (HAART), with excellent response.76,77 In conclusion: good and timmely clinical-epidemiologic research, and high-quality laboratory-work, with the appropriate use of chemotherapy are final products of an excellent medical education, team-working multidisciplinary research, and the rich production of the many good publications generated by IPEC/Fiocruz researchers.88-96 Clearly, zoonotic sporotrichosis is an important mycosis in Brazil, and there is much to be learned about the natural history, epidemiology and ecology of S. schenckii.

The goal of disease prevention is of particular interest for this low-iricome population of the «favelas», given the limited access to medical care and lack of affordable therapy.

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