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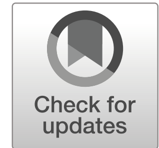

VETERINARY MICROBIOLOGY - RESEARCH PAPER

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Promising application of the SsCBF ELISA test to monitor the therapeutic response of feline sporotrichosis caused by *Sporothrix brasiliensis* from Brazilian epidemics

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Abstract

Sporotrichosis zoonotic transmission by cats has obtained hyperendemic magnitude in Rio de Janeiro, Brazil. Atypical cases, relapses, and reinfections as well as reduced diagnostic sensitivity of conventional methods have been reported. Previously, the anti-SsCBF enzyme-linked immunosorbent assay (ELISA) test was shown to be useful as a diagnostic tool for human sporotrichosis. Effective diagnosis and treatment are critical to interrupt the chain of transmission of this major pathogen in Brazilian Public Health. To evaluate its applicability for feline sporotrichosis diagnosis and/or therapeutic follow-up, 15 domestic cats from Rio de Janeiro were clinically and laboratory monitored by cytopathology, culture, *Sporothrix* genotyping, and anti-SsCBF IgG levels. Subsequently, animals were divided into satisfactory and non-satisfactory therapeutic responders. Averages of antibody serum levels obtained for diagnosis (first consultation) compared with the levels found after follow-up (last consultation) were significantly different in both groups ($p = 0.0002$ and $p = 0.038$, respectively). We conclude that the SsCBF ELISA test can predict feline sporotrichosis therapeutic responses even for animals with distinct clinical evolutions.

Keywords Antifungal treatment · *Felis catus* · Serology · Sporothichosis · *Sporothrix brasiliensis* · Zoonoses

Introduction

In the last decades, the zoonotic transmission of sporotrichosis by *Felis catus* has assumed, in the state of Rio de Janeiro, Brazil, a hyperendemic magnitude. Recently, a geographical expansion of its etiological agent, *Sporothrix brasiliensis* [1], to other states of the country has been reported with both human and animal cases [2, 3]. Furthermore, *S. brasiliensis* has been reported as the most virulent species [4–7].

Routine laboratory procedures for the diagnostic investigation of sporotrichosis in human and animal hosts, as well as the respective therapeutic protocols, are relatively well established [8]. However, the disease still affects both hosts resulting in alarming epidemiological data [3]. The laboratory diagnosis of the disease in felines begins with the cytology by imprint of the lesions, followed by isolation of the agent in culture. The latter is considered the gold standard [9]. Other methods, such as those based on investigation of serum antigens and antibodies, have been studied since the 1970s [10, 11], although none involved a laboratory routine or proved to be clinically validated.

Recently, the epidemic background of sporotrichosis has prompted the implementation of qualitative and quantitative, fast, sensitive, and specific diagnostic tests, based on immunochemical and/or molecular techniques [12–17]. Likewise,

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in 2015, a new test was validated for the serological diagnosis of human sporotrichosis [18], capable of diagnosing atypical cases, relapses, and reinfections. Nevertheless, such clinical adverse outcomes are even more frequently described for the feline host, in part due to the inadequate therapeutics and intermittent or abandonment of treatment. Altogether, these factors may reduce the diagnostic sensitivity of the conventional methods [19–21].

This new approach is based on the enzyme-linked immunosorbent assay (ELISA) with the SsCBF antigen purified from the cell wall of *S. schenckii* [18, 22]. The anti-SsCBF ELISA is a useful laboratorial resource for the diagnosis and therapeutic monitoring of this mycosis in human patients, including those that developed the clinically atypical and severe forms that are difficult to treat. It is also sensitive enough to monitor relapsing episodes [21, 23–25]. This test was also used in a preliminary study of feline sporotrichosis diagnosis without, however, establishing the etiological agent involved or its use in a therapeutic follow-up [13].

The aim of the present work was to perform a case-by-case clinical–serological study, including the molecular determination of the feline sporotrichosis etiological agent. In addition, clinical-diagnostic routines are proposed and evaluated complementary to the conventional methods. The study included healthy and sick felines from a high endemic area previously not studied in the state of Rio de Janeiro. To the best of our knowledge, this is the first study to investigate the SsCBF-based ELISA as a potential tool for the follow-up of feline sporotrichosis therapy.

