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Refractory feline sporotrichosis: a comparative analysis on the clinical, histopathological, and cytopathological aspects[1]

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ABSTRACT.- Silva F.S., Cunha S.C.S., Moraes V.A., Leite J.S. & Ferreira A.M.R. 2022. **Refractory feline sporotrichosis: a comparative analysis on the clinical, histopathological and cytopathological aspects.** *Pesquisa Veterinária Brasileira 42:e06923, 2022.* Departamento de Patologia e Clínica Veterinária, Faculdade de Veterinária, Universidade Federal Fluminense, Av. Alm. Ary Parreiras 503, Vital Brazil/Santa Rosa, Niterói, RJ 24220-000, Brazil. E-mail: francinesilva@id.uff.br

Sporotrichosis is a chronic fungal infection caused by *Sporothrix* species. The occurrence of cases that are resistant to long-term treatment, especially in the nasal planum of cats, emphasizes the importance of studying its pathogenesis. The purpose of this study was to analyze and compare the inflammatory process of cutaneous lesions of feline refractory sporotrichosis to clinical aspects through cytopathological and histopathological examination. Moreover, the study included 13 cats with cutaneous lesions that had been resistant to itraconazole treatment for more than a year. Cutaneous lesions samples were collected for cytopathological, histopathological, and fungal culture analyses. Tissue fragments were processed and stained with hematoxylin-eosin (HE) and Grocott methenamine silver (GMS). Further, two clinical presentations had the highest occurrence: the localized cutaneous form in animals with good general condition and stable disease (n=9, 69.2%) and the disseminated cutaneous form in cats with poor general condition (n=4, 30.8%). In cats with refractory sporotrichosis, the nasal planum (84.6%) was the most common location of lesions. In the cytopathological study, cats with fewer than two lesions and in good general condition (n=9, 69.2%) showed absence or mild yeast intensity (up to 5 yeasts per field), lower intensity of macrophages and neutrophils, and higher intensity of epithelioid cells, lymphocytes, plasma cells, and eosinophils. On the other hand, (n=4, 30.8%) of the cats with disseminated sporotrichosis and a poor general condition had a marked intensity of yeasts, which were mostly phagocytosed by an increased number of macrophages and neutrophils. Of those animals with good general condition, the majority (n=6, 66.7%) had higher eosinophil intensity. In histopathology, malformed suppurative granuloma was the predominant type (n=9, 69.2%) in feline sporotrichosis lesions, followed by well-formed granulomas (n=4, 30.8%). Malformed granulomas showed mild to moderate fungal intensity (55.6%) in animals with good general condition and localized lesions while marked fungal intensity (44.4%) in cats with the disseminated form of the disease and poor general condition. Well-formed granulomas (n=4, 30.7%) had mild to moderate intensity of fungal load, and 75% of the animals with this type of granuloma had only one lesion and were in good general condition.

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Long-term itraconazole treatment in these cats with refractory sporotrichosis can keep the infection under control and localized lesions stable; however, fungus reactivation can occur, resulting in an exuberant and inefficient immune response.

INDEX TERMS: Sporotrichosis, refractory sporotrichosis, histopathology, cytopathology, cats.

