Are *Aedes aegypti* mosquitoes potential vectors for leishmaniasis? – Case report

Mosquitos Aedes aegypti *são vetores potenciais de leishmaniose? – Relato de caso*

Willian Marinho Dourado COELHO[1,2]; Katia Denise Saraiva BRESCIANI[3];
Juliana de Carvalho Apolinário COÊLHO[4]; Luciano Alves dos ANJOS[1]; Wilma Aparecida Starke BUZETTI[1]

[1] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Engenharia, Departamento de Biologia e Zootecnia, Ilha Solteira – SP, Brazil
[2] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias, Departamento de Patologia Clínica e Patologia Animal, Andradina – SP, Brazil
[3] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina Veterinária, Araçatuba – SP, Brazil
[4] Faculdades Integradas de Três Lagoas, Três Lagoas – MS, Brazil

Abstract

In Brazil dipters of the *Lutzomyia* genus are the main vectors of leishmaniasis for humans and animals. However, other hematophagous insects such as ticks, fleas, and horse flies may also be considered potential vectors of this protozoon. This paper, regarding an endemic area for visceral leishmaniasis, is the the first description of the *Leishmania* spp. presence in *Aedes aegypti* mosquitoes. Two *A. aegypti* mosquitoes were captured: one of them was feeding on a polysymptomatic dog with leishmaniasis, confirmed by parasitic demonstration and positive PCR for *Leishmania* spp., and the other was collected in the environment where the dog was isolated. The mosquito engorged with dog's blood was crushed between two microscopic slides and the other one was processed by the polymerase chain reaction assay (PCR) searching for the presence of *Leishmania* spp. DNA. Amastigote forms of Leishmania sp, were observed in the smear prepared from one mosquito by microscopic examination, as well as other protozoa's flagellated forms. In the other insect it was observed *Leishmania* DNA amplification. This observation reinforces the role of dogs as sources of infection of *Leishmania* spp. even to other potential vector species.

Keywords: Amastigote. Canine. Parasite. Vectors. Zoonosis.

Resumo

No Brasil, os dípteros do gênero *Lutzomyia* são os principais vetores da leishmaniose para humanos e animais. No entanto, tem sido constatado que outras espécies de invertebrados hematófagos, como carrapatos, pulgas e mutucas, também podem ser vetores desse protozoário. Este trabalho, realizado em uma área endêmica de leishmaniose visceral, é a primeira descrição da presença de *Leishmania* spp. em mosquitos da espécie *A. aegypti*. Dois mosquitos *A. aegypti* foram capturados no local onde estava isolado um cão polissintomático acometido por leishmaniose visceral, confirmada pela demonstração do parasita em biópsias de órgãos e por resultado positivo na prova de PCR para *Leishmania* spp. Um dos mosquitos estava sugando o sangue do cão e o outro estava livre no ambiente. O mosquito ingurgitado com o sangue do animal foi esmagado entre duas lâminas de microscopia e o outro foi processado por meio da reação em cadeia pela polimerase (PCR) aplicada à pesquisa do ADN de *Leishmania* spp. Ao exame microscópico do esfregaço preparado com o mosquito que estava parasitando o cão foram observadas formas amastigotas de *Leishmania* spp., bem como formas flageladas de outra espécie de protozoário. No outro inseto foi detectada amplificação de ADN do gênero *Leishmania*. Esta constatação reforça o papel dos cães como fontes de infecção de *Leishmania* spp. até mesmo para outras espécies de vetores potenciais.

Palavras-chave: Amastigota. Canino. Parasita. Vetores. Zoonose.

DOI: 10.11606/issn.1678-4456.bjvras.2017.131768

Correspondence to:
Willian Marinho Dourado Coelho
Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Engenharia, Departamento de Biologia e Zootecnia
Rua Monção, 226
CEP 15385-000, Ilha Solteira, SP, Brazil
e-mail: willianmarinho@hotmail.com

Received: 03/05/2017
Approved: 19/10/2017

Introduction

In Brazil leishmaniasis is a serious vector-borne disease with a complex epidemiology (CAVALCANTE; VALE, 2014). Sandflies are the main transmission form of this parasite (GONTIJO; MELO, 2004). However, this protozoon has also been observed in other hematophagous arthropods such as ticks, fleas, and horseflies (COELHO et al., 2016), suggesting the existence of other potential vectors to this protozoon (CAMPOS; COSTA, 2014).

The present paper is the description of *Leishmania* spp. presence in *Aedes aegypti* mosquito collected during blood meal in a polysymptomatic dog with laboratory-confirmed visceral leishmaniasis.

A. aegypti is an important anthropophilic parasite, considered the main vector of dengue (TAUIL, 2001), urban yellow fever (BRAGA; VALLE, 2007), Zika virus (VASCONCELOS, 2015), and Chikungunya fever (DONALÍSIO; FREITAS, 2015). In dogs this parasite can act as a vector of heartworm disease (HENDRIX et al., 1986).

