

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

Detecção molecular e parasitológica de *Leishmania* spp. em díptero da espécie *Tabanus importunus*

Willian Marinho Dourado Coelho^{1*}; Katia Denise Saraiva Bresciani¹

¹Departamento de Apoio, Produção e Saúde Animal, Faculdade de Medicina Veterinária de Araçatuba – FMVA, Universidade Estadual Paulista – UNESP, Araçatuba, SP, Brasil

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Abstract

Leishmaniasis is an important chronic zoonosis caused by protozoa of the genus *Leishmania* spp. The major vectors of this protozosis 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.

Resumo

A leishmaniose é uma importante zoonose, de caráter crônico, causada por protozoários do gênero *Leishmania* spp. Esta protozoose tem como principal vetor os flebotomíneos, sendo que, no Brasil, o *Lutzomyia longipalpis* é a principal espécie incriminada na transmissão da leishmaniose Visceral Americana. A presença do ácido desoxirribonucleico (DNA) do parasito em ectoparasitos, como carrapatos e pulgas, tem gerado especulações quanto a existência de novos vetores no ciclo da leishmaniose. Foi objetivo deste estudo relatar a detecção molecular de *Leishmania* spp. em uma mutuca da espécie *Tabanus importunus* que parasitava um cão oligossintomático infectado por *Leishmania* spp. A análise molecular amplificou o DNA do protozoário na cabeça, na região torácica e no abdômen do tabanídeo, resultando como positivo para o complexo *Leishmania*. Este é o primeiro relato da presença de DNA de *Leishmania* spp. em insetos dípteros da espécie *T. importunus*.

Palavras-chave: Cães, leishmaniose visceral canina, Mutuca, PCR, protozoário, vetores.

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 (ROSPAL 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°,

*Corresponding author: Willian Marinho Dourado Coelho
 Departamento de Apoio, Produção e Saúde Animal, Faculdade de Medicina Veterinária de Araçatuba – FMVA, Universidade Estadual Paulista – UNESP, Rua Clóvis Pestana, 793, CEP 16050-680, Araçatuba, SP, Brasil
 e-mail: willianmarinho@hotmail.com

51.37944°, 405 m altitude). This animal lived in a peri-urban zone in a house with a large yard shaded by fruit and ornamental trees, in the company of other animals such as exotic caged birds and chickens in a chicken run.

The dog was clinically characterized as oligosymptomatic for leishmaniasis (onychogryphosis, weight loss, lymphadenomegaly, muscle atrophy and dermatitis). During anamnesis at the animal's place of origin, a horse fly was observed sucking blood on the dog's left dorsal thoracic region.

In this municipality and surroundings, these tabanids frequently attack farm animals such as cattle, horses and goats, and parasitize dogs, nestlings in their nests, and even people.

The insect was captured by hand, stored in a plastic tube and frozen at -20°C . The animal was diagnosed parasitologically as a carrier of *Leishmania* spp. infection, since numerous amastigote forms of *Leishmania* spp. were visible in the imprint of the right popliteal lymph node. The dog was euthanized according to Brazilian legislation, as per Resolution no. 1000/2012 of the Federal Council of Veterinary Medicine, and subjected to necropsy.

The dipteran collected from the animal was divided into three parts with a bistoury, and these parts were separated into aliquots and identified as follows: head (H_1), thorax (T_1) and abdomen (A_1). When stored in the tube, the insect ejected a droplet of blood-like material from the posterior end of its abdomen (Figure 1). The tube was washed with 0.5 mL of 0.9% sterile sodium chloride solution and the sample was classified as washed (W_1).

The insect parts were macerated in a porcelain crucible containing 0.5 mL of the same physiological solution. Similarly, the blood sample and the lymph node fragment from the dog were collected, divided into aliquots and stored at -20°C for analysis by polymerase chain reaction (PCR).

The aliquots were subjected to molecular analysis by PCR, using oligonucleotides that amplify the conserved region of the kinetoplast (kDNA) minicircle, using the primers 13A (5'-GTG GGG GAG GGG CGT TCT -3') e 13B (5'-ATT TTA CAC CAA CCC CCA GTT-3') (RODGERS et al., 1990). These analyses were performed by an outsourced laboratory known for its accurate diagnosis of animal and human leishmaniasis.

Part of W_1 was used to prepare the smear on a microscope slide. This material was fixed and stained using a "Panótico Rápido®" kit and was examined under 1000x magnification in a light



Figure 1. *Tabanus importunus* infected by *Leishmania* spp.

microscope equipped with a flat achromatic lens; 300 microscope fields were examined.

The molecular analysis indicated that all the samples from the arthropod (H_1 , T_1 , A_1 and W_1) 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_1 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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