J. Comp. Path. 2018, Vol. 160, 72–78

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER
www.elsevier.com/locate/jcpa

INFECTIOUS DISEASE

Comparison of the Sensitivity of Three Methods for the Early Diagnosis of Sporotrichosis in Cats

J. N. Silva*,†, L. H. M. Miranda†, R. C. Menezes†, I. D. F. Gremião†, R. V. C. Oliveira‡, S. M. M. Vieira†, F. Conceição-Silva§, L. Ferreiro* and S. A. Pereira†

** Laboratório de Micologia, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 9090, Agronomia, Porto Alegre, Rio Grande do Sul, † Laboratório de Pesquisa Clínica em Dermatozoonoses em Animais Domésticos, Instituto Nacional de Infectologia Evandro Chagas (INI), Fundação Oswaldo Cruz (Fiocruz), Av. Brasil 4365, ‡ Laboratório de Epidemiologia Clínica, INI/Fiocruz, Av. Brasil 4036/201A and § Laboratório de Imunoparasitologia, Instituto Oswaldo Cruz/Fiocruz, Av. Brasil 4365/Pavilhão 26/406C, Manguinhos, Rio de Janeiro, Brazil*

Summary

Sporotrichosis is caused by species of fungi within the *Sporothrix schenckii* complex that infect man and animals. In Rio de Janeiro, Brazil, an epidemic has been observed since 1998, with most of the cases being related to transmission from infected cats. Although the definitive diagnosis of feline sporotrichosis is made by fungal culture, cytopathological and histopathological examinations are used routinely, because the long culture period may delay treatment onset. However, alternative methods are desirable in cases of low fungal burden. Immunohistochemistry (IHC) has been described as a sensitive method for diagnosing human and canine sporotrichosis, but there are no reports of its application to cats. The aim of this study was to analyse the sensitivity of cytopathological examination (Quick Panoptic method), histopathology (Grocott silver stain) and anti-*Sporothrix* IHC by blinded comparisons, using fungal culture as the reference standard. Samples were collected from 184 cats with sporotrichosis that exhibited skin ulcers. The sensitivities of Grocott silver stain, cytopathological examination and IHC were 91.3%, 87.0% and 88.6%, respectively. Grocott silver stain showed the best performance. IHC showed high sensitivity, as did cytopathological examination and these may be considered as alternative methodologies. When the three methods were combined, the diagnosis was established in 180 (97.8%) out of 184 cases. Taken together, these findings indicate the need to implement these methods as routine tools for the early diagnosis of sporotrichosis in cats, notably when fungal culture is not available.

Keywords: histopathology; immunohistochemistry; feline sporotrichosis

Introduction

Sporotrichosis is caused by different species of thermodimorphic fungi within the *Sporothrix schenckii* complex. The disease has worldwide distribution, with reports from all continents (Barros *et al.*, 2011a; Chakrabarti *et al.*, 2015) and has become an emerging health problem over the last two decades because of the increasing numbers of human and animal cases, changes to epidemiology and case distribution and multiple outbreaks (López-Romero *et al.*, 2011; Chakrabarti *et al.*, 2015). This fact highlights the need for efficient diagnostic tools.

Transmission of *Sporothrix* spp. usually occurs by means of traumatic inoculation through the skin. Zoonotic transmission has been reported and is generally

Correspondence to: L.H.M. Miranda (e-mail: luisa.helena@ini.fiocruz.br).

0021-9975/$ - see front matter
https://doi.org/10.1016/j.jcpa.2018.03.002

associated with scratches and bites from infected cats (Rodrigues *et al.*, 2016; Gremião *et al.*, 2017). Cats are the main species involved in this kind of transmission due to the high numbers of yeast-like cells in their cutaneous lesions (Gremião *et al.*, 2015, 2017; Silva *et al.*, 2015), suggesting that, in contrast to other animals, cats act as reservoir hosts amplifying the organisms.