Methodology

Ethical aspects and inclusion criteria

This study was approved and conducted according to the norms of the Ethics Committee on Animal Use from the Federal Fluminense University (CEUA-UFF, protocol numbers 208/2012 and 7561040518/2018). The owners were informed of the objectives and methodology of the study and were asked to sign an informed consent form and answer questions related to the epidemiological variables. The animals were included in the study regardless of breed, age, or gender, and the study was conducted over 12 months (August 2015–2016).

The animals were selected from those assisted by the project entitled “Integrated actions for the prevention and control of the animal sporotrichosis” in the Reference Unit for the Diagnosis of Animal Sporotrichosis,” associated to the Center for Microorganisms Investigation of the Biomedical Institute of the Universidade Federal Fluminense and to the Niterói City Hall, PMN, Niterói, RJ, as long as clinical reviews were performed in, at least, two return visits from 2015 to 2017. The animals included were felines from the municipalities of Niterói and São Gonçalo, belonging to the Metropolitan Region II or Leste Fluminense, a hyperendemic area for the zoonotic transmission of sporotrichosis [20].

All animals, clinically assisted by the veterinarian staff, were evaluated for gender, age, castration, and contact with soil/plants and other domestic animals. All data were registered on a standardized data collection form.

Clinical evaluation

To determine the severity of sporotrichosis, the criteria used were modified from those established by Miranda and colleagues [26]. For this purpose, clinical parameters such as apathy, weight loss, lack of appetite, ocular secretion, epistaxis, respiratory distress, nasal secretion, sneezing, and hyporexia were associated with the clinical aspects, including lesion number, size, and location. Consequently, the felines were classified as (Supplementary material) follows: (i) “mild” clinical presentation, for those with a good general clinical condition and carriers of one or two lesions up to 5 cm in diameter, in any part of the body, except in the nasal area, or (ii) “severe” clinical presentation, for those with clinical parameters indicative of systemic impairment, with three or more lesions, equal to or larger than 5 cm in diameter, and/or with nasal lesions and/or breathing impairment. To determine the body condition score, the outline of the animal was analyzed as well as palpation performed, to correlate subcutaneous and abdominal fat and the superficial musculature. This resulted in categories that varied from cachectic to obese, with scores from 1 to 5, according to Ettinger and Feeldman [27].

The therapeutic protocol adopted was modified from Reis et al. [28]. The felines were classified as follows: (i) Mild were treated with the itraconazole, at 10 mg/kg for those up to 3 kg with a maximum dose of 100 mg/cat for those weighting above 3 kg (ii) and those with the severe form were treated with itraconazole according to the former protocol, together with orally administered potassium iodide at 2.5 to 5 mg/kg/day.

Cytological and mycological diagnostics

Biological samples were collected based on the clinical conditions, type of lesion, and availability at the time of the study, consisting of a swab from the lesion exudate and from the oral/nasal cavities. The cytopathological analysis was conducted by impression smears of the skin lesion prepared on glass slides, subsequently, stained by the quick panoptic method (Laborclin, Pinhais, PR, Brazil), a Romanowsky-type stain. The slides were analyzed by light microscopy for the identification of yeast-like structures suggestive of *Sporothrix* spp. The swabs (from the lesion exudates and oral/nasal cavities) were seeded onto Sabouraud dextrose agar 2% (BD, Franklin

Lakes, NJ, USA) and Mycosel® (BD, Franklin Lakes, NJ, USA), incubated at room temperature and observed over 4 weeks. Colonies suggestive of the *Sporothrix* genus were subcultivated on Sabouraud agar 2% dextrose (BD, Franklin Lakes, NJ, USA) at room temperature, for colony isolation, as previously described [29]. The fungi were initially identified by macroscropic and microscopic characteristics and then, subcultured in brain–heart infusion agar (BHI; BD, Franklin Lakes, NJ, USA) at 37 °C for conversion to the yeast stage of *Sporothrix*.