RESUMO.- [**Esporotricose felina refratária: uma análise comparativa sobre os aspectos clínico, histopatológico e citopatológico.**] A esporotricose é uma infecção fúngica crônica causada por espécies do gênero *Sporothrix.* A ocorrência de casos refratários ao tratamento de longo prazo, especialmente na região nasal de gatos, alerta para a importância do estudo de sua patogênese. Este estudo teve como objetivo analisar o processo inflamatório das lesões cutâneas de esporotricose felina refratária ao tratamento, por meio de exames citopatológico e histopalógico, e comparar com aspectos clínicos. Treze gatos com lesões cutâneas refratárias ao tratamento com Itraconazol por mais de um ano foram incluídos no estudo. Amostras de lesões cutâneas foram coletadas para análises citopatológicas, histopatológicas e cultura fúngica. Fragmentos de tecidos foram processados e corados pela hematoxilina-eosina (HE) e Prata Metenamina de Grocott (GMS). Duas apresentações clínicas tiveram maior ocorrência: a forma localizada cutânea em animais com bom estado geral e doença estável (n=9, 69,2%); e a forma disseminada cutânea em gatos com estado geral ruim (n=4, 30,8%). A região nasal (84,6%) foi a localização mais frequente das lesões nos gatos com esporotricose refratária ao tratamento. No estudo citopatológico, felinos com menos de duas lesões, e em bom estado geral, (n=9, 69,2%) revelaram ausência ou leve intensidade de leveduras (até 5 leveduras por campo), menor intensidade de macrófagos e neutrófilos, e maior intensidade de células epitelióides, linfócitos, plasmócitos e eosinófilos. Enquanto, (n=4, 30,8%) dos felinos que apresentavam a forma disseminada da esporotricose associada a um estado geral ruim, revelaram acentuada intensidade de leveduras em sua maioria fagocitadas por acentuado número de macrófagos e neutrófilos. Dos animais com bom estado geral, a maioria (n=6, 66,7%) apresentava maior intensidade de eosinófilos. Na histopatologia, o granuloma supurativo mal formado foi o tipo predominante (n=9, 69,2%) nas lesões de esporotricose felina, e (n=4, 30,8%) foram de granulomas bem formados. Granulomas mal formados apresentaram leve a moderada intensidade fúngica (55,6%) nos animais com bom estado geral e lesões localizadas, e acentuada intensidade fúngica (44,4%) nos gatos com a forma disseminada da doença e estado geral ruim. Granulomas bem formados (n=4, 30,7%) apresentaram leve a moderada intensidade de carga fúngica, sendo 75% dos animais com esse tipo de granuloma com apenas uma lesão e bom estadogeral. O tratamento de longo prazo com itraconazol, nesses gatos com esporotricose refratária, pode manter a infecção controlada e lesões localizadas estáveis de forma temporária, contudo a reativação do fungo pode ocorrer levando a uma resposta imunológica exuberante e ineficiente.

TERMOS DE INDEXAÇÃO: Esporotricose, esporotricose refratária, histopatologia, citopatologia, gatos, felinos.

INTRODUCTION

Sporotrichosis is a subcutaneous mycosis caused by the species of the pathogenic clade of the genus *Sporothrix* (Beer et al. 2016). There have been several cases of zoonotic transmission, and feline sporotrichosis is currently considered hyperendemic in the state of Rio de Janeiro (Beer et al. 2016).

The genus *Sporothrix* contains several species, the vast majority of which are environmental and nonpathogenic. Moreover, *Sporothrix brasiliensis*, *Sporothrix schenckii*, *Sporothrix globosa*, and *Sporothrix luriei* are part of the pathogenic group (Beer et al. 2016, Rodrigues et al. 2016). Among the *Sporothrix* species, *S. brasiliensis* is the zoonotic pathogen with the highest virulence, and feline populations have shown a high susceptibility to this agent (Arrillaga-Moncrieff et al. 2009, Gremião et al. 2017).

Cats are primarily responsible for the zoonotic transmission of *S. brasiliensis* in southern and southeastern Brazil (Rodrigues et al. 2016). The cat transmits the fungus through scratching or biting by inoculating yeasts rather than conidia, a more virulent morphotype for mammals (Gremião et al. 2017).

Feline sporotrichosis can manifest as a single skin lesion or as several disseminated lesions with systemic and even fatal involvement. The most common types of lesions are nodules and ulcers, which can be found in three or more anatomical sites associated with lymphangitis and lymphadenitis. Respiratory symptoms and mucosal involvement are common, and they are linked to a worse course of disease and treatment failure (Almeida-Paes et al. 2014, Gremião et al. 2015). Feline nasal lesions have been linked to antifungal resistance and relapse after clinical cure (Gremião et al. 2011).

The definitive diagnosis is based on the isolation of the etiologic agent in culture and the identification of the species using a tissue sample from the lesion. Other diagnostic aid tools, such as cytology, serology, histopathology, and PCR with calmodulin gene sequencing, can be used (Rodrigues et al. 2014).

