Diagnostic accuracy assessment of cytopathological examination of feline sporotrichosis

Jessica N. Silva - DVM, MSc1,2,*, Sonia R. L. Passos - MD, PhD1, Rodrigo C. Menezes - DVM, PhD1, Isabella D. F. Gremião - DVM, PhD1, Tânia M.P. Schubach - DVM, PhD1, Jeferson C. Oliveira - MD, PhD2, Anna B. F. Figueiredo - DVM, MSc1 and Sandro A. Pereira - DVM, PhD1

1Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Avenida Brasil, 4365. Manguinhos, Rio de Janeiro, RJ. 21040-900, Brasil and 2Instituto Biomédico, Universidade Federal Fluminense, Rua Professor Hernani Melo, 101–São Domingos, Niterói–RJ, 24210-130

*To whom correspondence should be addressed. Jéssica Nunes Silva, Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Av. Brasil, 4365, Manguinhos, Rio de Janeiro, Brasil CEP: 21040-900. Tel/Fax: +55 21 3865-9553; E-mail: jessicanunes7@gmail.com

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Abstract

Sporotrichosis is an implantation mycosis caused by pathogenic species of Sporothrix schenckii complex that affects humans and animals, especially cats. Its main forms of zoonotic transmission include scratching, biting and/or contact with the exudate from lesions of sick cats. In Brazil, epidemic involving humans, dogs and cats has occurred since 1998. The definitive diagnosis of sporotrichosis is obtained by the isolation of the fungus in culture; however, the result can take up to four weeks, which may delay the beginning of antifungal treatment in some cases. Cytopathological examination is often used in feline sporotrichosis diagnosis, but accuracy parameters have not been established yet. The aim of this study was to evaluate the accuracy and reliability of cytopathological examination in the diagnosis of feline sporotrichosis. The present study included 244 cats from the metropolitan region of Rio de Janeiro, mostly males in reproductive age with three or more lesions in non-adjacent anatomical places. To evaluate the inter-observer reliability, two different observers performed the microscopic examination of the slides blindly. Test sensitivity was 84.9%. The values of positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and accuracy were 86.0, 24.4, 2.02, 0.26 and 82.8%, respectively. The reliability between the two observers was considered substantial. We conclude that the cytopathological examination is a sensitive, rapid and practical method to be used in feline sporotrichosis diagnosis in outbreaks of this mycosis.

Key words: Sporothrix sp., cat, zoonosis, diagnosis, cytopathology.
Introduction

Sporotrichosis is an implantation mycosis caused by thermomodimorphic pathogenic species of the genus *Sporothrix* [1]. In Brazil, *Sporothrix brasiliensis* has been considered the most prevalent species of this genus in humans and cats [2]. Since 1998, it has occurred as an epidemic disease affecting human beings and animals in Rio de Janeiro [3]. By December 2012, approximately 4,120 feline cases had been diagnosed at the ‘Instituto Nacional de Infectologia Evandro Chagas’ (INI)/‘Fundação Oswaldo Cruz’ (Fiocruz), Rio de Janeiro, Brazil. The cat is the animal species most affected by this fungal disease, and skin ulcers are the main clinical signs observed [4]. Cats present high zoonotic potential for the transmission of this fungus because their skin lesions contain high numbers of yeast-like cells [5], which demonstrates the importance of these animals in the epidemiological chain of this mycosis [6].

The reference standard for the diagnosis of sporotrichosis is the isolation of the *Sporothrix* in culture media. Nevertheless, despite the high sensitivity of this diagnostic method in feline cases [7] the time required to obtain results of this examination can take up to four weeks [8]. Cytopathological examination has been used in the presumptive diagnosis of feline sporotrichosis due to its good sensitivity, but its accuracy is still unknown to date [9]. Analysis of the exudate from skin lesions of cats infected with *Sporothrix* often reveals numerous round, oval or cigar-shaped yeast-like forms inside macrophages and neutrophils, or in the extracellular medium. These structures measure 3–5 μm in diameter and 5–9 μm in length, and are surrounded by a clear halo [10]. Other techniques such as histopathology [11], serology [12] and polymerase chain reaction [13] have also been used to detect *Sporothrix* infection in cats, with the former requiring more processing time and the latter two being used for research purposes.

