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Feline sporotrichosis: Histopathological profile of cutaneous lesions and their correlation with clinical presentation

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ABSTRACT

Cutaneous lesions of feline sporotrichosis show high fungal load and are associated with severe disease and elevated zoonotic potential. The present study describes the histopathology and fungal load of the lesions in different clinical presentations of feline sporotrichosis. Cats with sporotrichosis were separated into groups L1, L2 and L3 (lesions in one, two and three or more locations, respectively) and subjected to skin biopsies for histopathology. Eighty-six cats were included in the study. Lesions were suppurative granulomatous in 84 cases and poorly formed granulomas were predominant. The well-formed granulomas were associated with group L1. The high fungal load was predominant in group L3 and in poorly formed granuloma cases and did not occur in well-formed granulomas cases. The good general condition was associated with low fungal load. These findings suggest that the fungal load control in animals with more localized lesions and well-organized response is linked with the improvement in the outcome of infected cats.

1. Introduction

Sporotrichosis is a mycosis that affects humans and many animals worldwide, and is caused by *Sporothrix schenckii* complex species [1]. Transmission occurs through a traumatic inoculation through the skin with soil, plants, and organic matter contaminated with the fungus [2]. Zoonotic transmission may also occur through a bite, scratch or contact with the exudate from infected lesions. Cats have a high zoonotic potential, since their skin lesions contain plenty of yeast [3].

Since 1998, sporotrichosis has appeared as an epidemic disease in Rio de Janeiro, Brazil, and by 2009, there have been 2200 human cases, 3244 feline cases and 120 canine cases diagnosed by the Evandro Chagas Clinical Research Institute (IPEC) – FIOCRUZ [4]. Among the human cases reported up till 2004, 83% have reported contact with sporotrichosis infected cats, and of these, 56% resulted from a scratch or bite of the animal [5]. Zoonotic transmission appears to explain the implementation of the epidemic and its maintenance in an endemic form [6].

Several species of the *S. schenckii* complex have been isolated from human and feline lesions in Rio de Janeiro, Brazil and *Sporothrix brasiliensis* has been considered the most prevalent species in this current epidemic [4,7], with a prevalence of more than 90.0% of cases. *S. schenckii* has also been described in few cases (6.0%). *Sporothrix mexicana* and *Sporothrix globosa* were observed in a single case by each one [8].

Currently, the cat is the animal species most affected by sporotrichosis [9] and their cutaneous lesions are characterized by subcutaneous nodules which develop into

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ulceration. The clinical signs in feline sporotrichosis are variable, with subclinical infections, single lesions, and fatal disseminated systemic forms [3,10].

Experimental studies in mice suggest that the histopathologic lesions of sporotrichosis vary with the stage of disease, showing first the formation of abscesses and the participation of macrophages and lymphocytes, associated with a high fungal load. When the macrophages activate and begin to differentiate, thereby forming granulomas, the fungal load decreases, accompanied by the reduction of abscesses and increase in plasma cells [11–13].

The occurrence of severe sporotrichosis, including skin lesions without granulomas and rich in fungal elements in cats that are apparently immunocompetent [3,10] show the high susceptibility of these animals to infection by *S. schenckii* and emphasize the need to better understand the immune response against this fungus and its association with the fungal load and general condition of the animals. The acquired results could help in the design of new drugs and in the treatment and control of feline sporotrichosis, further reducing its transmission to humans and other cats.

This present study aims to describe a histopathological profile of feline sporotrichosis lesions and to verify the differences in pathological expression and fungal load, according to clinical presentation, age and general condition of the animals.

2. Material and methods

2.1. Sampling

The study was cross-sectional and samples were obtained from outpatient routines at the Domestic Animal Dermatozoonosis Laboratory of Clinical Research (LAPCLIN/DERMZOO) IPEC – FIOCRUZ, during the period of July 2009–August 2011. The animals considered eligible were cats with sporotrichosis (confirmed by isolation and identification of *Sporothrix* sp. complex in culture medium), showing ulcerated skin lesions, with no previous antifungal or corticosteroid therapy.

Cases were excluded when the biopsy sample was considered superficial (not including at least the reticular dermis) or not representative (not exhibiting inflammatory infiltration covering more than one third of the sample).

The general condition of the animals was classified as good, fair or poor. Good general condition was considered with the absence of extra-cutaneous signs (0). The presence of signs such as dyspnea, conjunctivitis, weight loss, dehydration, pale mucous membranes and prostration, when mild, indicate fair general condition (1 or 2), and when accented, poor general condition (3 or 4).

The cats were divided into three groups according to the distribution of skin lesions: L1, L2 and L3 [3]. In L1, cats with lesions in one location were included; in L2, cats with lesions in two non-contiguous locations were included, and in L3, cats with lesions in three or more non-contiguous locations were included.

Samples were obtained from exudates of skin ulcers by means of sterile swabs (isolation and identification of fungi in culture medium) [14] and from fragments of the edge of the ulcerated lesion by means of biopsy, using a punch of 0.4 cm, after sedation and local anesthesia. The biopsy specimens were fixed in 10% buffered formalin for histological examination at the Department of Pathology, IPEC – FIOCRUZ.

The procedures performed on the cats and the use and storage of their biological samples, were approved by the Animal Ethics Committee (CEUA), FIOCRUZ. License number LW-28/10.

2.2. Histopathology

Sections (5 μ) were stained with haematoxylin and eosin (HE) and Grocott silver stain for microscopic analysis.

Histopathological analysis was performed in an optical microscope by a trained technician. The lesions were described considering the type of inflammation – granulomatous or nonspecific. In cases of granulomatous inflammation, the cell activation degree of the mononuclear phagocytic system in granulomas – macrophages or epithelioid cells – and organization of the granuloma were also verified. Table 1 is adapted from a study of Miranda et al. [15] and represents definitions of the histopathological variables used in this study.

In the Grocott silver stain analysis, the cases in which yeast-like forms (YLF) were present were considered positive. The cases were considered negative when no YLF was observed after the analysis of 50 high power microscopic fields (400×).

2.3. Statistical analysis

Data were stored and analyzed with the Statistical Package for Social Sciences (SPSS) for Windows, version 16.0 (SPSS Inc.). For descriptive analysis, we calculated the frequencies of the variables. The different groups were compared regarding the clinical findings and histopathological analysis.

The chi-square test of independence was used to determine a significant association between the variables studied. Fisher's exact test was applied to the comparison of variables with only two categories.

In all specified analyses, a p-value < 0.05 was considered as the indication of a statistically significant association.

3. Results

3.1. Sampling

One hundred and nine cats, seventy-nine males and thirty females, were observed from July 2009 to August 2011 and eighty-six were included in the study. Seventeen cats were not included due to previous anti-fungal or corticosteroid therapy. One cat was excluded for no isolation of fungi and five because biopsy samples were considered non-representative or superficial.

3.2. Histopathological findings

Microscopically, 84 (97.7%) showed suppurative granulomatous inflammation and two (2.3%) showed nonspecific inflammation.