In Rio de Janeiro, Brazil, an epidemic of sporotrichosis affecting man and animals has been described since 1998 (Schubach *et al.*, 2004; Pereira *et al.*, 2014; Gremião *et al.*, 2017). In this scenario, *Sporothrix brasiliensis* is the most prevalent aetiological agent in man and cats (Rodrigues *et al.*, 2013; Boechat *et al.*, 2018). Most of the cases in this epidemic are related to transmission from infected cats (Gremião *et al.*, 2017), which emphasizes the importance of these animals in the epidemiology of this mycosis. Cats are the animal species most affected by sporotrichosis and skin ulcers are the main lesion observed (Pereira *et al.*, 2014, 2015). By 2015, 4,703 feline cases had been diagnosed at the Instituto Nacional de Infectologia Evandro Chagas (INI), Fundação Oswaldo Cruz (Fiocruz), which is a referral centre for the diagnosis and treatment of human and animal sporotrichosis and other mycoses in Rio de Janeiro (Gremião *et al.*, 2017).

The reference standard method for diagnosing sporotrichosis is isolation of *Sporothrix* spp. in culture media (Rippon, 1988). However, this method is not 100% sensitive and *Sporothrix* spp. growth may not be observed (Silva *et al.*, 2015). This is generally due to inadequate collection or transportation of the material or to contamination with saprophytic microorganisms (Moore and Ackerman, 1946; Schwarz, 1992). In some cases, this method can delay the start of antifungal treatment because the isolation of the fungus may require up to 30 days (Silva *et al.*, 2015). As a consequence of the delay in the antifungal treatment, sporotrichosis can become severe, increasing the risk of transmission and reducing the chances of clinical cure. In addition, sporotrichosis is unlikely to be the first clinical suspicion in non-endemic regions and the general procedures of sample collection and processing for isolation of the fungus may therefore not be properly implemented. In such cases, other diagnostic methods are required, in particular those that are useful for making the differential diagnosis between neoplasia and other infectious diseases (Miranda *et al.*, 2013; Silva *et al.*, 2015).

Cytopathological examination (CPE) is used routinely as a screening method for the diagnosis of feline sporotrichosis because of its high sensitivity, which enables the detection of approximately 78–85% of feline cases. CPE involves simple non-invasive sample collection and is a rapid and inexpensive method. It is therefore suitable for routine use by trained veterinary practitioners, even in small facilities, leading to rapid diagnosis and early initiation of the treatment in endemic areas (Pereira *et al.*, 2011; Silva *et al.*, 2015).

Histopathology is an ancillary diagnostic tool that is also used as a rapid, inexpensive and widely available alternative for diagnosing feline sporotrichosis (Miranda *et al.*, 2013). Special histochemical stains, such as Grocott's silver stain (GSS), have been described for diagnosing human and animal sporotrichosis and are usually applied to enhance the visualization of the yeast-like cells in tissues (Miranda *et al.*, 2009, 2013; Quintella *et al.*, 2011, 2012).

Although the detection of yeast-like cells in tissues or cytological preparations from lesions of cats does not generally pose a problem in the diagnosis of sporotrichosis, there are cases in which the fungal burden is low and the use of more accurate methods is required (Miranda *et al.*, 2013). In this sense, immunohistochemistry (IHC) allows the detection of antigen in tissue through the assessment of antigen–antibody interactions. This method has already been shown to improve the sensitivity of the histological diagnosis of human and canine sporotrichosis (Marques *et al.*, 1992; Miranda *et al.*, 2011); however, it has not yet been applied to feline sporotrichosis.

Convenient methods enabling rapid and reliable results are highly desirable for the diagnosis of feline sporotrichosis and for the implementation of early treatment and control measures (Pereira *et al.*, 2011), thereby potentially reducing the transmission of *Sporothrix* spp. to man and other animals (Silva *et al.*, 2015). The aim of the present study was to compare the sensitivity of CPE, GSS and anti-*Sporothrix* spp. IHC with fungal culture as the reference standard.

