



INFECTIOUS DISEASE

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

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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.

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Introduction

Sporotrichosis is caused by different species of thermophilic 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

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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.

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