The histopathology of lesions in the nose of cats associated with *Sporothrix* spp. shows a high frequency of poorly organized granulomas, with severe pyogranulomatous inflammation infiltrating subcutaneous tissue, mucosa, muscle, cartilage, and bone, as well as a high fungal load (Gremião et al. 2015). Well-organized granulomas and presence of epithelioid cells were associated with control of fungal load and good general condition (Miranda et al. 2013).

The purpose of this study is to analyze and compare histopathological and cytopathological characteristics of 13cases of feline sporotrichosis, which were resistant to antifungal treatment, to clinical aspects.

MATERIALS AND METHODS

The study was conducted with consent (CEUA-UFF) and in accordance with protocol no. 810. Moreover, the study included 13 cats with refractory cutaneous lesions. Refractory cats were those which received itraconazole 100mg daily for more than a year and did not achieve

complete clinical remission or had lesion recurrence after clinical cure in the same location. Animal samples were collected for cytopathology, fungal culture, and histopathology. Further, the distribution of skin lesions was classified according to Schubach et al. (2004).

For the cytopathological examination, collection was performed either by imprint in ulcerated lesions or by fine needle aspiration (FNA) in nodular lesions. The samples were air-dried, stained with Romanowsky dye (Diff-Quik), and examined under a 40× magnification optical microscope (Nikon Eclipse® E 200). In addition, the percentage for each cell type was determined. Then, the percentages of inflammatory cells were categorized as 0 (0%), 1 (≥1%<10%), 2 (≥10%<50%), 3 (≥50%<85%), and 4 (≥85%). At 1000× magnification, yeasts were classified as mild + (up to 5 yeasts per field), moderate ++ (from 6 to 50 yeasts per field) and accentuated +++(more than 50 yeasts per field) (Bazzi et al. 2016).

Samples for fungal culture were obtained using a sterile swab in ulcerated lesions or a FNA in nodular lesions. *Sporothrix* spp. were isolated and identified in Sabouraud Dextrose 2% Agar (BD, New Jersey, USA) and Mycosel® (BD, New Jersey, USA) medium at 25°C for 4 weeks. Membranous, flattened, pleated colonies ranging in color from white to cream or black were considered suggestive and subcultured in brain heart infusion (BHI) medium at 37°C to test for thermal dimorphism.

Samples for histopathology were collected using an 8-mm punch skin biopsy technique, under previous anesthesia with meperidine (3mg/kg IM) and propofol (4mg/kg IV). The fragments were fixed in 10% formalin. The analysis was carried out in sections (5µ) and using optical microscopy (400× and 1000×). Hematoxylin-eosin (HE) staining was used to classify and determine the intensity of the inflammatory response, while GMS staining was used to identify and quantify yeasts. Lesions were classified based on the following criteria: type of inflammation (granulomatous or nonspecific); organization of granulomas (well organized or poorly organized); cellular activation of the monocytic phagocytic system (macrophages or epithelioid cells); and amount of neutrophil, lymphocyte, and plasma cell infiltration performed in a 400× field representative of the lesion. The following categories were considered according to Gremião et al. (2015): absent, mild to moderate (between 1 and 30 cells), and accentuated (over 30 cells). Moreover, yeasts were classified according to Bazzi et al. (2016).

Information such as breed, sex, age, and rapid test for detecting FIV antibodies and FeLV antigens (ELISA, SNAP® Combo Plus, IDEXX) were analyzed.

Data from the study were subjected to descriptive statistical analysis, which was processed using the SAS program (Statistical Analysis System, Version 5, Microsoft®, 2001).

RESULTS

Most of the cats in the study were mixed breed (SRD) (n=10, 76.9%) and male (n=10, 76.9%) and had a mean age of five years. Thirteen (100%) of the animals were spayed. All animals were residents of the state of Rio de Janeiro and lived in a domiciled lifestyle but had a history of rescue at the onset of the disease (Table 1). Figure 1-4 (disseminated lesions) and Figure 5 (localized lesion) depict the two clinical presentations observed.