Case report

Two mosquitoes of *A. aegypti* species were analyzed for the presence of *Leishmania* spp. The insects were captured in the urban area in the municipality of Andradina (20.8961º, 51.37944º, altitude 405 m), São Paulo State, Brazil, which is an endemic area of visceral leishmaniasis (VL). One of these insects, captured inside a portable dog house, was engorged with the dog's blood. The other mosquito was captured in the environment near the place where the dog was hospitalized. The dog (Figure 1) was a male adult, non-pure breed, and had alopecia with generalized dermatitis, onychogrifosis, emaciation, lymphadenomegaly, hepatomegaly, and epistaxis. The investigation of dog's infection with *Leishmania* spp. was performed by biopsies of sternum bone marrow and of the left popliteal lymph node. A fragment of ear skin was removed for histopathological analysis. A blood sample was collected from the cephalic vein in a tube containing EDTA for parasitological analysis and PCR reaction to *Leishmania* DNA amplification using the pair of oligonucleotides described by Rodgers et al. (1990).

One mosquito was crushed between microscopic slides, and the smear was submitted to haematological staining with Panótico Rápido® kit and observed using a 10x wide field micrometer eyepiece (Bioval®) with 100-fold magnification; 300 microscope fields were examined.

The other mosquito was macerated in a porcelain crucible containing 0.5 mL of sterile physiological solution and DNA was extracted using the GFX genomic blood kit®

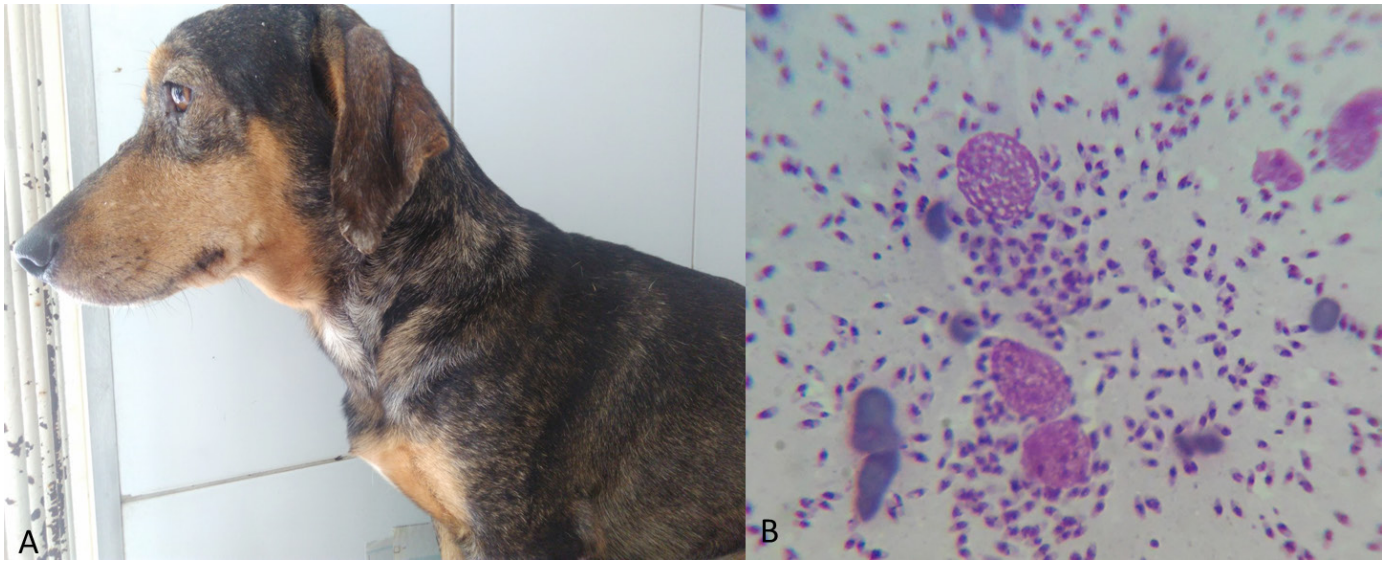

Figure 1 – (A) Dog with leishmaniasis presenting skin lesions. The animal was parasitized by *Aedes aegypti* mosquitoes. (B) Sample of dog's popliteal lymph node presenting large amount of *Leishmania* spp. amastigote forms (100x)

(Amersham Biotech, Piscataway, New Jersey, USA) for a final volume of 100 µL (NUNES et al., 2007).

DNA amplification by PCR was performed using oligonucleotides that amplify the conserved region of the *Leishmania* kinetoplast (kDNA) minicircle, using the primers 13A (5'-GTG GGG GAG GGG CGT TCT -3') and 13B (5'-ATT TTA CAC CAA CCC CCA GTT-3') (RODGERS et al. 1990), which amplifies a 120 pb kDNA fragment. A PCR-negative control (ultrapure water) was used. This analysis was performed by an outsourced laboratory known for its accurate diagnosis of animal and human leishmaniasis (Hermes Pardini, Belo Horizonte, Minas Gerais, Brazil).

