Research Note

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Conjunctival swab PCR to detect *Leishmania* spp. in cats

Uso da PCR de suabe conjuntival para detecção de *Leishmania* spp. em gatos

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Abstract

The relevance of the dog as a source of visceral leishmaniasis infection is known, but the role of cats as reservoir hosts for leishmaniasis is not yet fully clear. This study assessed the efficacy of conjunctival swab PCR (CS-PCR) in the detection of cats infected by *Leishmania* spp. The results were seven (13.5%) cats positive for *Leishmania* spp. in the PCR, in 52 cats tested from Pirassununga-SP and Ilha Solteira-SP. From the city of Pirassununga – SP 28.6% (2/7) were positive and from the city of Ilha Solteira – SP 11.1% (5/45) were positive. The results showed that CS-PCR was capable of detecting cats infected by this protozoan. Conjunctival swab samples proved easier to perform in cats, which might facilitate studies on the frequency and distribution of feline leishmaniasis.

Keywords: Conjunctival swab, PCR, cats.

Resumo

A importância do cão como fonte de infecção da leishmaniose visceral já é conhecida, mas o papel dos gatos como reservatórios das leishmanioses ainda não está totalmente esclarecido. O presente estudo avaliou a eficácia da PCR de suabe conjuntival (PCR-SC) na detecção de gatos infectados por *Leishmania* spp. Foram encontrados sete (13,5%) gatos positivos para *Leishmania* spp. na PCR de suabe conjuntival, dentre 52 animais de Pirassununga - SP e Ilha Solteira - SP testados. Sendo positivos 28,6% (02/07) dos gatos do município de Pirassununga e 11,1% (5/45) dos gatos do município de Ilha Solteira. Os resultados demonstraram que o suabe de conjuntiva ocular foi capaz de detectar gatos infectados por esse protozoário. A coleta de amostras da conjuntiva mostrou ser um método simples, menos invasivo e pouco estressante para os gatos e seus proprietários, o que pode facilitar estudos sobre a frequência e distribuição da leishmaniose felina.

Palavras-chave: Suabe conjuntival, PCR, gatos.

Introduction

The infection by *Leishmania infantum* (syn. *L. chagasi*) (KUHLS et al., 2011) in domestic cats (*Felis catus domesticus*) has been reported in several European countries, Israel, and Brazil, where this zoonosis is considered endemic (POLI et al., 2002; PRATLONG et al., 2004; SAVANI et al., 2004; MAROLI et al., 2007; MARTÍN-SÁNCHEZ et al., 2007, MAIA et al., 2008). In this context, some studies of feline leishmaniasis (FL) facilitate the hypothesis that cats are susceptible to infection by *Leishmania* spp., like most of the canine population residing in endemic areas. However, the true level of susceptibility/resistance of felines to the infection and its role in the leishmaniasis cycle is still controversial (MAIA et al., 2010). Information regarding leishmaniasis involving felines has been increasing, but there are still many questions to be answered by new studies, especially regarding the pathogenesis and the true role of the cat as a reservoir host for *Leishmania* spp. (AYLLON et al., 2008). The evidence that there is a transmission of feline parasites to the disease vector was proven by Maroli et al. (2007) and Silva et al. (2010), by xenodiagnosis in Italy and Brazil, respectively, thus suggesting that cats can be a secondary reservoir host for *L. infantum*. Such scenarios confirm the necessity of increased attention to the role of cats in the leishmaniasis urban cycle and investigation of these animals' participation as sources of disease transmission (POLI et al., 2002; SOLANO-GALLEGO et al., 2007; SHERRY et al., 2011).

This study reports the detection of *Leishmania* spp. DNA in conjunctival cells of cats, by PCR.

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Materials and Methods

The procedures and content of this study were approved by the Ethics Committee of the Veterinary and Animal Sciences School of the University of São Paulo (FMVZ-USP), number 2361, on August 23, 2013.

Conjunctival swabs (CS) were collected from 52 cats (male and female, young and adult) from Pirassununga-SP (n=07); and from Ilha Solteira-SP (n=45), in 2012, in partnership with the Pirassununga Center of Zoonosis Control (CCZ) and the Ilha Solteira Animal Protection Association (APAISA), respectively. During the collection, the clinical condition of each cat and the presence of clinical manifestations compatible with leishmaniasis were evaluated (SILVA et al., 2010).