Materials and Methods

Sampling

Samples were obtained from cats seen at the Laboratório de Pesquisa Clínica em Dermatozoonoses em Animais Domésticos (Lapclin-Dermzoo)/INI/Fiocruz, Rio de Janeiro, Brazil, between 2009 and 2011. Cats with skin ulcers not previously given antifungal treatment were eligible for this study. All cases included were confirmed as sporotrichosis by isolation of *Sporothrix* spp. in culture from skin ulcers. The medical procedures performed on the cats were approved by the Animal Ethics Committee of Fiocruz, under license number L-041/06.

All specimens were obtained at the same time from a single skin ulcer, including those for fungal culture.

In cats with multiple skin ulcers, the one with the largest diameter was selected for sampling.

Fungal Culture

Material collected with a sterile swab was seeded onto Sabouraud's dextrose agar and Mycobiotic agar (Difco, Becton-Dickinson, Sparks, Massachusetts, USA) and incubated at 25°C. Dimorphism was confirmed by conversion to the yeast phase in brain–heart infusion broth at 37°C.

Cytopathological Examination

For CPE, impression smears of skin ulcers on a glass slide were analysed in triplicate. The slides were air-dried and then stained using the Quick Panoptic method (Instant Prov Kit; Newprov, Pinhais, Brazil), a Romanowsky-type stain. All slides were then analysed under an optical microscope at ×1,000 magnification. The results were considered positive when at least one yeast-like structure suggestive of *Sporothrix* spp. was detected (Silva *et al.*, 2015). These structures measured 3–5 μm in diameter and were surrounded by a clear halo (Welsh, 2003).

Histopathology with Grocott Silver Stain

For the histopathological analysis, one fragment from the edge of the ulcer was obtained through biopsy, using a punch of 4 mm, after sedation of the animals by intramuscular injection of ketamine hydrochloride (10 mg/kg) and acepromazine maleate (0.1 mg/kg) and local anaesthesia of the biopsy region with 2% lidocaine hydrochloride without adrenaline. The biopsy specimens were fixed in 10% neutral buffered formalin and then processed routinely at the Serviço de Anatomia Patológica, INI/Fiocruz. Three serial sections of the same tissue sample were subjected to GSS (Carson and Hladick, 2009), for examination under an optical microscope at ×400 magnification.

Cases were considered positive when black or dark brown yeast-like cells consistent with *Sporothrix* spp. were found. These were round to oval or cigar-shaped yeasts measuring 2–6 μm, usually presenting with elongate buds with a narrow base (Barros *et al.*, 2011b).

Anti-Sporothrix *spp. Immunohistochemistry*

The procedures for antigen preparation and production of anti-*Sporothrix* spp. antiserum have been described previously (Lopes Alves *et al.*, 1994). The assay in feline tissue was carried out based on the conditions previously documented for canine sporotrichosis (Miranda *et al.*, 2011).

Formalin-fixed, paraffin wax-embedded samples were sectioned onto silanized slides, followed by dewaxing and rehydration. Three serial sections of the same tissue sample were prepared. The following steps were then performed: inhibition of endogenous peroxidase with 30% H_2O_2 in 40 ml/100 ml (v/v) methanol; blocking of non-specific binding with normal swine serum (NCL-SSERUM, Novo Castra, Newcastle, UK) in 1.5% bovine serum albumin (BSA) (1 in 20 dilution); followed by incubation with a solution of 0.1 g/ml milk powder in 3.0% BSA. The histological sections were then incubated overnight in a humid chamber at 4°C with anti-*Sporothrix* spp. antiserum diluted 1 in 4,000 in 1.5% BSA. For each run, sections from a sample previously confirmed to be positive by isolation of *Sporothrix* spp. in culture and for containing yeast-like cells on histopathological analysis were included as a positive control. Additional sections from the same positive sample were incubated with normal rabbit serum (X0902; Dako, Carpinteria, California, USA) as a negative control.