The purpose of the present study was to evaluate the accuracy and reliability of cytopathological examination in the diagnosis of feline sporotrichosis.

Material and methods

A blinded, cross-sectional diagnostic survey was carried out with cats assisted at the ‘Laboratório de Pesquisa Clínica em Dermatozoonoses em Animais Domésticos’, INI/Fiocruz, Rio de Janeiro, Brazil, between October 2007 and December 2010. The animals considered eligible for this study were cats with clinical suspicion of sporotrichosis showing at least one ulcerated skin lesion. Animals that had received prior systemic antifungal treatment for sporotrichosis were not included in the study.

The animals’ age, breed, gender, and origin were assessed. During clinical examination, the cats were classified into three groups according to the distribution of skin lesions: L1 (presence of cutaneous lesions in one location); L2 (cutaneous lesions in two nonadjacent locations), and L3 (cutaneous lesions in three or more nonadjacent locations) [7].

Samples for cytopathy were obtained from three impression smears on one glass slide of the same ulcerated skin lesion of the largest diameter. The slides were air-dried and then stained by the Quick Panoptic method (Instant Prov; Newprov)—a Romanowsky-type stain similar to DiffQuik [9]. Subsequently, all slides were analyzed with a light microscope at a magnification of 1000×. Two different observers performed the microscopic examination of the slides blinded to the result of the other observer and to the fungal culture results. One observer (JNS-DVM) had experience in the field of veterinary mycology. The other observer (RCM-DVM, PhD) was a pathologist and had more experience than JNS in the fields of microscopy and cytopathology. Results were considered positive when at least one yeast-like structure suggestive of *Sporothrix* was verified.

Concomitantly, a single collection of exudates from the edge of the same ulcerated skin lesion was performed with a sterile swab for fungal culture. The samples were seeded onto Sabouraud dextrose agar with chloramphenicol and Mycobiotic agar (Difco), incubated at 25°C, and observed for four weeks. The isolates were subcultured on potato dextrose agar at 25°C and dimorphism was confirmed by conversion to the yeast phase in brain-heart infusion broth at 37°C [8]. The test results were stored in a database developed using the software program *Statistical Package for the Social Sciences* for Windows (SPSS WIN), version 16.0.

To study the inter-observer variability, it was estimated that the double-reading of the 180 slides would be enough, considering 0.90 (excellent) for *Kappa*, 0.05 for alpha, and 80% for power to reject the null hypothesis of the *Kappa* index, less than or equal to 0.79 (good), in an expected sporotrichosis prevalence of 80%. For accuracy assessment, the sample was calculated for 169 animals to estimate a sensitivity of 75% with an absolute error of 0.10.

Fungal culture was used as the reference standard. Overall accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated at intervals of 95% confidence using the WinPepi 11.2 software [14]. The results were compared by the Chi-square test of independence at 5% significance level. Statistical analysis was conducted using SPSS WIN 16.0 and MedCalc 12 software programs. The inter-observer variability was estimated by the *Kappa* index [15].
Results

A total of 244 cats with clinical suspicion of sporotrichosis were included in the study; most of them were male (78.7%) and cross-breed (89.8%). The median age of the cats was 2 years old. One hundred forty-two cats (64.5%) were from the city of Rio de Janeiro. Two hundred thirty cats were classified according to the distribution of cutaneous lesions: 52 (22.6%) belonged to group L1, 52 (22.6%) to group L2, and 126 (54.8%) to group L3.