Molecular identification of Sporothrix

All isolates ($n = 15$) were submitted to molecular identification according to a species-specific PCR targeting the calmodulin gene (CAL) [16]. Briefly, fungal cells were recovered from 10-day colonies and used for genomic DNA extraction with the Fast DNA kit (MPBiomedicals, Vista, CA, USA), according to the manufacturer's protocol. The DNA extract concentration was estimated with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and stored at − 20 °C until use. For PCR, a 25-μL reaction mix consisted of 12.5 μL PCR Master Mix buffer (2X), composed of 3 mM MgCl2, 400 mM each dNTPs, and 50 U/mL TaqPolymerase (Promega Corporation, Madison, WI, USA); 9.5 μL water; 1 μL each of forward and reverse primers (10 pmol/μL; Integrated DNA Technologies, USA); and 1 μL of target DNA (100 ng/μL), using the touchdown PCR method on a Eppendorf Mastercycler Pro machine (Eppendorf, Hamburg, Germany). The resulting PCR amplicons were separated and visualized on 1.2% agarose gel electrophoresis for 1 h at 100 V in the presence of GelRed (Biotium, Hayward, CA, USA). The L-Pix Touch (Loccus Biotecnologia, São Paulo, Brazil) imaging system was used to visualize the stained bands under UV light.

SsCBF ELISA

This test was previously validated by Fernandes et al. (2011) [13] with sensitivity and specificity of 90% and 96%, respectively. The positive predictive value was 93%, while negative predictive value was 94%, with global efficiency of 93%. The immunoglobulin G (IgG) titration was determined by ELISA with the SsCBF antigen (*Sporothrix schenckii* ConA Binding Fraction), as previously described by Penha and Bezerra [22], with slight adaptations to feline samples, as described below.

One to three milliliter of peripheral blood was collected, per cat, preferentially from the left or right femoral or the jugular veins. Serum was separated by centrifugation at 2428×*g* and stored at − 20 °C, for serological evaluation. The optimal concentration of the antigen and of the secondary antibody and the feline anti-IgG HRP (Mybiosource, San Diego, California, USA) were determined by testing a serum pool from healthy animals as a control and a serum pool from animals diagnosed with sporotrichosis by the mycological test (gold standard). Briefly, serial dilutions from 1:2 were performed of the feline serum, starting with the 1:100 dilution. High binding ELISA microtiter plates were sensitized with the SsCBF antigen (250 pg/mL in 0.1 M carbonate–bicarbonate buffer pH 9.6) for 2 h at 37 °C. After a blocking step, the plates were washed three times with PBS + 0.05% Tween 20 (PBS-T) and incubated with the diluted feline sera for 2 h at 37 °C. After washing with PBS-T, the plates were incubated with an anti-feline IgG HRP conjugate (1:20,000 in PBS-T) [13]. The plates were developed with the substrate (0.25 ng/mlO-phenylenediamine and 0.006% of H_2O_2 in citrate–phosphate buffer, pH 5.6) for 20 min. The reaction was stopped with 3 M H_2SO_4, and the absorbance was determined at 492 nm (A492) in an ELISA reader. The cutoff (A492 = 0.230) was established by calculating the mean plus two times the standard deviation of the absorbance values of the serum samples from the healthy felines. Based on the cutoff at 1:400 dilution a positive reaction was determined (Table 1).

Statistical analysis

Data were processed and analyzed with the aid of the GraphPad Prism Software, version 6.0. The comparison of the means between different groups was performed using Student unpaired *t* test and the Pearson correlation coefficient. To homogenize the OD data obtained in the different experiments, the reactivity index (RI) was calculated for each sample by dividing the mean OD of the sample tested by the OD cut-off value of each experiment. Samples with RI > 1.0 were considered positive. For all tests, the level of significance was set at $p < 0.05$.

Results

ELISA standardization

The results showed that 250 pg/mL of the SsCBF antigen was the optimum concentration to detect anti-SsCBF IgG antibodies in the pooled serum of cats infected with *S. brasiliensis* ($n = 10$; Fig. 1a) Control sera ($n = 10$) provided negative reactivity in the same assay conditions (Fig. 1b). These results indicate that SsCBF ELISA was useful for diagnosing feline sporotrichosis caused by *S. brasiliensis*, since high serum reactivity was observed at 1:400 dilution (Fig. 1b). Based on these data, 1:400 was established as the minimum discriminative point to distinguish between healthy and sick animals using, as a cutoff point, the absorbance corresponding to the mean plus two times the SD (A492 = 0.230) of the healthy animal serum pool.

