Original article

Mycological evaluation of bronchoalveolar lavage in cats with respiratory signs from Rio de Janeiro, Brazil

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Summary

Twenty-three cats with respiratory signs who had domiciliary contact with cats with sporotrichosis were studied. Sneezing was the predominant extracutaneous sign. Twelve cats had no skin lesions and 11 had ulcerated skin lesions. Mycological culture of material obtained from the nasal cavity, oral cavity, bronchoalveolar lavage (BAL) and skin lesions, when present, was performed for all cats. In the case of autopsy, lung fragments were cultured. *Sporothrix schenckii* was isolated from four of the 12 cats without skin lesions: BAL (one cat) and oral and/or nasal cavity (three cats). The latter three animals developed nasal and distant skin lesions within the following 2–4 weeks. The cat with *S. schenckii* isolated from BAL did not develop skin lesions or lower respiratory tract symptoms during the 6 months of follow-up. *S. schenckii* was isolated from one or more biological samples of all 11 cats with skin lesions: oral cavity (five), nasal cavity (eight), BAL fluid (four), skin lesions (eight), and blood culture (one). No yeast-like structures were observed upon BAL cytology in any of the 23 cats. The results suggest that *S. schenckii* can cause infection of skin contiguous to the natural facial orifices through colonisation of the mucosal surfaces of the upper airways.

Key words: bronchoalveolar lavage, cat, respiratory tract diseases, sporotrichosis.

Introduction

Sporotrichosis is a subcutaneous mycosis caused by the dimorphic fungus *Sporothrix schenckii*, which infects humans and different animal species.[1] Infection occurs through traumatic inoculation via the skin, decomposing organic matter and fungus-contaminated plants or through the scratch or bite of cats with sporotrichosis.[2] Less frequently, inhalation of conidia can cause pulmonary sporotrichosis and the infection might become disseminated.[1]

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The first case of sporotrichosis in cats in Brazil was reported in 1956.[3] In 1965, the same author described a series of eight cats with sporotrichosis,[4] which became the largest series of feline sporotrichosis reported in the international literature until 1996.[5]

Although feline disease is rare, since 1998 an epidemic of sporotrichosis involving cats, dogs and humans has been detected in the metropolitan region of Rio de Janeiro.[6] During the epidemic in Rio de Janeiro, 347 cats were studied. Respiratory signs, mainly sneezing (36.8%), were the most frequent extracutaneous symptoms (44.4%). These observations, together with the report of some owners that the respiratory signs had preceded skin lesions, suggest that both cutaneous and inhalatory infection play an important role in feline disease.[7]

The specific diagnosis of pulmonary diseases in small animal clinical practice is limited and several techniques such as bronchial brushing, transthoracic pulmonary

aspiration and bronchoalveolar lavage (BAL) are being used.[8,9]

Fungal agents causing systemic mycoses, when inhaled, can spread through blood from the lungs to different organs. *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans* and *Histoplasma capsulatum* have been diagnosed by BAL analysis in dogs and cats, indicating the usefulness of this technique in the diagnosis of pulmonary mycoses.[8,10,11] The objective of the present study was to investigate the bronchoalveolar lavage in cats with respiratory signs and epidemiological evidence of sporotrichosis.

Materials and methods

Cats with upper respiratory tract signs with or without skin lesions, who had domiciliary contact with other cats with sporotrichosis, were seen at the Zoonosis Service of the Evandro Chagas Clinical Research Institute (IPEC), Fiocruz. After clinical and dermatological examination, superficial secretion was collected with sterile swabs from ulcerated skin lesions, oral cavity and nasal cavity. Blood was collected by aseptic venipuncture for mycological blood culture and the determination of feline immunodeficiency virus (FIV) antibodies and feline leukaemia virus (FeLV) antigens. The last two tests were carried out according to manufacturer instructions (IDEXX, Wesbrook, MA, USA).

Bronchoalveolar lavage fluid was obtained using an sterile endotracheal tube (3 or 3.5 mm) after tranquilisation of the animals with 10 mg kg^{-1} acepromazine and 0.1 mg kg^{-1} ketamine, IM, anaesthesia induction with 10–12.5 mg kg^{-1} thiopental, IV, and topical anaesthesia of the larynx with 10% lidocaine. A volume of 2–5 ml sterile physiological saline was instilled in each animal and immediately aspirated for three consecutive times.[12] The samples collected for mycological culture (BAL fluid, and oral, nasal and lesion swabs) were seeded onto Sabouraud dextrose agar (Difco, Detroit, USA) supplemented with chloramphenicol and onto Mycobiotic agar (Difco) and incubated at 25 °C for 30 days. BAL fluid was directly examined (slides prepared with 20% potassium hydroxide and Indian ink) and seeded onto Petri dishes containing Niger seed agar (*Guizotia abyssinica*). The suspected isolates of *S. schenckii* were subcultured on dextrose-potato agar (Difco) at 25 °C for morphological identification and dimorphism was demonstrated by the growth of yeast-like structures in brain–heart infusion medium at 37 °C. The slides for cytological analysis of BAL were obtained by cytological centrifugation of 400 μl of the sample at 1000 rpm (720 g) for 5 min[13] and stained with Grocott's silver methenamine for the detection of fungal structures.