Two hundred twenty-five animals (92.2%, n = 244) tested positive in the fungal culture (Fig. 1). Among the 19 cases without isolation of Sporothrix, in 12 there was no growth of any fungal species, in one there was growth of Cryptococcus neoformans, in one there was growth of Malassezia pachydermatis and in 5 there was growth of fungal contaminants. In 199 cases (81.6%, n = 244) (Table 1), yeast-like forms compatible with Sporothrix were observed in the cytopathological examination (Fig. 2). Eight negative cases in culture were considered positive in cytopathological examination by JNS; six of these were considered negative by RCM (Table 1).

Among the 19 cats without isolation of Sporothrix, eight were diagnosed with pyoderma, three with flea-allergy dermatitis/pyoderma, one with cryptococcosis, one with eosinophilic disease, and one with cutaneous malasseziosis. One cat was subsequently re-examined and diagnosed with sporotrichosis by mycological culture. Four cases were lost to follow-up and the definitive diagnosis was not established.

The accuracy parameters are described in Table 2, and their association with the distribution of cutaneous lesions is shown in Table 3. The sensitivity of cytopathology was higher than the specificity, and a positive test occurred twice as often in cats with sporotrichosis. The inter-observer reliability was substantial, with simple Kappa index of 0.61 (0.51 to 0.72–95% CI).

Discussion

The use of cytopathological examination for the diagnosis of feline sporotrichosis has already been described;
Table 3. Sensitivity, specificity, predictive values, likelihood ratios, and accuracy of cytopathology in relation to the distribution of cutaneous lesions for the diagnosis of infection with Sporothrix in cats.

<table>
<thead>
<tr>
<th></th>
<th>L1 (52)</th>
<th>95% CI</th>
<th>L2 (52)</th>
<th>95% CI</th>
<th>L3 (126)</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>75.0%</td>
<td>59.7–86.8%</td>
<td>83.3%</td>
<td>69.8–92.5%</td>
<td>88.5%</td>
<td>81.5–93.6%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>75.0%</td>
<td>34.9–96.8%</td>
<td>25.0%</td>
<td>63–80.6%</td>
<td>75.0%</td>
<td>19.4–99.4%</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>94.3%</td>
<td>80.8–99.3%</td>
<td>93.0%</td>
<td>80.9–98.5%</td>
<td>99.1%</td>
<td>95–99.9%</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>35.3%</td>
<td>14.2–61.7%</td>
<td>11.1%</td>
<td>28–48.3%</td>
<td>17.7%</td>
<td>3.8–43.4%</td>
</tr>
<tr>
<td><strong>LR+</strong></td>
<td>3.00</td>
<td>0.89–10.1</td>
<td>1.11</td>
<td>0.62–1.98</td>
<td>3.54</td>
<td>0.65–19.4</td>
</tr>
<tr>
<td><strong>LR-</strong></td>
<td>0.33</td>
<td>0.17–0.64</td>
<td>0.67</td>
<td>0.11–4.08</td>
<td>0.15</td>
<td>0.07–0.32</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>75.0%</td>
<td>0.61–0.86</td>
<td>54%</td>
<td>0.4–0.68</td>
<td>82%</td>
<td>0.74–0.88</td>
</tr>
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</table>

Note: PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR-: negative likelihood ratio; CI: confidence interval.

However, only one study evaluated the sensitivity of this method in the diagnosis of this mycosis in cats to date [9]. The present study is the first to describe the accuracy and reliability of this diagnostic method using fungal culture as a reference standard.

The feline population included in this study was composed mostly of male, crossbred animals at reproductive age presenting cutaneous lesions in three or more nonadjacent anatomic places. This profile was not different from those previously described in other surveys conducted in the same area [3,7,16].