Table 1 Distribution of 15 domestic felines with sporotrichosis according to the laboratory results by conventional methods, severity of the lesions, sporotrichosis evolution, and clinical-serological follow-up

Animal (Groups)	Cytopathology	Culture	Sporotrichosis	Clinical and serological follow-up	
				Pro-diagnose/Therapeutic response	ELISA
HO-064 (STR)[a]	−	+	Severe	Pro-diagnose	1:600
				Last follow-up	1:800
HO-095 (STR)	+	+	Severe	Pro-diagnose	1:800
				Follow-up no. 1	Borderline
				Last follow-up	1:400
HO-097 (STR)	+	+	Severe	Pro-diagnose	1:3200
				Follow-up no. 1	Borderline
HO-276 (STR)[a]	−	+	Severe	Pro-diagnose	1:3200
				Last follow-up	1:6400
HO-132 (STR)	+	+	Severe	Pro-diagnose	1:3200
				Follow-up no. 1	Borderline
				Follow-up no. 2	1:3200
				Last follow-up	1:3200
HO-317 (STR)	+	+	Severe	Pro-diagnose	1:400
				Last follow-up	1:600
HO-151 (STR)	+	+	Severe	Pro-diagnose	1:800
				Follow-up no. 1	1:800
				Last follow-up	1:1600
HO-210 (STR)[b]	+	+	Severe	Pro-diagnose	1:6400
				Follow-up no. 1	–
				Follow-up no. 2	1:800
				Last follow-up	1:400
HO-265 (STR)	+	+	Severe	Pro-diagnose	1:1600
				Follow-up no. 1	1:1600
				Last follow-up	1:800
HO-116 (STR)[c]	+	+	Severe	Pro-diagnose	1:3200
				Last follow-up (Clinical cure)	1:600
HO-321 (STR)	+	+	Mild	Pro-diagnose	1:3200
				Last follow-up	1:1600
HO-211 (NSTR)	+	+	Mild	Pro-diagnose	1:800
				Last follow-up (Relapse)	1:6400
HO-239 (NSTR)[a,d]	−	+	Severe	Pro-diagnose	1:1600
				Follow-up no. 1	1:1600
				Last follow-up (aggravation of the nasal lesion)	1:12800
HO-244 (NSTR)[a,e]	+	+	Mild to severe	Pro-diagnose (under ATM subdose)	-
				Follow-up no. 1	Borderline
				Follow-up no. 2 (Nasal involvement)	1:25600
				Follow-up no. 3	1:25600
				Last follow-up	1:25600
HO-082 (NSTR)[e]	+	+	Mild to severe	Pro-diagnose	1:6400
				Follow-up no. 1 (treatment interruption)	1:12800
				Follow-up no. 2	1:6400
				Last follow-up (treatment Interruption)	1:25600

ELISA: (−) negative

STR clinical-therapeutic evolution defined as satisfactory therapeutic response, *NSTR* clinical-therapeutic evolution defined as non-satisfactory therapeutic response

[a] These cats were included in the present study under previously prescribed antifungal drug treatment, with different presentations (topical and/or systemic) and dose

[b] Figure 3a, c, and e

[c] Clinical cure determined by complete sporotrichosis lesion healing followed by 3 months under therapy and another 6-month period in the absence of antifungal drugs

[d] Figure 3b, d, and f

[e] These animals were first clinically evaluated as carriers of "Mild" sporotrichosis but during their follow-up evolved to a "severe" form of the disease

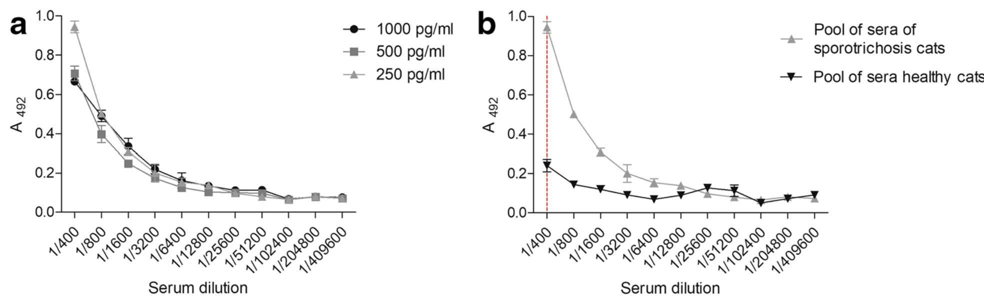

Fig. 1 Reactivity of the pool of sera from healthy and diseased cats with sporotrichosis tested with the ELISA SsCBF-based test. **a** The SsCBF antigen concentrations, ranging from 250 to 1000 pg/mL, were tested with a pool of sera from cats with laboratory-confirmed sporotrichosis ($n = 10$; gold standard). **b** Comparison between the reactivity for the pool of sera from healthy and sporotrichosis diagnosed cats using the optimum antigen concentration (250 pg/mL). The red dotted line indicates the cutoff serum dilution (1/400)