Good clinical condition was observed in nine (69.2%) animals, while four (30.8%) animals had the disseminated cutaneous form, which was associated with apathy, dehydration, and lymphadenitis. The four cats with the disseminated form of the disease and poor general condition were male. The most common extracutaneous clinical signs were sneezing and nasal discharge (53.8%), followed by conjunctivitis (38.5%) and inspiratory dyspnea (23.1%). In assessing the distribution of lesions, five (38.4%) cats were classified as L1, three (23.1%) as L2, and five (38.5%) as L3. The sites

Table 1. Information on breed, sex, age, lifestyle and reproductive status of cats with sporotrichosis which were refractory to treatment

Animal (no.)	Breed	Sex	Age (years)	Lifestyle	Neutering
1	Mixed	M	9	Domiciled	Yes
2	Mixed	M	6	Domiciled	Yes
3	Siamese	F	5	Domiciled	Yes
4	Siamese	M	5	Domiciled	Yes
5	Mixed	M	U	Domiciled	Yes
6	Mixed	M	4	Domiciled	Yes
7	Siamese	M	U	Domiciled	Yes
8	Mixed	M	U	Domiciled	Yes
9	Mixed	M	U	Domiciled	Yes
10	Mixed	F	4	Domiciled	Yes
11	Mixed	F	2	Domiciled	Yes
12	Mixed	M	3	Domiciled	Yes
13	Mixed	M	U	Domiciled	Yes

Mixed = Mixed breed, M = male, F = female, U = unknown.

Fig.1-4. Clinical evolution of a cat with nasal sporotrichosis refractory to treatment with itraconazole for a long period. (**1**) Presents clinical signs of ulcerated nasal lesion at diagnosis and initiation of treatment. (**2**) Clinical presentation of the lesion with reduction in size and reestablishment of the epidermis, leaving nodular granulomatous lesion. (**3**) Return of the lesion enlargement to a tumoral presentation and spread to other sites with nodular lesions in the edge of the pinna and periocular region. (**4**) Increase in the size of the nasal lesion, spread to other regions of the body and worsening of the general condition.

with the highest occurrence of skin lesions were the nasal planum (84.6%), limbs (53.8%), pinna (38.5%), and periocular region (23.1%). The most common skin lesions were ulcers and nodules, which were mostly located in the nasal region (84.6%). Of these 11 animals with nasal lesions, five (45.4%) had ulcers, three (27.3%) had nodules, two (18.2%) had tumors and ulcers, and one (9.1%) had a nodule and ulcer. Other patterns also observed were papules, erythema, crust, excoriation, and scar (Table 2). When compared to the other cats in the study, only one animal had a positive FIV serological test and showed no clinical and laboratory alterations.

In the cytopathological study, there was a higher occurrence of the type of pyogranulomatous inflammation. Four (30.8%) cats with disseminated sporotrichosis and poor general condition had marked yeast intensity (more than 50 yeasts per field), which was mostly phagocytosed by macrophages and neutrophils. Most of the cats with the most severe presentation of the disease had a predominance of neutrophil and macrophage cell types, with an absence or a slight percentage of epithelioid cells, lymphocytes, plasma cells, and eosinophils. In those cats with disseminated sporotrichosis, neutrophils were reported in category 4 (≥85%) in 75% of the cats, and 25% of the animals were in category 3 (≥50%<85%). Macrophages occurred in category 2 (≥10%<50%) in three (75%) cats and category 1 (≥1%<10%) in one (25%) cat. Epithelioid cells revealed category 2 in one (25%) cat, category 1 in two (50%) cats, and absence in one (25%) cat. Category 1 (≥1%<10%) lymphocytes were observed in the four (100%) cats with disseminated disease. Plasma cells presented category 1 (≥1%<10%) in two (50%) animals and absence (0) in two (50%) cats; and category 1 eosinophils (≥1%<10%) were present in only one (25%) cat.