In this study, the sensitivity of cytopathology compared to fungal culture was considered to be satisfactory (84.9%). In a study previously conducted at the same institution, 806 cats with sporotrichosis were evaluated and the reported test sensitivity was 78.9%; however, it was used a larger sample size, less stringent eligibility criteria, a distinct method of sample collection, and the microscopic examination was performed by several observers [9]. Despite the different methodological designs used, the sensitivity levels reported in both studies were similar.

The sensitivity of the cytopathological examination in cats from group L3 was higher (88.5%) than in cats from groups L1 and L2. These results can be attributed to the higher fungal load found in cats from group L3. Similar findings were observed by other authors in histopathological examination of skin lesions using the Grocott’s silver stain method [11].

In this study, the specificity of cytopathology was considered low, with an unstable confidence interval, probably owing to the inclusion of few cats that did not present sporotrichosis lesions. The finding that six out of the eight cases positive by cytopathological examination and negative by fungal culture were not confirmed by the most experienced observer suggests that they are false positive. However, fungal culture is not 100% sensitive, despite being a reference method. The predictive values of a test are influenced by the prevalence of the disease in the study population and the results should be interpreted in this context [15]. Overall, high PPV and low NPV were found, reflecting a sample with high prevalence of sporotrichosis (92%) and few negative cases selected in a referral outpatient clinic during the outbreak. However, in group L3 (Table 3), which includes the majority of cats with sporotrichosis in this epizootic, both LR+ and specificity are good (0.15 and 75%, respectively), suggesting a better performance in severe cases.

In some situations, the yeast-like forms of Sporothrix observed in the cytopathology may be mistaken for other species, such as Histoplasma capsulatum and Cryptococcus neoformans. Furthermore, the observation of artifacts (e.g., stain precipitate), cell debris and colonizing microorganisms can also lead to false-positive diagnosis [17]. The six discordant results from the eight cases supposed to be false-positive may be related to the reduced experience of the observer JNS, who may have confused technical artifacts or cellular debris with yeast structures of Sporothrix spp. False-negative results can be associated with the low fungal load verified in the lesions of animals from groups L1 and L2.

Despite the degree of agreement between the two observers that examined the slides be considered substantial, it was lower than the expected result for this kind of study. Inter-observer variation commonly occurs, because two observers do not always produce the same results [15]. Therefore, the discrepancies between the results found by the observers in this study can be explained, partially, by the distinct levels of experience among them, which can be minimized by training.

Cytopathology is widely used to diagnose and/or differentiate infectious, inflammatory, proliferative, and neoplastic diseases [18]. Although fungal culture is the reference standard to diagnose feline sporotrichosis, in some cases, this method delays the beginning of antifungal treatment because of the time required for isolating the fungus.
Compared with fungal culture and histopathological examination, cytopathology is simple to perform, inexpensive, and presents immediate results. Considering these advantages, in addition to the high accuracy of cytopathology observed in this study, a positive result in this method could allow the beginning of antifungal treatment before fungal isolation in epidemic situations [9]. The early treatment of cats may increase the chance of clinical cure and reduce the risk of transmission of Sporothrix to humans and other animals.

INI/Fiocruz is a national reference center for the diagnosis and treatment of fungal infections [3]. Because of the outbreak of feline sporotrichosis that has occurred since the late 1990s, most cats seen at this institution presented this mycosis, and this larger prevalence was a limitation of this study, although calculations could have been performed to estimate post-test chances according to each prevalence setting. Studies of inter-observer variability including cats with skin ulcers assisted at different non-specialized clinics may inform about the accuracy and reliability of this test in those settings, compared with the good results herein verified in a specialized treatment center. Additionally, conducting a multicentric study involving other centers that perform clinical assistance to cats can be an alternative to reduce this limitation.

The results of the present study reinforce the fact that, in endemic regions of sporotrichosis where there are difficulties in performing fungal culture, the use of cytopathological examination in cats is strongly recommended. This method is as a viable diagnostic tool able to identify approximately 80% of cases and therefore allow the implementation of early preventive and control measures for this zoonosis.

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Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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