Clinical–therapeutic–serological follow-up

All the animals had sporotrichosis confirmed by isolation of *Sporothrix* spp. in mycological culture and later genotyped as *S. brasiliensis*. The results revealed that the SsCBF-based ELISA was able to diagnose the mycosis at the first visit in UDEA (pro-diagnose), animals with sporotrichosis caused by *S. brasiliensis*, even when one of the classical methods was negative and/or the feline was receiving antifungal therapy at the point of inclusion in the study. In contrast, the serum from only one animal (HO-244) gave a negative serological result while it was positive by both cytology and culture. This animal was included in the present study under a previously prescribed therapeutic protocol with a monoazolic subdose. It subsequently presented a worsening of the clinical condition, as recorded by the simultaneous increase in the titration of the anti-SsCBF IgG.

Since the SsCBF-based ELISA discriminated between sick and healthy animals, this method was analyzed as a follow-up serological tool for clinical–therapeutic response monitoring. All animals in the present study were clinically followed-up, whenever possible, from admittance ("pro-diagnose") until discharge ("last follow-up"), with a minimum of two and a maximum of five samples collected. According to Table 1, among the 15 felines analyzed, 13 presented the severe form of sporotrichosis, while two were classified as carriers of the mild form of the disease. Regardless of the clinical severity, established in the first visit, the clinical–therapeutic evolution of the felines was different, allowing their division into two other groups, defined as satisfactory therapeutic response (STR group ($n = 11$)) and non-satisfactory therapeutic response (NSTR ($n = 4$)).

STR group

Most of the STR group was formed by male cats (72.7%), half of them neutered, with ages varying from 9 months to 10 years (mean = 4.67; SD ± 3.13 years). Almost all were classified with severe sporotrichosis, with a single cat presenting the mild form (HO-321) of the disease. During the therapeutic follow-up, the average time between the first and the last visits, with their respective serological analyses, was 8 months. The arithmetic mean of serum antibodies in the first visit (pro-diagnose), determined according to the readings at A492 (OD), was 2.636 (SD = ± 1722.37), while for the last follow-up (either during or post-treatment or discharge; Table 1) the mean was 1.800 (SD = 2316.89). Most cats had high IgG antibody titers in pro-diagnose, with a strong correlation between the serological result and sporotrichosis clinical evolution. As shown in Fig. 2a, anti-SsCBF IgG serum levels obtained in the pro-diagnose differed significantly ($p = 0.0002$) to those detected in the last follow-up, within the STR group. Figure 3 shows the feline HO-210, with severe sporotrichosis, presenting disseminated lesions in the first visit (Fig. 3a) and

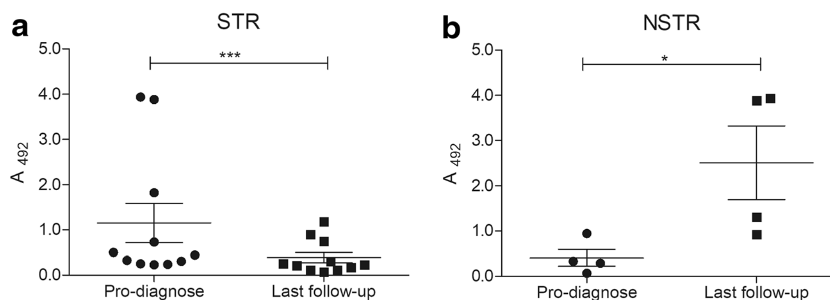

Fig. 2 **a** Comparative chart of the IgG anti-SsCBF (A 492) reactivity in the sera of 11 animals with a satisfactory therapeutic response (STR), between the pro-diagnose and last follow-up periods (***$p = 0.0002$). **b** Comparative chart of the IgG anti-SsCBF reactivity (A 492) in the sera of four animals with non-satisfactory therapeutic response (NSTR), between the pro-diagnose and last follow-up periods (*$p = 0.0380$)

after clinical healing (Fig. 3c). Figure 3e displays the therapeutic follow-up chart, showing an important marked reduction in the IgG titers between the pro-diagnose and the last follow-up.