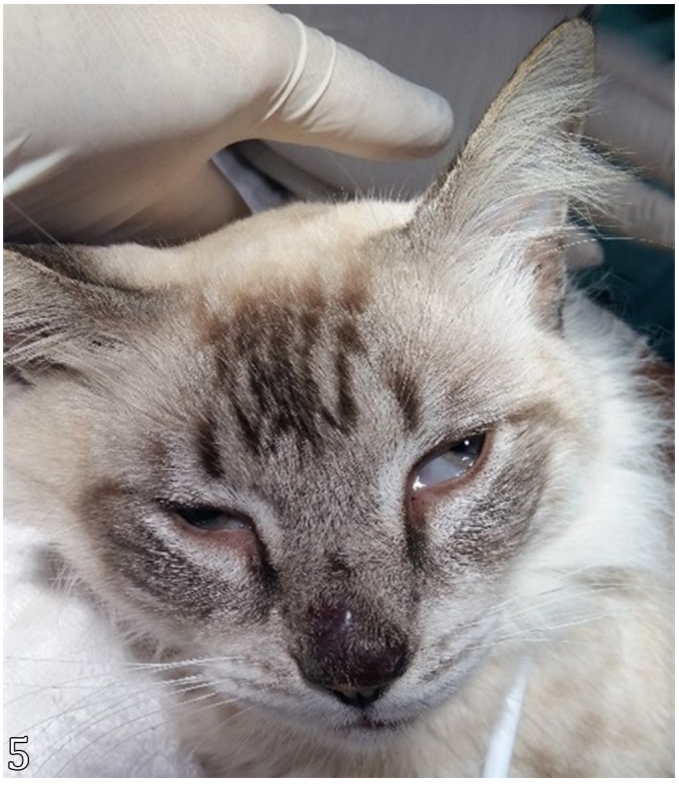

Fig.5. Nodular nasal lesion in a cat with feline sporotrichosis refractory to long-term treatment with itraconazole.

However, nine (69.2%) cats with good general condition and with one to two lesions showed absence or mild intensity of fungal structures (up to 5 yeasts per field). These yeasts had an opacified color, were predominantly extracellular, and were arranged in lumps. In the cytopathology of these cats, a lower percentage of macrophages and neutrophils and a higher percentage of epithelioid cells, lymphocytes, plasma cells, and eosinophils were observed. Of those animals with good general condition, the majority (n=6, 66.7%) had higher percentages of eosinophils (categories 1 and 2). Regarding the fungal structures in this group, four (44.4%) animals had no yeast (0), and five (55.6%) had mild intensity (up to 5 yeasts per field). Of the cats in this group, five (55.6%) animals revealed category 2 (≥10%<50%) for macrophages, while eight (88.9%) cats revealed category 3 (≥50%<85%) for neutrophils. Epithelioid cells had higher percentages in four (44.4%) of these felines, the majority (75%) having category 2. Five (55.6%) cats had lymphocytes for category 2 (≥10%<50%), and four (44.4%) cats had eosinophils in this category. Plasma cells were classified as category 1 (≥1%<10%) in six (66.6%) animals (Table 3).

In the study, the fungal agent was identified based on its morphological and tinctorial characteristics through histopathological diagnosis using GMS staining. Among these 13 study animals, eight (61.5%) cats had positive fungal culture results, while five (38.5%) cats had negative results. Of the five negative cases in the mycological culture, three (60%) animals had negative cytopathology, while two (40%) cats had mild intensity of fungal structures.

Histopathological examination of the 13 lesions revealed marked suppurative granulomatous inflammation located in the dermis. The pattern of the inflammatory infiltrate was distributed diffusely in eight cases (61.5%) and nodularly in five cases (38.5%). Most of the differentiation of mononuclear cells was from macrophages, with a marked distribution in

Table 2. Clinical characteristics of cats with sporotrichosis regarding location, distribution, type and number of lesions

Animal (no.)	Site	Type	Number
1	Nasal, pinna	Ulcer, papule, excoriation, crust, erythema	Multiple
2	PL	Ulcer, scab	1
3	Nasal	Nodule	1
4	Nasal, pinna	Ulcer	2
5	Nasal, pinna, PO, TL, PL, flank, tail	Ulcer	Multiple
6	TL, PL	Ulcer	2
7	Nasal	Nódulo	1
8	Nasal, pinna, PL, TL	Ulcer, scar, scab	Multiple
9	Nasal, TL	Ulcer	2
10	Nasal	Nodule, ulcer	1
11	Nasal	Nodule	1
12	Nasal, PO, TL, PL, tail	Nodule, tumor, ulcer, crust	Multiple
13	Nasal, pinna, PO, PL	Nodule, tumor, ulcer, crust	Multiple

PO = Periocular, TL = thoracic limb, PL = pelvic limb.

