

## Histochemical and molecular evaluation of the prevalence of *Leishmania* spp. in hematophagous insects



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### ABSTRACT

The prevalence study of *Leishmania* spp. in hematophagous insects captured from the environment in bat roosts and pigeon nests, or feeding their hosts (cattle, pigs, horses, dogs and humans) in urban, peri-urban and rural areas, between 2012 and 2014. For this study, the amastigotes present in these insects were detected by histochemical and PCR techniques. Positive gene amplification for *Leishmania* was found in two horseflies of the species *Tabanus importunus* collected in the environment, and amastigote forms of *Leishmania* spp., as well as erythrocytes and leukocytes, were histochemically detected in one of that insect. The other analyzed insects were not positive by PCR nor by direct parasitological examination. Only horseflies captured in urban and peri-urban areas were positive. During the collection, no phlebotomine sand flies were captured in rural areas far from the city limits. It can be concluded that the discovery of horseflies positive for *Leishmania* spp. in urban and peri-urban areas indicates the likelihood that urban areas and their surroundings provide vector parasites with an environment suitable for the spread and consequent perpetuation of the biological cycle of this protozoan.

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## 1. Introduction

Leishmaniasis is a zoonotic disease widely distributed throughout Brazil, and phlebotomine sand flies act as its main vector (Savani et al., 2009; Silva et al., 2008). This disease, whose epidemiological characteristics are predominantly rural, finds ideal conditions to urbanize its cycle in the precarious sanitary conditions of urban and peri-urban environments (Bevilacqua et al., 2001; Da Silva and Cunha, 2007; Góes et al., 2013).

The northwest region of the state of São Paulo is an endemic area for the occurrence of this protozoan, and natural infection in cats (Coelho et al., 2010, 2011) as well as coinfection by *Leishmania chagasi* and *Trypanosoma evansi* in a dog from this same region have been described. The presence of amastigote forms of *Leishmania* spp. in a horsefly in this area was also reported for the first time (Coelho and Bresciani, 2013).

In view of the existence of new forms of transmission (Da Silva et al., 2009; De Freitas et al., 2006) and the possible action of new vectors (Coutinho and Linardi, 2007; Coutinho et al., 2005; Otranto and Dantas-Torres, 2010; Paz et al., 2010a, 2010b), the

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purpose of this study was to use histochemical and polymerase chain reaction (PCR) techniques to evaluate the prevalence of *Leishmania* spp. in hematophagous dipterans captured in the environment in bat roosts and pigeon nests, or parasitizing cattle, pigs, horses, dogs and humans.

## 2. Material and methods

### 2.1. Study sites

In the period of October 2012 to October 2014, hematophagous insects were captured in urban, peri-urban and rural areas in the municipality of Andradina (20.8961°, 51.37944°, altitude 405 m) (Figs. 1 and 2), in areas close to the Tietê River in the municipalities of Pereira Barreto (20.3818°, 51.0633°, altitude 347 m) and Itapura (20.3846°, 51.3032°, altitude 318 m), and in areas close to the Paraná River in the municipality of Castilho (20.5220°, 51.2915°, altitude 365 m), all in the state of São Paulo, Brazil (Fig. 2).

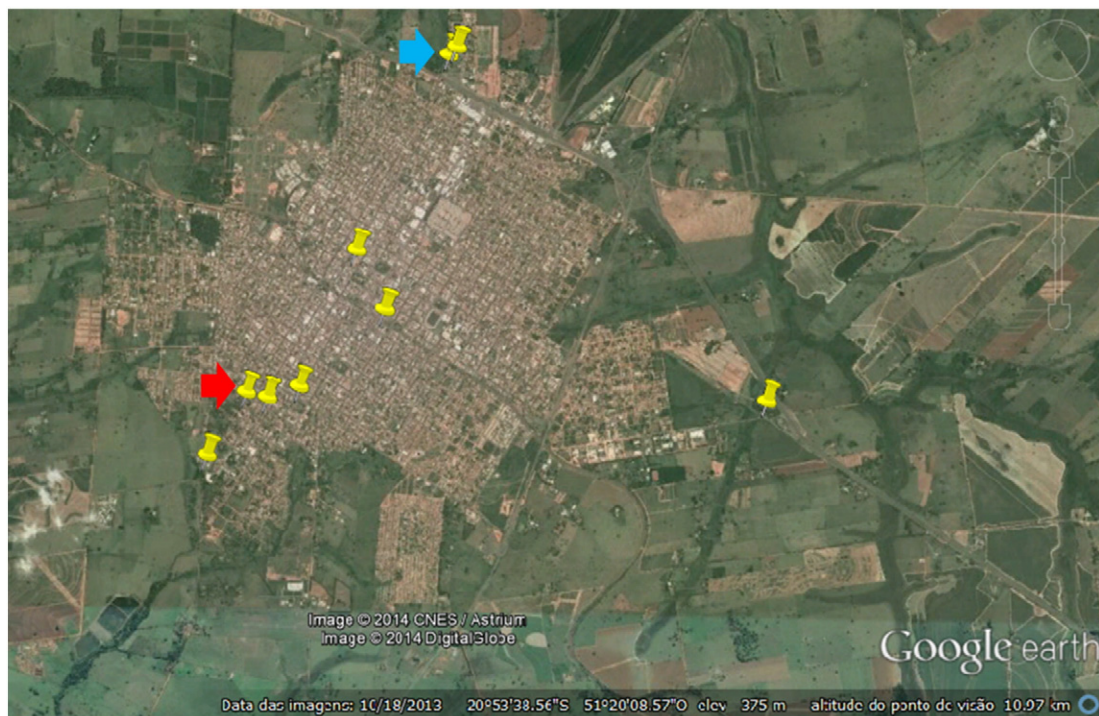
### 2.2. Insect capture

A total of 187 horseflies were collected, 55.61% (104/187) of the genus *Tabanus* spp., 31.5% (59/187) of *Chrysops* spp., 2.13% (4/187) of *Chlorotabanus* spp., 4.27% (8/187) of *Dicladocera* spp. and 6.41% (12/187) of *Lepiselaga* spp. The insects were captured manually and with entomological nets, during the daytime until twilight, directly from the environment in the proximities of bat roosts and pigeon nests, or on cattle, horses, pigs, dogs and humans. The tabanid flies were classified by consulting taxonomic articles (Benchimol and Sá, 2005; Coscarón and Papavero, 2009).

The 83 black flies (*Simulium* spp.) that were collected were found only in the vicinity of forests and areas of pastureland close to rural homes as they are parasites of humans during the day and at night.

A total of 77 stable flies (*Stomoxys calcitrans*) were collected from the environment in residential urban, peri-urban and rural areas, and on horses and humans.

To capture phlebotomine sand flies, light traps and carbon dioxide traps were placed in rural areas in the proximities of permanent preservation areas, forests, horse stables, cattle sheds, pigsties, chicken coops and pastures. Collection efforts were carried out at least twice a week. However, no insect was captured in these areas during the period of this study.



**Fig. 1.** Collection sites of hematophagous insects (yellow markers) in the urban and peri-urban perimeters of the municipality of Andradina, SP, from Oct. 2012 to Oct. 2014. Legend: yellow markers (collection sites); red arrow (pool of horseflies with gene amplification positive for *Leishmania* spp.); blue arrow (horsefly with amastigote form of *Leishmania* spp.).

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