Serial washes in Tris-buffered saline were performed and the sections were incubated with a universal biotinylated secondary antibody and streptavidin-conjugated horseradish peroxidase (LSAB+ System Kit K0690, Dako). The reaction was 'visualized' with 3, 3′ diaminobenzidine (SIGMAFAST™ 3, 3′ diaminobenzidine tablets; Sigma–Aldrich, St. Louis, Missouri, USA) as chromogen. The sections were counterstained with Mayer's haematoxylin and dehydrated.

The presence of brown-stained yeast-like cells consistent with *Sporothrix* spp. was evaluated by examination under an optical microscope at ×400 magnification. Positive and negative controls were included in each run.

Data Analysis

The results (positive/negative) obtained with the three different methods (GSS, CPE and IHC), either alone or combined, were compared using the McNemar test for paired samples. $P < 0.05$ was considered to indicate a significant difference.

Microscopical examination of the CPE, IHC and GSS slides was performed blindly by a single observer (JNS) with experience in the microscopical diagnosis of sporotrichosis.

Results

One hundred and eighty-four cats were included in this study. Fig. 1 illustrates a cat with sporotrichosis presenting with multiple ulcerated skin lesions. Most

Fig. 1. Feline sporotrichosis. (A) Cat with multiple ulcerated skin lesions on the forelimb and face. (B) Ulcer on the bridge of the nose.

animals were male (n = 150; 81.5%), mixed-breed (n = 152; 82.6%) and in good general condition (n = 143; 77.7%). The age ranged from 6 to 108 months (median 24 months).

One hundred and forty-four (78.3%) cases tested positive for yeast-like cells of *Sporothrix* spp. by all three methods, four (2.2%) tested negative by all the three methods, 23 (12.5%) tested positive by two methods and thirteen (7.0%) tested positive by just one method (Figs. 2A, B). Among cases that tested negative by CPE (n = 24), yeast-like cells were detected in 18 and 15 cases by GSS and IHC, respectively. IHC and CPE alone diagnosed sporotrichosis in two and six cases, respectively. Combining the three methods increased the diagnostic sensitivity to 98.3%. The difference in sensitivity between the three techniques was not significant. Figs. 3A-C show yeast-like cells of *Sporothrix* spp. detected by the three methods used.

Discussion

The evaluation of alternative tools for diagnosing sporotrichosis is useful, particularly in situations in which fungal culture is not available or when the results are delayed due to slow growth. The present study compares for the first time the sensitivity of three different methods for diagnosing sporotrichosis in 184 cats, using fungal culture as the reference standard.

The results described in this study confirm that CPE has a high sensitivity (87.0%) in detecting yeast-like cells of *Sporothrix* spp. in skin lesions of cats, as previously described (Pereira *et al.*, 2011; Silva *et al.*, 2015). Despite its low specificity and the fact that false-positive cases have been identified in endemic areas by less experienced observers (Silva *et al.*, 2015), we still recommend the routine use of CPE for the presumptive diagnosis of feline