61.5% of cases, and epithelioid cells in 46.1% of cases. The neutrophil was the most frequently observed polymorphonuclear cell, with marked intensity in 84.6% of the cases.

Malformed suppurative granuloma was the predominant type (n=9, 69.2%), with four cases (30.8%) being well-formed granulomas. Malformed granulomas with mild to moderate fungal load (n=5, 55.6%) were observed in animals with good general condition and localized lesions (L1 and L2), whereas malformed granulomas with a marked fungal load (n=4, 44.4%) were reported in cats with the disseminated form of the disease and poor general condition. In this group of malformed granulomas with marked fungal load (100%) and poor general condition of the animal, they found a marked intensity of neutrophils (100%) and macrophages (100%), as well as mild to moderate presence of lymphocytes (100%) and plasma cells (75%) (Table 4).

Well-formed granulomas (n=4, 30.7%) presented mild to moderate intensity of fungal load. Most (n=3, 75%) animals with this type of granuloma had only one lesion (L1) and were in good general condition. Well-formed granulomas predominated, with a marked intensity of epithelioid cells (75%) forming well-defined aggregates and surrounded by lymphocytes (75%) and plasma cells (50%) (Fig. 6).

DISCUSSION

Cases refractory to itraconazole monotherapy have been described in Rio de Janeiro (Gremião et al. 2011, 2015, Silva et al. 2015). The cat's increased sensitivity to *Sporothrix* spp. may be related to immune response factors, the amount of innocuous, the depth of inoculation, and the fungus's virulence (Arrillaga-Moncrieff et al. 2009, Gremião et al. 2017, Terra et al. 2017). In the present study, cats with sporotrichosis that had been resistant to itraconazole treatment for more than a year were clinically evaluated, and their lesions were analyzed using a cytohistopathological comparison.

In the 13 cases of feline sporotrichosis refractory to long-term antifungal therapy, two clinical presentations had the highest occurrence: the localized cutaneous form (L1 and L2) in animals with good general condition and stable disease (n=9, 69.2%) and the disseminated cutaneous form in cats with poor general condition (n=4, 30.8%). Corroborating the findings of Miranda et al. (2013) discovered that all cats

Table 3. Distribution of cell types in the cytopathological examination of cats with refractory sporotrichosis

Cellular type	Grading of intensity*												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Macrophage	2	2	1	1	1	2	1	2	2	2	1	2	2
Epithelioids	2	2	0	0	0	3	1	0	0	2	1	1	1
Neutrophils	4	3	3	4	4	3	3	3	3	3	3	3	4
Lymphocytes	1	2	1	1	1	1	2	2	2	1	2	1	1
Plasmocyte	1	0	0	0	1	0	1	1	0	1	1	0	0
Eosinophil	0	0	2	0	0	0	1	2	2	1	2	0	1
Yeasts	+++	0	+	0	+++	+	+	+	0	0	+	+++	+++

*Grading of intensity of cell types: 0 (0%), 1 (≥1%<10%), 2 (≥10%< 50%), 3 (≥50%<85%) and 4 (≥85%); Yeasts were quantified as mild + (up to 5 yeasts per field), moderate ++ (from 6 to 50 yeasts per field) and accentuated +++ (above 50 yeasts per field) at 1000x magnification.