When the owner of the animal refused treatment, euthanasia was performed with a lethal dose of intravenous thiopental after the owner had signed a consent form. The animals were submitted to autopsy and a lung fragment was removed from each animal and divided into two portions: one was stained with periodic-acid Schiff and Grocott's silver methenamine for histopathological analysis and the other was used for mycological culture as described above.

The animals in which *S. schenckii* was isolated from skin lesion fragments were treated orally with 5–10 mg ketoconazole, twice a day (BID), or 5–10 mg itraconazole, once a day (QD). All procedures of this study were approved by the Ethics Committee on Animal Use of the Oswaldo Cruz Foundation (protocol 223–04).

Results

Between May 2003 and December 2004, 23 cats from the State of Rio de Janeiro were submitted to BAL (Table 1). Of these, 21 cats were not castrated, 19 were mongrels and four were siamese. Male cats predominated ($n = 16$). Age ranged from 5 to 66 months (median of 18) and weight ranged from 1.3 to 4.2 kg (median of 3.2). The respiratory signs observed were sneezing ($n = 23$), breathlessness ($n = 4$), cough ($n = 3$) and nasal secretion ($n = 2$).

Twelve cats had no skin lesions neither history of cutaneous lesions. In this group, *S. schenckii* was isolated from the oral cavity ($n = 2$), nasal cavity ($n = 3$) and BAL fluid ($n = 1$). No growth of microorganisms in blood culture was observed for any of the 12 cats, and no FIV antibodies or FeLV antigens were detected. The three cats in which *S. schenckii* was isolated from the nasal cavity developed skin lesions on the nose (Fig. 1), followed by distant lesions within 2–4 weeks after the exam. The three animals were discharged with healed lesions after oral treatment with 50 mg ketoconazole, BID (two cats), or 50 mg itraconazole, QD (one cat), for 4–8 months.

The other 11 cats had skin lesion at the following sites: nose ($n = 10$), trunk ($n = 2$), head ($n = 8$), forelimbs ($n = 5$), and hind limbs ($n = 5$). *S. schenckii* was isolated from the oral cavity ($n = 5$), nasal cavity ($n = 8$), BAL fluid ($n = 4$), skin lesions ($n = 8$), and blood culture ($n = 1$).

One cat was positive for FIV and two were positive for FeLV. Three cats started treatment with 50 mg ketoconazole, BID, orally. Two of them were discharged after 6–8 months of treatment and the other cat died due to

Table 1 Clinical signs, mycological culture and clinical outcome of 23 cats submitted to bronchoalveolar lavage

		Mycological culture						
Case	Skin lesions	Oral cavity	Nasal cavity	Skin lesions	BAL	Blood culture	Lung fragment	Clinical outcome
1	Absent	–	–	0	+	–	0	a
2	Absent	–	+	0	–	–	0	b, c
3	Absent	–	–	0	–	–	0	a
4	Absent	–	–	0	–	–	0	a
5	Absent	+	+	0	–	–	0	b, c
6	Absent	–	–	0	–	–	0	a
7	Absent	–	–	0	–	–	0	a
8	Absent	–	–	0	–	–	0	a
9	Absent	–	–	0	–	–	0	a
10	Absent	–	–	0	–	–	0	a
11	Absent	–	–	0	–	–	0	a
12	Absent	+	+	0	–	–	0	b, c
13	Present[2,5]	–	–	–	+	–	0	d
14[6]	Present[1,2,3,4]	0	0	+	–	0	+*	e
15	Present[1,2]	+	+	+	–	+	0	e
16	Present[1]	–	–	+	–	–	+	e
17	Present[2,5]	–	+	+	–	–	0	c
18	Present[2]	–	+	+	–	–	0	c
19[7]	Present[1,2,4,5]	+	+	–	+	–	+	e
20	Present[1,2,3,4,5]	+	+	–	–	–	+	e
21	Present[1,2]	+	+	+	+	–	+*	e
22	Present[1,2,4]	+	+	+	+	–	+	e
23[6]	Present[1,2,4,5]	–	+	+	–	–	–	e

BAL, bronchoalveolar lavage; [1]Head; [2]nose; [3]trunk; [4]forelimbs; [5]hind limbs; [6] FeLV infection, [7]FIV infection; +, isolation of *Sporothrix schenckii*; –, negative mycological culture; 0, not performed

*Visualisation of yeasts suggestive of *Sporothrix schenckii* upon histopathological examination of the lung; a, asymptomatic; b, progression to cutaneous sporotrichosis; c, satisfactory therapeutic response; d, intoxication unrelated to treatment; e, euthanasia.

