Molecular and parasitological detection of *Leishmania* spp. in a dipteran of the species *Tabanus importunus*

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Abstract

Leishmaniasis is an important chronic zoonosis caused by protozoa of the genus *Leishmania* spp. The major vectors of this protozoosis are sand flies, and *Lutzomyia longipalpis* is considered the main species implicated in the transmission of American Visceral Leishmaniasis in Brazil. The presence of the parasite’s deoxyribonucleic acid (DNA) in ectoparasites such as ticks and fleas has prompted speculations about the existence of new vectors in the cycle of leishmaniasis. The aim of this paper is to report the molecular detection of *Leishmania* spp. in a horse fly of the species *Tabanus importunus* which parasitized an oligosymptomatic dog infected with *Leishmania* spp. Molecular amplification of the protozoan’s DNA in the head, thoracic region and abdomen of the tabanid tested positive for *Leishmania* complex. This is the first report of the presence of DNA from *Leishmania* spp. in dipterous insects of the species *T. importunus*.

Keywords: Dogs, canine visceral leishmaniasis, horse fly, PCR, protozoan, vectors.

Introduction

Leishmaniasis are an important disease, with a range of clinical and epidemiological features, more frequently reported in regions where several species of sand flies exist, particularly the species of *Lutzomyia longipalpis* (LAURENTI et al., 2009; SOARES et al., 2010; COSTA et al., 2013).

Although leishmaniasis is transmitted by sand fly bites infection via other routes have been reported, including congenital transmission in dogs (ROSYPAL et al., 2005; DA SILVA et al., 2009) and blood transfusions (DE FREITAS et al., 2006; GOODNOUGH, 2013). Among other possibilities of transmission, researchers have reported the presence of DNA from this parasite in ixodids of the species *Rhipicephalus sanguineus* and in fleas of the species *Ctenocephalides felis felis*, suggesting that these arthropods may act positively in the epidemiology of this disease (OTRANTO; DANTAS-TORRES, 2010). Horse flies are hematophagous insects known to be important vectors of several etiologic agents that cause diseases, particularly trypanosomiasis (SILVA et al., 1996).

The aim of this paper is to report the molecular detection of *Leishmania* spp. in a horse fly of the species *Tabanus importunus* captured in the region of Andradina, state of São Paulo, Brazil.

Findings

An adult male dog of undefined breed, short-haired and predominantly black, was sent to the Veterinary Hospital of Andradina Educational College in the state of São Paulo (20.8961°,
T. importunus infected by Leishmania spp.

The molecular analysis indicated that all the samples from the arthropod (H, T, A, and W) and from the dog were positive for Leishmania complex, and the reference value adopted was the negative result. Microscopic analysis of the smear from W showed amastigotes forms of Leishmania spp. (Figure 1).

Leishmaniasis is an important zoonosis whose cycle is classically characterized by the presence of dipterous vectors of the genus Lutzomyia (GALATI et al., 2003; KAMHAWI, 2006; SILVA et al., 2008). However, entomological studies carried out in different Brazilian municipalities did not confirm the presence of this phlebotomine in areas endemic to L. chagasi (DANTAS-TORRES, 2006). Thus, the possible existence of a new vector has been suggested in the epidemiology of this parasitosis (PAZ, 2010a).

Several researchers have mentioned the possibility that R. sanguineus acts as a vector of leishmaniasis (PAZ et al., 2010b), either through its bite (DANTAS-TORRES et al., 2010) or through the ingestion of infected ticks (COUTINHO et al., 2005). Similarly, fleas have been reported as vectors of Leishmania spp. (COUTINHO; LINARDI, 2007; PAZ, 2010a). However, the detection of Leishmania spp. DNA does not suffice to imply a species as vector (SAVANI et al., 2009).

In this sense, coinfection by Leishmania (Leishmania) chagasi and Trypanosoma (Trypanozoon) evansi has already been found in a dog from the state of Mato Grosso do Sul (SAVANI et al., 2005).

In this region, the non-selective behavior of these tabanids for host species is worthy of note, as it is an endemic area for human and canine visceral leishmaniasis. This fact is worth highlighting because tabanids are considered important vectors of trypanosomiasis in animals (NUNES, 1996; FRANKE et al., 1994; HERRERA et al., 2004).

The epidemiology of leishmaniasis is a constantly expanding theme and the action of new vectors in the cycle of this disease must be considered. Thus, this paper offers the first report of the presence of amastigotes forms and DNA of Leishmania spp. in a horse fly of the species T. importunus parasitizing a dog that was a carrier of canine visceral leishmaniasis, suggesting the possibility that the dipteran of species Tabanus importunus act as mechanical vectors in the cycle of this zoonosis.

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Figure 1. Tabanus importunus infected by Leishmania spp.
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