Table 4. Distribution of cell types in the histopathology examination of cats with refractory sporotrichosis

Cellular type	Intensity	Animals
Macrophage	Absent	
	Mild/moderate	3,6,7,8,10
	Accentuated	1,2,4,5,9,11,12,13
Epithelioid Cells	Absent	1
	Mild/moderate	2,3,4,5,7,13
	Accentuated	6,8,9,10,11,12
Neutrophils	Absent	
	Mild/moderate	3,9
	Accentuated	1,2,4,5,6,7,8,10,11,12,13
Lymphocytes	Absent	3
	Mild/moderate	1,4,5,6,7,8,9,11,12,13
	Accentuated	2,10
Plasmocytes	Absent	
	Mild/moderate	1,3,4,5,7,8,9,13
	Accentuated	2,6,10,11,12
Yeasts	Absent	
	Mild/moderate	2,3,4,6,7,8,10,11
	Accentuated	1,5,9,12,13

Graduation of intensity of cell types: Absent, mild/moderate (between 1 and 30cells), accentuated (over 30 cells); Yeasts were quantified as mild + (up to 5 yeasts per field), moderate ++ (from 6 to 50 yeasts per field) and accentuated +++ (above 50 yeasts per field) at 1000x magnification.

in groups L1 and L2 had good general condition. Gremião et al. (2009) linked the highest occurrence of therapeutic failure and poor prognosis to cats with nasal lesions of sporotrichosis, which corresponded to the large percentage (84.6%) of cats with refractory nasal lesions in our study. In all groups, male and mixed breed cats were predominant (n=10, 76.9%). According to the findings of Gremião et al. (2009), nodules and ulcers were the most common types of nasal lesion (n=10, 76.9%).

Cats (n=4, 30.8%) with disseminated sporotrichosis and a poor general condition showed marked intensity of yeasts, extracellular or phagocytosed by macrophages and neutrophils in cytopathology. The high fungal load may be an indicator of treatment failure (Silva et al. 2015). In these cats, with the most severe presentation, a moderate to accentuated number of neutrophils and macrophages were observed, as well as absence or slight percentage of epithelioid cells, lymphocytes, plasma cells, and eosinophils. The presence of a high number of monocytes and neutrophils may suggest a collaborative activity between Th1 and Th17 responses (Carnero et al. 2018). However, studies in mice show that *Sporothrix brasiliensis* causes longer-lasting infections with chronic systemic dissemination, as well as greater stimulation of Th17 and Treg responses, especially in the later stages of infection. The Treg response is induced to combat excessive inflammation, promotes the inhibition of Th1e activity of macrophage activation, and stimulates Th17 lymphocytes (Batista-Duharte et al. 2018). In these study cases, the high presence of macrophages and neutrophils, as well as the high intensity of yeasts in the chronic phase, may demonstrate an imbalance of the immune response, which still needs to be established in feline sporotrichosis.

However, most of the cats studied (n=9, 69.2%) had one to two lesions, were in good general condition, and revealed an absence or slight number of fungal structures in the cytopathology. These yeasts had an opacified color, were predominantly extracellular, and were arranged in lumps. The cytopathology of these cats revealed a lower intensity of macrophages and neutrophils, as well as a higher percentage of epithelioid cells, lymphocytes, plasma cells, and eosinophils. Of those animals with good general condition, the majority (n=6, 66.7%) had higher percentages of eosinophils (categories 1 and 2). To the best of our knowledge, no cytopathological study of sporotrichosis has found an increase in the occurrence of eosinophils in nasal lesions. However, after the eighth week of *Sporothrix* spp. infection, the Th2-type cell response tends to the growth and increase of interleukins IL-4, IL-6, IL-10, and IL-5, which induces the production and activation of eosinophils (Romani 2011).

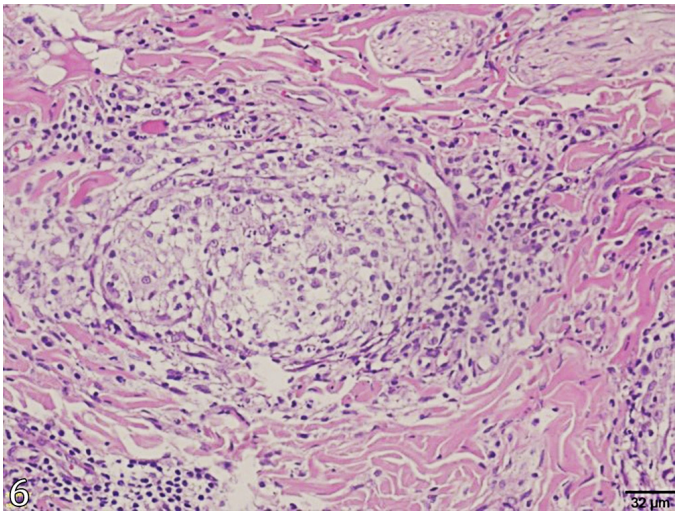

Fig.6. Biopsy of feline sporotrichosis lesion in the nasal region. Well-formed granuloma with lymphoplasmacytic infiltrate in the deep dermis. HE, obj.40x.