STR Group case report

In this STR group, an undefined breed (UDB) HO-210 feline is highlighted as a neutered male, 2 years old, presenting a 1-week evolution of severe sporotrichosis in a clinical cutaneous disseminated form with a nasal lesion and respiratory signs (sneezing), along with many ulcerated lesions (Score 2) in the dorsum, and in the pelvic and thoracic limbs (Figs. 3a), and without former treatment. The laboratory tests showed positive results for cytology and culture, and a positive serological pro-diagnose result (IgG titer 6400). During the therapeutic follow-up a marked reduction in the IgG titer was observed until clinical cure (Table 1), which occurred after 9 months of treatment, with complete healing of all lesions (IgG titer 400; Fig. 3c and e).

NSTR group

The NSTR group ($n = 4$) was formed by two females with a neutered Siamese and the other an unneutered UDB, both with Severe sporotrichosis, and two UDB neutered males, with mild and severe sporotrichosis. The ages ranged from 4 months to 12 years (mean = 3.81; SD ± 5.05). The average time between the first and the last visit to UDEA, with their respective serological analyses, was 9 months. The arithmetic mean of antibodies in the pro-diagnose, expressed according to the readings at A492 (OD), was of 2.050 (SD = ± 2.904), while for the last follow-up it was 17,600 (SD = ± 9.600). The average of the IgG titers obtained in the ELISA pro-diagnose tests (A492) that was compared with the average obtained for the last follow-up sera (with at least three and a maximum of