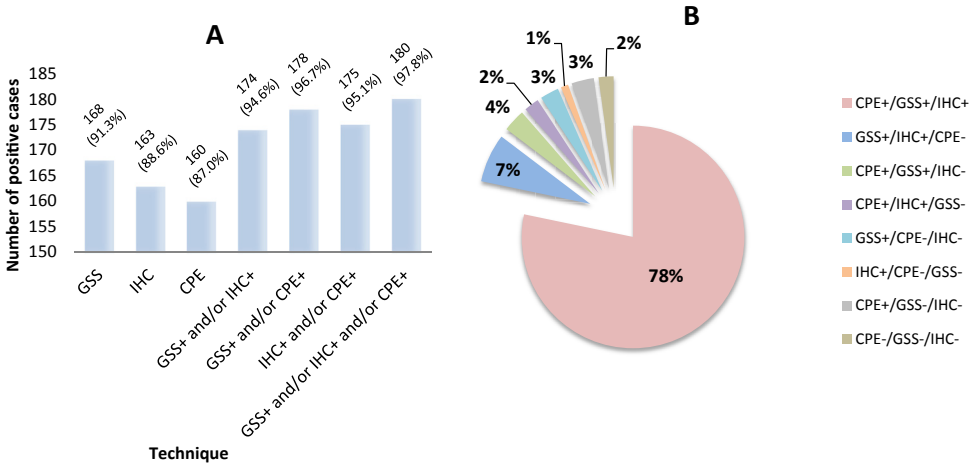

Fig. 2. Evaluation of three different methods for detecting yeast-like cells of *Sporothrix* spp. in skin lesions of cats with sporotrichosis. (A) Sensitivity values of cytopathological examination (CPE), histopathology with Grocott silver stain (GSS) and anti-*Sporothrix* spp. immunohistochemistry (IHC) and the combined sensitivity of the methods. Note: and/or indicates that either one or all of the referred methods is positive. (B) Distribution of positive cases detected by each technique.

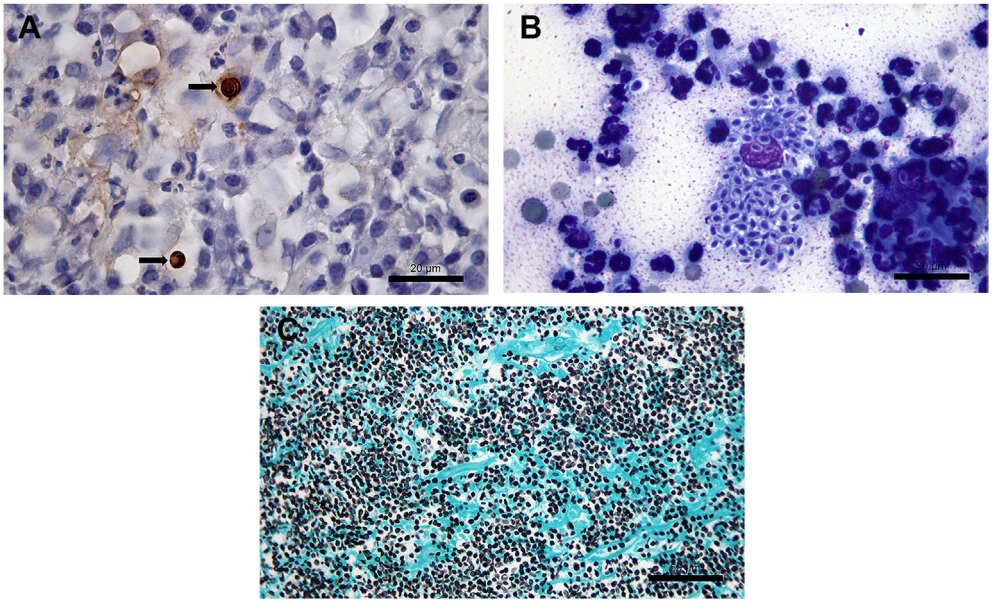

Fig. 3. Detection of yeast-like cells of *Sporothrix* spp. in skin lesions of cats with sporotrichosis by different techniques. (A) Histological section from a cat with cutaneous sporotrichosis. Two brown *Sporothrix* spp. yeast-like cells. IHC. (B) Impression smear of a skin lesion from a cat with sporotrichosis showing numerous cigar-shaped or oval yeast-like cells with a single round pink nucleus surrounded by blue cytoplasm and an unstained cell wall, within macrophages and extracellular medium. Quick Panoptic stain. (C) Histological section from a cat with cutaneous sporotrichosis. Numerous dark-brown *Sporothrix* spp. yeast-like cells. GSS.

sporotrichosis within veterinary practice, especially in outbreaks or endemic situations. In this respect, we advocate advanced training of these professionals in order to reduce false-negative (i.e. lack of identification of yeast-like cells) and false-positive (i.e. misidentification of yeast-like cells) results. CPE is inexpensive, rapid and easy to use. Furthermore, this method was positive in six cases that tested negative by the other two methods in this study, which supports the use of CPE as a first-line option, contributing to a quick diagnosis and to the early treatment of sick cats, increasing the chances of clinical cure and consequently reducing the risk of transmission of *Sporothrix* spp. to man or other animals (Silva *et al.*, 2015).