These cats were refractory to treatment and had stable disease, as evidenced by few lesions and good general condition, but they had a low fungal load, making cytopathological diagnosis difficult. Silva et al. (2015) reported a reduction in fungal burden in cats after 12 weeks of itraconazole treatment, which was accompanied by a decrease in fungal culture positivity. The author recommends combining cytopathology, histopathology, and immunohistochemistry to improve diagnostic sensitivity.

Discordant results in mycological culture and cytopathology highlights the complexities of diagnosing feline sporotrichosis that is resistant to treatment. Itraconazole 100mg/day, regardless of treatment duration, reduces the sensitivity of cytopathology and fungal culture (Macêdo-Sales et al. 2018). The GMS allowed for the diagnosis of inconclusive cases in cytopathology and fungal culture, where lesions with a low fungal load made identifying the fungal agent difficult. Although fungal culture is the gold standard method for diagnosing sporotrichosis (Schubach et al. 2004), in cats, fungal organisms are considered highly characteristic in tissues, and histopathology is a viable and faster option for diagnosing this mycosis in cats, especially when fungal isolation is not possible (Pereira et al. 2011, Gremião et al. 2015).

Corroborating with Miranda et al. (2013), the lesions found in this study were suppurative granulomatous. Malformed suppurative granuloma was the most common type, accounting for 69.2% of all granulomas, while 30.8% were well-formed granulomas. Previous studies found a higher frequency of well-formed granulomas as well as better control in refractory cases (Miranda et al. 2013, Gremião et al. 2015).

Malformed granulomas showed a mild to moderate fungal load in animals with good general condition and localized lesions (L1 and L2), and a marked fungal load in cats with the disseminated form of the disease and poor general condition. Predominance of neutrophils (53.8%) and macrophages (53.8%) and mild to moderate presence of lymphocytes (53.8%) and plasma cells (46.1%) were the main characteristics of malformed granulomas. The higher occurrence of malformed granulomas in the refractory cases reported here, especially in these animals with long treatment times, may suggest that the stability acquired in these chronic nasal lesions may be lost due to immunological factors of the host, consistency of the stimulus developed by the fungus, and irregularity in treatment. Such changes may allow the resumption of multiplication of *Sporothrix*'s yeast, which, despite stimulating an immune response with an increase in macrophages, neutrophils, and phagocytosis in the granulomatous lesion (Carnero et al. 2018), appears to be exuberant and ineffective in refractory feline sporotrichosis lesions with disseminated lesions, since yeasts show large growth.

Well-formed granulomas (n=4, 30.7%) had mild to moderate intensity of fungal load. Most (n=3; 75%) animals with this type of granuloma had only one lesion (L1) and were in good general condition. Well-formed granulomas predominated, with a marked intensity of epithelioid cells (75%) forming well-defined aggregates and surrounded by lymphocytes and plasma cells, correlating with previous findings by (Miranda et al. 2013, Gremião et al. 2015). These findings suggest that a lower fungal load is associated with the presence of well-formed granulomas, suggesting that infection is controlled by an adequate immune response, leading to better control of the lesion; in addition, the antifungal may have contributed to a lower parasite load and a more effective immune response.

CONCLUSION

Given the above findings, it is reasonable to conclude that the two clinical presentations observed, as well as the cytohistopathological alterations, show that cats with localized refractory nasal lesions and good general condition have a low fungal load and a large number of epithelioid cells in a well-formed granuloma, providing stability during the illness. However, refractory cats with nasal lesions, after a long time of treatment, and a poor general condition with disseminated lesions demonstrate a high fungal load in malformed granuloma, indicating an exuberant and inefficient pro-inflammatory immune response.

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