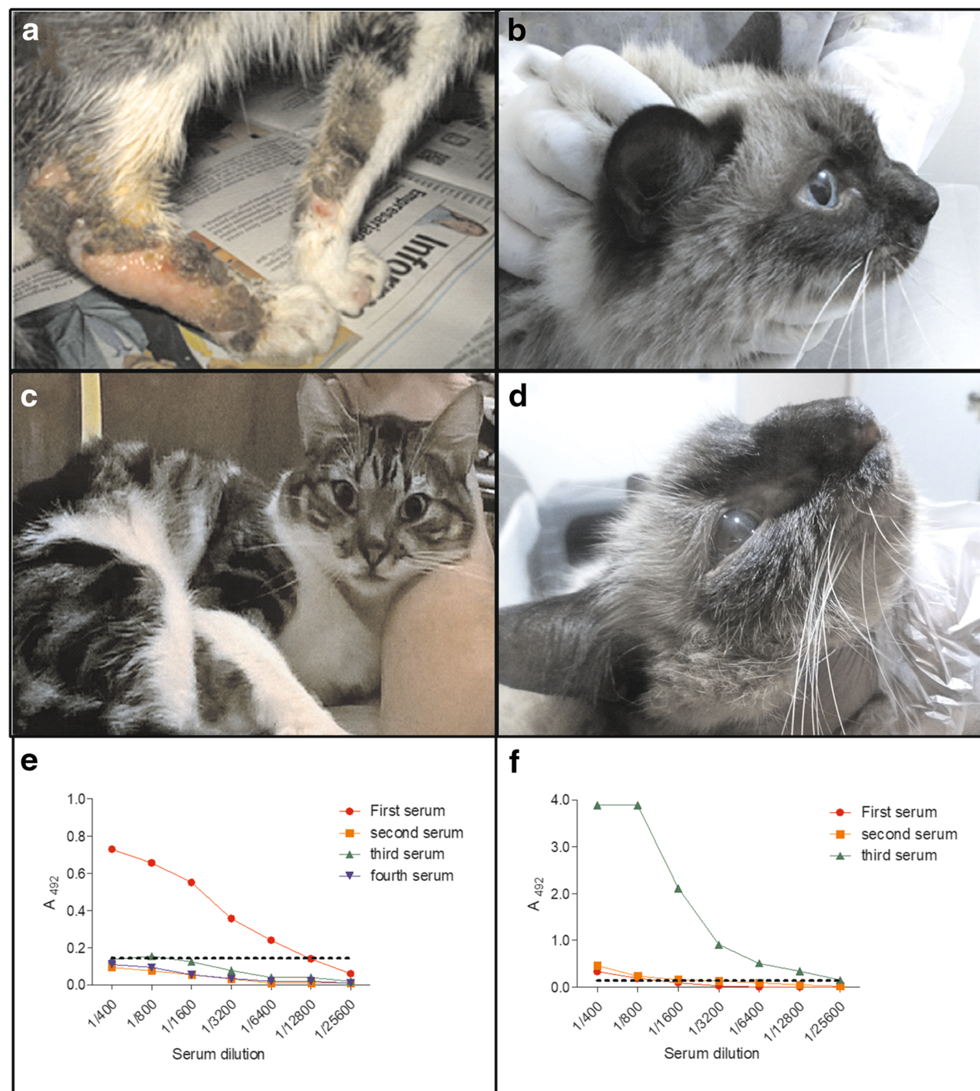

Fig. 3 STR group: **a** Feline HO-210, with Severe sporotrichosis, presenting disseminated lesions in the first visit and **c** after clinical healing. **e** Therapeutic follow-up chart, showing marked reduction in the IgG titers between the pro-diagnose and the last follow-up. **b** Feline HO-239 from the NSTR group with Severe sporotrichosis, exhibiting nasal swelling in the first visit (Pro-diagnose) with improvement after 60 days (Follow-up no. 1) and **d** major aggravation of the nasal lesion after 6 months (Follow-up no. 2;), as indicated by the elevated IgG after the third therapeutic follow-up (**f**). The dotted line indicates the cut-off point

41 months of treatment) allowed the detection of a higher serum levels of anti-SsCBF IgG in the latter, corresponding to a non-satisfactory clinical evolution ($p = 0.0380$; Fig. 2b).

NSTR group case reports

Among the felines of the NSTR group, HO-211 was classified as carrier of a mild form of this zoonosis, with a single lesion, located in the inguino-scrotal region. This was first detected 10 days after castration at the clinical evaluation of the animal's first visit to UDEA. In pro-diagnose, the anti-SsCBF ELISA showed a positive result (IgG titer 800). According to the owner, the cat rubbed the surgical wound daily in the garden soil, which was also accessed by other animals with sporotrichosis. The treatment protocol was implemented, accompanied by isolation of the feline. However, in the follow-up visit, the serum antibodies titer was higher (IgG titer 6400), corresponding to worsening of the inguino-scrotal lesion. However, sometimes the animal was not confined and therefore frequently exposed to the garden soil. The animal is still being followed-up since the lesion has not healed.

Domestic feline HO-239, a Siamese neutered female of 12 years old, presented to UDEA with severe sporotrichosis, following a 10-year unsuccessful treatment with a sub-dose of azolic monotherapy (20–60 mg/cat/day of itraconazole; Fig. 3b). There was serious nasal swelling and severe respiratory impairment (Score 1). The cytopathology was negative, while culturing as well as pro-diagnose ELISA (IgG titer 1600), were positive. The therapeutic protocol was adopted (refer to methods), with an adjustment of the azole dose in combination with potassium iodide. A favorable therapeutic response was observed after 2 months, with the reduction of the nasal swelling and improved respiration, Score 3, and maintenance of the previous serum antibody titers (Table 1, Follow-up no. 1). Unfortunately during the 6-month clinical evaluation a relapse of the nasal involvement was detected, even more severe than the first visit, along with respiratory impairment and regression to Score 1 (Fig. 3d). As shown in Table 1, “Last follow-up,” there was a concomitant and corresponding increase in the titration of anti-SsCBF IgG (IgG titer 12,800; Fig. 3f). The animal remains under observation by the UDEA veterinarian team.

Finally, animal HO-082 presented serological positive peaks with different IgG titers during treatment corresponding to episodes of intermittent therapeutic abandonment. This UDB neutered male feline, 2 years old, presented untreated mild sporotrichosis in the first visit to UDEA, with lesions in the cephalic region and in the thoracic limbs. The standardized therapeutic protocol was prescribed, and positive laboratory results were obtained by cytology and culture, with isolation of *S. brasiliensis*, along with a corresponding anti-SsCBF serology (IgG titer 6400). After 9 months, the owner reported two interruptions in the antifungal therapy, with a major recrudescence of the lesions (number and size) correlated with an increase in serum IgG levels (IgG titer 12.800; Table 1, Follow-up no. 1). The feline was therefore reclassified as carrier of a severe sporotrichosis. Seventeen months later, with the respective therapeutic readjustment, the animal showed a significant clinical improvement, along with the reduction in the serum antibody titers (IgG titer 6400; Table 1, Follow-up no. 2). However, after a further 3 months, a new blood sample revealed the highest IgG titer (IgG titer 25,600; Table 1, Last follow-up). Again, the owner confirmed the abandonment of the intended therapy with evident clinical aggravation of the pre-existing lesions. Therefore, the levels of the anti-SsCBF IgG were variable, although always corresponding to episodes of therapy interruption.