When it comes to non-endemic areas, it should be kept in mind that sporotrichosis might not be the first clinical suspicion, even in common clinical presentations. Additionally, the observers may not be familiar with the microscopical appearance of the pathogen in such areas, impairing the prompt identification of yeast-like cells of *Sporothrix* spp. by **CPE**. In such cases, the smear could be forwarded for interpretation by a specialist pathologist. Alternatively, histopathology may be considered for diagnosing sporotrichosis, since it enables retrospective analysis of formalin-fixed, paraffin wax-embedded tissues, which is particularly desirable when sporotrichosis is not initially considered as a differential diagnosis and no sample is collected for fungal culture.

In fact, histopathological analysis by means of haematoxylin and eosin staining is a decisive tool and should be used routinely by pathologists. While the histopathological presentation is consistent with sporotrichosis (i.e. suppurative granulomatous inflammation), special histochemical stains like GSS should be used for the detection of yeast-like cells (Miranda *et al.*, 2013). In this study, GSS had high sensitivity for detecting yeast-like cells in histological sections from lesions of cats with sporotrichosis, as previously found by Miranda *et al.* (2013). In human and canine sporotrichosis, GSS is not considered a high-sensitivity method, because the skin lesions in those species typically have low fungal burdens. Previous studies reported a sensitivity of GSS of 35.3% and 65.5% in human and canine sporotrichosis, respectively (Miranda *et al.*, 2009; Quintella *et al.*, 2011). Significant improvement in diagnostic sensitivity has been described with the use of IHC (Marques *et al.*, 1992; Miranda *et al.*, 2011) in human and canine samples. However, this was not the case for feline sporotrichosis, as demonstrated in this study, in which GSS had the highest sensitivity of the techniques evaluated.

These opposing findings can be partially due to the high fungal burden that is usually seen in skin lesions caused by *Sporothrix* spp. in cats. Therefore, yeast-like cells are easily found, irrespective of the method used. However, particular attention should

be directed to cases with low fungal burdens that can be found occasionally in feline sporotrichosis, especially in animals with localized lesions or those already under antifungal treatment. In these cases, even repeated sampling may fail to isolate *Sporothrix* spp. in culture and IHC should therefore be used as an alternative for GSS-negative samples. Microscopical analysis of multiple sections from the same sample should also be carried out in order to increase the chance of detecting sparse yeasts in cases of low fungal density.

Potentially relevant is the fact that not only is *S. brasiliensis* the most prevalent causative species of feline sporotrichosis in Rio de Janeiro (Boechat *et al.*, 2018), but it has also been reported to be highly virulent and correlated with high fungal burden (Almeida-Paes *et al.*, 2015). Within this context, we hypothesize that infection with less virulent species of the *Sporothrix* complex, outside the Brazilian endemic region for example, could lead to lower fungal burden and related diagnostic difficulties.

The combination of CPE, GSS and IHC increased the diagnostic sensitivity in comparison with each technique alone. Although analysis of the fungal burden was not the objective of this study, it is likely that the disagreement between the methods occurred in cases with low fungal burden, in which evaluation of different methods is particularly important.

In view of the high potential of cats for transmitting *Sporothrix* spp. and the increasing numbers of reports of sporotrichosis worldwide, we highlight the importance of including this disease in the differential diagnosis of cases in which the clinical and histopathological presentations resemble that of sporotrichosis. Since the methods described here permit the diagnosis in almost 100% of cases, we emphasize the importance of their implementation as regular tools, notably when fungal culture is unavailable. Furthermore, we strongly encourage the application of these methods in non-endemic areas, together with the assessment of fungal burden in order to determine their adequacy in different epidemiological scenarios.