Leishmania spp. amastigotes forms were observed in bone marrow, lymph node, and dog skin samples. No parasitic evolutionary forms were found in the blood

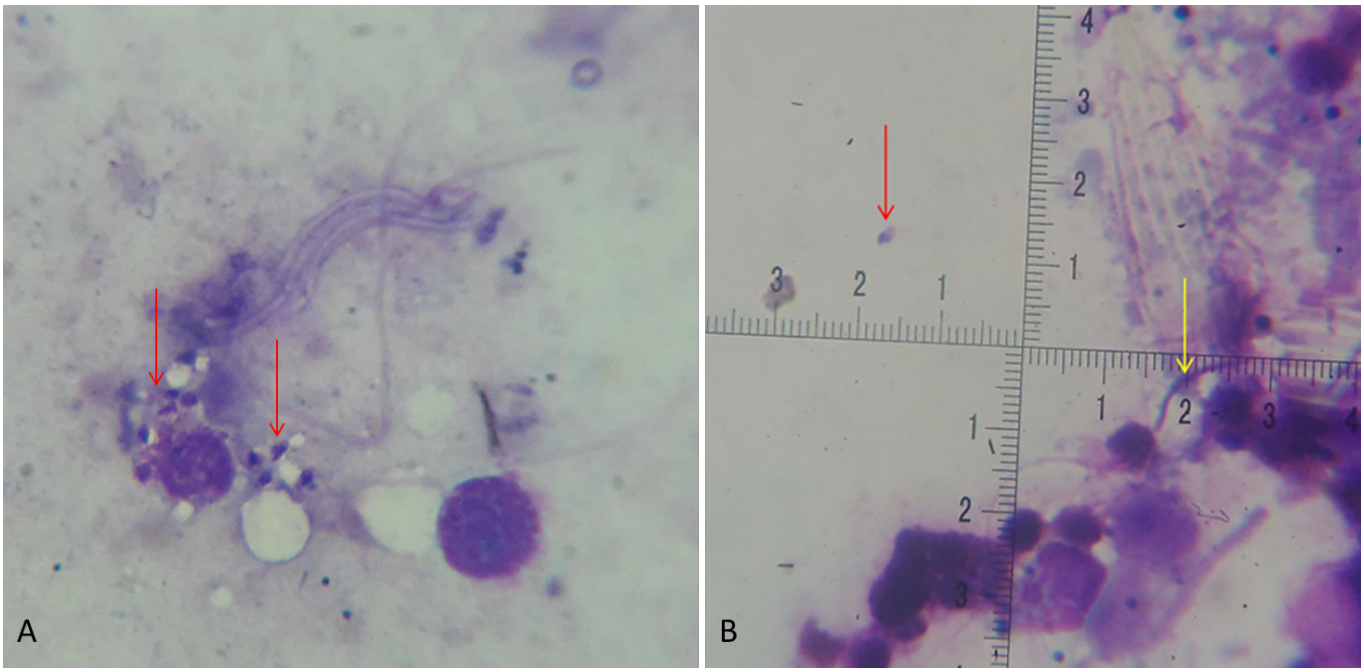

Figure 2 – Amastigote forms of *Leishmania* spp. (red arrow) found in a mosquito of the species *Aedes aegypti* along with another flagellate protozoan species (yellow arrow) (100 x)

smear. *Leishmania* spp. DNA amplification was obtained in the dog blood sample.

Amastigote forms of the genus *Leishmania* were observed in the smear prepared from the first mosquito, as well as flagellated forms of other protozoa species (Figure 2). These protozoa presented an average body size of 21 µm with flagellum of up to 73 µm. The molecular analysis indicated that the sampled mosquito was positive for *Leishmania spp.*

One of the limitations of this study, besides the sample size, is that *Leishmania* amastigote forms or amplified DNA were observed within the mosquitoes that had been fed on the dog but could be not infected by the parasite. Other studies must be done to show the infection of *A. aegypti*, for instance, by its gut microscopy evaluation.

A research carried out in this same region also observed horse flies of *Tabanus importunus* species with the presence of amastigote forms of *Leishmania* spp. (COELHO; BRESCIANI, 2013; COELHO et al., 2016). Viol et al. (2016) also found forms of the *Leishmania* genus in ticks. These findings indicate that other ectoparasites, including culicidae (as in the present study) may also become infected by *Leishmania* spp, due to their feeding habits. This is the first description of the presence of *Leishmania* spp. in *A. aegypti* mosquitoes after feeding in a dog with visceral leishmaniasis, demonstrating that dogs may act as a source of infection of this parasite even to other potential vector species.

Conflict of Interest Statement

There was no conflict of interest during the execution of this study.

Acknowledgments

We thank the veterinarians Aziz Abdelnour and Antonio Gilberto Gasparelli Junior, owners of CLINICÃO, for the use of the veterinary clinic where the study was developed and for the financial support.

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