Discussion

In the sporotrichosis epidemic via zoonotic transmission in Brazil, distinct clinical forms, as well as unique therapeutic responses for the feline [28, 30] and the human hosts, have been described [21, 31–33]. These differ from the profile observed at the beginning of the 1990s when the outbreak was first described [34] so that alternative/complementary fast, sensitive, and specific diagnostic methods of investigation are required. Once standardized and validated, such methods might enable the laboratory confirmation of the clinical suspicion, crucial for commencement of treatment [35]. Equally important is the ability to perform therapeutic follow-up, since one of the major challenges of sporotrichosis treatment is the identification of the appropriate time for interruption, continuation, or adequacy of the adopted therapy for the feline patient. To the best of our knowledge, this is the first report showing promising data on the application of the SsCBF ELISA to both diagnose *S. brasiliensis* feline infection and to monitor the respective therapeutic follow-up.

In 2005, Bernardes-Engemmann and colleagues [12] validated the anti-SsCBF ELISA method as a tool capable of diagnosing human sporotrichosis regardless of the sex or age of the host, or clinical form of the disease, with a high sensitivity and specificity, as well as reproducibility. The applicability of the ELISA SsCBF-test for the diagnosis of feline sporotrichosis was previously investigated [13] in a single study, which again confirmed its efficacy after comparing this antigen to the results obtained with a crude exoantigen. In the present study, the ELISA with the SsCBF antigen detected distinct clinical evolutions, characterized by satisfactory and non-satisfactory responses. In addition, the clinical instability resulting from therapeutic interruption, in mild and severe cases, as well as during the term of both therapeutic protocols adopted, was described. Furthermore, this ELISA was sensitive at pro-diagnose, independently from concomitant or former antifungal therapy during the admission in this study, or

even from the laboratory results obtained by conventional methods (cytology and culture). As expected, all animals were infected by *S. brasiliensis*, since this species is the main actor in the Brazilian epidemic scenario [1, 3, 20].

All the domestic felines from the STR group presented, in pro-diagnose, positive results both by the gold standard method and by ELISA. Further, the serological method showed proportionally decreasing titers of anti-SsCBF antibodies confirming the satisfactory evolution evidenced by the clinical improvement, regardless of the sporotrichosis severity. Also, domestic felines from the NSTR group showed that IgG anti-SsCBF titers reflected the good or poor therapeutic responses to the adopted protocols. Nevertheless, a limitation the authors acknowledge is the fact that the number of samples for these conclusions is still slight, especially in the group NSTR.

An important limitation of this study was the inability to identify a serological scar as an indicator of sporotrichosis cure, since most of the domestic felines were not followed until the establishment of a clinical cure. This resulted from the reluctance of many tutors to provide specialized clinical care after animal healing. Nevertheless, among the successful returns to the outpatient clinic one female domestic feline was highlighted with a severe form of the disease and excellent therapeutic response, which were followed until the sixth month after antifungal therapy (HO-116). In the last follow-up, blood was collected, and the ELISA showed an IgG antibody titer of 600. Future research with a higher number of animals, followed by determination of ELISA anti-SsCBF titers after clinical cure, will confirm whether this titer is the serological scar or if it represents a high titer, resulting from continuous exposure to *S. brasiliensis* in its environmental saprophytic phase. This last hypothesis is supported by the fact that this cat lives with other felines previously treated for sporotrichosis, all in the same garden.

Lastly, although there is a good correlation between therapeutic response and anti-SsCBF IgG titers, each animal shows a particular profile of antibody decrease during sporotrichosis therapy, probably due to *S. brasiliensis* isolate-related virulence or to the distinct feline immune response to this fungus [7] and/or particular drug pharmacodynamics.

In conclusion, the SsCBF ELISA shows potential to be an important tool not only for feline sporotrichosis diagnosis but, more importantly, to predict the therapeutic response. The efficient diagnosis and treatment of feline sporotrichosis are critical to break the chain of transmission of this disease, a major problem in Brazilian Public Health. Once validated by subsequent studies, such a method will contribute to the establishment of safer clinical healing, with a consequent reduction of therapeutic failure and relapses, both very common in the current epidemic scenario of this zoonosis.

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Conflict of interest The authors declare that they have no conflict of interest.

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