Acknowledgments

The authors are grateful to the staff of 'Laboratório de Pesquisa Clínica em Dermatozoonoses em Animais Domésticos' (INI/Fiocruz), to 'Laboratório de Micologia' and to 'Serviço de Anatomia Patológica' for the excellent technical assistance. We especially acknowledge T. M. Pacheco Schubach and R. Mexas for processing the figures. This study was partially supported by grants from the 'Programa de Apoio 'a Pesquisa Estratégica em Saúde' (PAPES VI) Fiocruz/'Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico' (CNPq)- Grant number: 422099/2017-1; and 'Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro' (FAPERJ)/ Grant number: E-23/203.303/2016 – 'Jovem Cientista do Nosso Estado'. RCM, LF and SAP are recipients of productivity fellowships from CNPq. JNS held a PhD scholarship from CNPq. SMMV held a scholarship from scientific initiation from Programa Institucional de Bolsas de Iniciação Científica/CNPq.

Conflict of Interest Statement

The authors report no conflicts of interest with respect to the publication of this manuscript.

References

Almeida-Paes R, de Oliveira LC, Oliveira MM, Gutierrez-Galhardo MC, Nosanchuk JD *et al.* (2015) Phenotypic characteristics associated with virulence of clinical isolates from the *Sporothrix* complex. *BioMed Research International*, **2015**, 1–10.

Barros MB, de Almeida Paes R, Schubach AO (2011) *Sporothrix schenckii* and sporotrichosis. *Clinical Microbiology Reviews*, **24**, 633–654.

Barros MBL, Schubach TMP, Coll JO, Gremião IDF, Wanke B *et al.* (2010) Sporotrichosis: development and challenges of an epidemic. *Revista Panamericana de Salud Pública*, **27**, 455–460.

Boechat JS, Oliveira MME, Almeida-Paes R, Gremião IDF, Machado ACS *et al.* (2018) Feline sporotrichosis: associations between clinical-epidemiological profiles and phenotypic-genotypic characteristics of the etiological agents in the Rio de Janeiro epizootic area. *Memórias do Instituto Oswaldo Cruz*, https://doi.org/10.1590/0074-02760170407.

Carson FL, Hladick C (2009) *Histotechnology: A Self Instructional Text*, 3rd Edit., American Society for Clinical Pathology Press, Chicago, pp. 239–243.

Chakrabarti A, Bonifaz A, Gutierrez-Galhardo MC, Mochizuki T, Li S (2015) Global epidemiology of sporotrichosis. *Medical Mycology*, **53**, 3–14.

Gremião IDF, Menezes RC, Schubach TMP, Figueiredo AB, Cavalcanti MC *et al.* (2015) Feline sporotrichosis: epidemiological and clinical aspects. *Medical Mycology*, **53**, 15–21.

Gremião IDF, Miranda LHM, Reis EG, Rodrigues AM, Pereira SA (2017) Zoonotic epidemic of sporotrichosis: cat to human transmission. *PLoS Pathogens*, **13**, e1006077.

Lopes Alves L, Travassos LR, Previato JO, Mendonça-Previato L (1994) Novel antigenic determinants from peptido rhamnomannans of *Sporothrix schenckii*. *Glycobiology*, **4**, 281–288.

López-Romero E, Reyes-Montes M del R, Torres AP, Ruiz-Baca E, Villagómez-Castro JC *et al.* (2011) *Sporothrix schenckii* complex and sporotrichosis, an emerging health problem. *Future Microbiology*, **6**, 85–102.

Marques ME, Coelho KI, Sotto MN, Bacchi CE (1992) Comparison between histochemical and immunohistochemical methods for diagnosis of sporotrichosis. *Journal of Clinical Pathology*, **45**, 1089–1093.