For the harvesting of conjunctival cells, sterile swabs manufactured for use in bacteriological isolation were used. A sterile swab was rubbed on the lower conjunctiva of both eyes of each cat to collect cells. The extremities of these swabs were separated and stored in 1.5mL microtubes, without DNAse and RNAse, at 4 °C until processing.

The purification of the DNA from swabs was performed using the salting-out technique (LAHIRI & NURNBERGER, 1991), with certain modifications: instead of Triton X-100 (CAS n°: 9002- 93- 1, Sigma), Nonidet P-40 (CAS n°: 9036-19-5, Sigma) was used, and the DNA was stored at –20 °C until use. A first PCR reaction, previously described by Rodgers et al. (1990), in the amplification of 120 bp, for *Leishmania* spp. detection, were tested in triplicate. In a second reaction, also performed in triplicate, the swab samples were tested with the oligonucleotides MC1 and MC2, in the amplification of 447 bp (CORTES et al., 2004). The positive control for the reactions was a DNA sample from *L. infantum* (syn. *L. chagasi*) MCAN/BR/1984/CCC-17.481 provided by the Laboratory of Leishmaniasis of the Centro de Pesquisas em Leishmanioses, Fiocruz (Rio de Janeiro, Brazil) and the negative control was sterile, deionized water.

Results and Discussion

Fifty two cats were analyzed using conjunctival swab PCR (CS-PCR), of these, 28.6% (2/7) from the city of Pirassununga and 11.1% (5/45) from the city of Ilha Solteira - SP were positive for generic 13A and 13B primers (RODGERS et al., 1990). In total, 13.5% of the cats were positive for *Leishmania* spp. None of the animals was positive for the primers MC1 and MC2 (*L. infantum*).

Although there is a single full article about the use of ocular conjunctiva swabs to detect *Leishmania* in felines (CHATZIS et al., 2014), the use of conjunctival swabs in this species has had its efficacy proven in the diagnosis of several etiological agents. CS-PCR has been successfully used to detect diseases such as feline herpesvirus (FHV-1), *Chlamydophila felis*, and *Mycoplasma felis* (HILLSTRÖM et al., 2012).

This method utilizing the swab to collect epithelial cell samples from the conjunctiva has been tested in canine VL detection by PCR and has been proven highly sensitive in diagnosing the disease in symptomatic (STRAUSS-AYALI et al., 2004; FERREIRA et al., 2008; PILATTI et al., 2009) and asymptomatic dogs (ALMEIDA FERREIRA et al., 2012). Thus, non-invasive sampling is very desirable because obtaining the samples is less complex, does not require skilled labor, and facilitates the bulk collection of samples.

Furthermore, PCR has demonstrated consistent results compared with other diagnostic tests, such as microscopy and parasite culture, especially when PCR is used with samples containing a low amount of the parasite, such as blood (GARCIA et al., 2005) and conjunctival cells (ALMEIDA FERREIRA et al., 2012). This type of sample collection has particular importance in cats because they may have a low parasitic load, which can complicate the parasitological diagnosis (QUINNELL & COURTENAY, 2009).

In all felines (n=52) from which samples were collected in this study, no clinical signs that suggest leishmaniasis were observed, which corroborates the results of Chatzis et al. (2014), in which the detection CS-PCR was compared in two groups, 50 clinically normal cats and 50 with clinical signs, CS-PCR detected more cats without clinical signs (4%). APAISA and Pirassununga´s CCZ were informed about positive cats.

According to data from the Epidemiological Surveillance Center of São Paulo State (CVE-SP), from 2007 to 2014, 8 cases of human cutaneous leishmaniasis were confirmed in Pirassununga (CVE, 2014). No official data were found regarding the occurrence of VL in humans in this city. In Ilha Solteira, which is an endemic area for canine VL (ASSIS et al., 2010; QUEIROZ et al., 2010), there was one case of human cutaneous leishmaniasis and one case of human VL. In our study, none of the samples was positive for the specific primers for *L. infantum*. Such information suggests that different *Leishmania* spp. species circulate both in endemic and non-endemic areas of VL, thus emphasizing the importance of using PCR, because it allows identification of the species that infects the animal (relating it to the phylogenetic and phylogeographic origins) and its implication in prevention of the disease.

Conclusion

The swab collection proved to be practical, easier, and less invasive, which confirms the potential use of this type of sample collection in studies using cats. The detection of *Leishmania* spp. infection in cats, combined with the absence of clinical signs, might indicate that these animals are healthy carriers of these parasites.

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