Figure 1 Photograph of a cat with nose and nasal mucosa lesions.

exogenous intoxication unrelated to treatment. The other eight cats were killed at the option of the owners. Seven of these cats were submitted to autopsy and *S. schenckii* was isolated from lung fragments in six animals. In three of them the fungus was also isolated from BAL culture. Yeast-like structures compatible with *S. schenckii* were visualised in the lungs of two cats upon histopathology. No structures suggestive of fungi were observed in any of the 23 Grocott-stained BAL fluid samples and *S. schenckii* was the only pathogenic fungus isolated from all samples.

Discussion

Twenty-three cats with sporotrichosis were studied whose sex, age, race and site of the lesions were similar to those reported by Schubach *et al.* [7] Non-castrated male mongrel animals with a mean age of about 2 years predominated and the lesions were preferentially located on the limbs, face and neck.

In three cats without skin lesions in which *S. schenckii* was isolated from the nasal cavity but not from BAL fluid, the subsequent occurrence of nasal and distant skin lesions suggests that inhalation of *S. schenckii* may have led to colonisation of the mucosae of the upper aerodigestive tract, followed by infection of the skin

around the natural facial orifices and probably of the lungs. *S. schenckii* may have then spread through licking from these locations to other sites on the skin and, possibly, through the bloodstream to the internal organs. These findings agree with Schubach *et al.* [14], who detected *S. schenckii* in peripheral blood of both felines with multiple skin lesions and those in good general health with localised lesions, suggesting that blood dissemination may occur early in cats infected with *S. schenckii*. Sethi *et al.* [15] experimentally demonstrated the dissemination of *S. schenckii* to internal organs after intranasal inoculation of yeasts in rats. Mycotic rhinitis is usually an important characteristic of natural infection with *Cryptococcus neoformans* in cats, dogs, horses, goats and koalas, starting as an infection of the upper respiratory tract which can disseminate through blood or extend by contiguity to skin adjacent to the nasal cavity. In human cryptococcosis, involvement of the upper respiratory tract is uncommon and the lung is the primary focus of disseminated infections.[16] hinitis observed in the present study contrasts with respiratory sporotrichosis in humans, which is characterised by pulmonary infection acquired through the inhalatory route, with eventual dissemination to other organs.[17]

In one cat without skin lesions, BAL fluid was the only clinical sample positive for *S. schenckii*. The animal continued to be free of lesions and presented no systemic symptoms or lower respiratory tract signs over the 6 months of follow-up. This fact might be explained by spontaneous resolution of the infection, transient colonisation of the bronchial tree or eventual contamination of the lower airways with content from the oral cavity during the intubation procedure. Evidence suggests that pulmonary infection with *S. schenckii* in humans may be asymptomatic and self-limited or that the microorganism exists as a pulmonary saprophyte.[17]

The presence of respiratory signs in cats without skin lesions in which *S. schenckii* could not be isolated and which did not develop sporotrichosis might be related to other etiological agents such as feline rhinotracheitis virus, feline calicivirus and bacteria such as *Bordetella bronchiseptica*, *Chlamydophila felis* and *Mycoplasma felis*.[18]

The higher frequency of isolation of *S. schenckii* from the nasal and oral cavities of cats with skin lesions compared with those without lesions might be due to blood dissemination, direct contact of the mucosae with wounds or inhalation of fungal structures already present in the environment or derived from contamination of skin lesions. Schubach *et al.* [19] studied 148 cats with sporotrichosis and *S. schenckii* was isolated from skin lesions in 100% of cases, from the nasal cavity in 31.8% and from the oral cavity in 22.3%. In a subsequent study, the same authors, analysing 347 cats with sporotrichosis, isolated *S. schenckii* from the nasal cavity of 79 (22.8%) animals.[7] These studies suggest that involvement of the respiratory tract can precede skin lesions or be secondary.

In the present study, *S. schenckii* was isolated by BAL culture from few cats. Schubach *et al.* [20] isolated *S. schenckii* from lung fragments obtained from 10 autopsied cats and identified yeasts upon histopathological examination in six lung fragments from nine cats.

The higher positivity obtained with the analysis of lung fragments compared with BAL, both in terms of culture and visualisation of the agent upon histopathological examination, might be related to the parenchymatous location of the fungus in the lungs since BAL material is obtained from the epithelial surface of the lungs.

Our results suggest that *S. schenckii* can cause infection of skin contiguous to the natural facial orifices through colonisation of the mucosal surfaces of the upper airways, and then disseminates by skin licking and through blood. According to the study presented, respiratory signs without skin lesion may be sporotrichosis in an early state before skin lesions, but are more often due to other causes even in cats with a history of contact to sporotrichosis. However, if respiratory signs in addition to skin lesions are present, it is most probable that these are caused by sporotrichosis.

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