Miranda LH, Conceição-Silva F, Quintella LP, Kuraiem BP, Pereira SA *et al.* (2013) Feline sporotrichosis: profile of cutaneous lesions and their correlation with clinical presentation. *Comparative Immunology, Microbiology and Infectious Diseases*, **36**, 425–432.

Miranda LH, Quintella LP, dos Santos IB, Menezes RC, Figueiredo FB *et al.* (2009) Histopathology of canine sporotrichosis: a morphological study of 86 cases from Rio de Janeiro (2001–2007). *Mycopathologia*, **168**, 79–87.

Miranda LH, Quintella LP, Menezes RC, dos Santos IB, Oliveira RV *et al.* (2011) Evaluation of immunohistochemistry for the diagnosis of sporotrichosis in dogs. *Veterinary Journal*, **190**, 408–411.

Moore M, Ackerman LV (1946) Sporotrichosis with radiate formation in tissue: report of a case. *Archives of Dermatology and Syphilology*, **53**, 253–264.

Pereira SA, Gremião IDF, Kitada AAB, Boechat JS, Viana PG *et al.* (2014) The epidemiological scenario of feline sporotrichosis in Rio de Janeiro, State of Rio de Janeiro, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, **47**, 392–393.

Pereira SA, Gremião IDF, Menezes RC (2015) Sporotrichosis in zoonotic transmission. In: *Sporotrichosis: New Developments and Future Prospects*, IZ Carlos, Ed., Springer International Publishing, Basel, pp. 83–112.

Pereira SA, Menezes RC, Gremião IDF, Silva JN, Honse C O *et al.* (2011) Sensitivity of cytopathological examination in the diagnosis of feline sporotrichosis. *Journal of Feline Medicine and Surgery*, **13**, 220–223.

Quintella LP, Passos SR, de Miranda LH, Cuzzi T, Barros MB *et al.* (2012) Proposal of a histopathological predictive rule for the differential diagnosis between American tegumentary leishmaniasis and sporotrichosis skin lesions. *British Journal of Dermatology*, **167**, 837–846.

Quintella LP, Passos SR, do Vale AC, Galhardo MC, Barros MB *et al.* (2011) Histopathology of cutaneous sporotrichosis in Rio de Janeiro: a series of 119 consecutive cases. *Journal of Cutaneous Pathology*, **38**, 25–32.

Rippon J (1988) Sporotrichosis. In: *Medical Mycology – the Pathogenic Fungi and Pathogenic Actinomycetes*, 3rd Edit., J Rippon, Ed., WB Saunders, Philadelphia, pp. 325–352.

Rodrigues AM, de Hoog GS, de Camargo ZP (2016) *Sporothrix* species causing outbreaks in animals and humans driven by animal–animal transmission. *PLoS Pathogens*, **12**, e1005638.

Rodrigues AM, Teixeira MM, Hoog GS, Schubach TM, Pereira SA *et al.* (2013) Phylogenetic analysis reveals a high prevalence of *Sporothrix brasiliensis* in feline sporotrichosis outbreaks. *PLoS Neglected Tropical Diseases*, **7**, e2281.

Schubach TMP, Schubach AO, Okamoto T, Barros MB, Figueiredo FB *et al.* (2004) Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998–2001). *Journal of the American Veterinary Medical Association*, **224**, 1623–1629.

Schwarz J (1992) The diagnosis of deep mycoses by morphologic methods. *Human Pathology*, **13**, 519–533.

Silva JN, Passos SRL, Menezes RC, Gremião IDF, Schubach MPT *et al.* (2015) Diagnostic accuracy assessment of cytopathological examination of feline sporotrichosis. *Medical Mycology*, **53**, 1–5.

Welsh RD (2003) Sporotrichosis. *Journal of the American Veterinary Medical Association*, **223**, 1123–1126.

[*Received, May 8th, 2017*
Accepted, March 13th, 2018]