

## Miltefosine susceptibility of isolates of *Leishmania (Leishmania) infantum* from dogs of the municipality of Embu-Guaçu, Brazil

Bianca A. Ferreira\*, Edite H. Y. Kanashiro, Mussya C. Rocha, Paulo C. Cotrim, Adriano C. Coelho

### Abstract

Visceral leishmaniasis (VL) is a parasitic disease caused by the protozoan *Leishmania (L.) infantum*. In Brazil, the number of cases of the disease has increased in the last years. The treatment of leishmaniasis in Brazil consists of the use of pentavalent antimonials and amphotericin B. Recently, miltefosine has been shown to be highly effective against VL in Asia. Although, this drug is not used in the treatment of VL in Brazil, miltefosine is approved for use in the treatment of canine visceral leishmaniasis (CVL). In this study, we evaluate the susceptibility to miltefosine *in vitro* of isolates of *L. (L.) infantum* from dogs of municipality of Embu-Guaçu, located in the metropolitan region of the city of São Paulo.

**Key words:** visceral leishmaniasis, *Leishmania infantum*, miltefosine, drug susceptibility.

### Introduction

Visceral leishmaniasis is a parasitic disease caused by the protozoan *L. (L.) infantum*. The disease is the most severe clinical form of leishmaniasis that can lead to death if it is not treated. In Brazil, about 3,000 new cases of the disease are reported annually, with an increasing number of cases in urban and periurban areas. Since VL is zoonotic in Brazil, domestic dogs constitute the main reservoir for the parasite, playing an essential role in transmission of disease to humans. The treatment of VL in Brazil consists in the use of pentavalent antimonials and amphotericin B, drugs that are considered expensive, toxic and that require parenteral administration. In CVL, the only drug used for treatment is miltefosine, a drug already used in the treatment of VL in Southeast Asia. In this study, we aim to evaluate the susceptibility to miltefosine *in vitro* of isolates of *L. (L.) infantum* from dogs of the municipality of Embu-Guaçu. Considering the potential of miltefosine be used in the chemotherapy of VL in the near future, it is urgent to investigate the susceptibility of *L. (L.) infantum* from dogs that are potential reservoirs of the disease in Brazilian endemic regions.

### Results and Discussion

Previously, isolates were typed by Instituto Oswaldo Cruz, FIOCRUZ, RJ, Brazil according to the protocol described by Cupolillo et al., 1994<sup>1</sup> and then confirmed by polymerase chain reaction (PCR) of *hsp70* gene followed by digestion with the restriction enzyme *Hae* III<sup>2</sup> (Fig. 1A and 1B). The *in vitro* susceptibility of isolates and the reference strains of *L. (L.) infantum* to miltefosine in promastigotes were determined as previously described<sup>3</sup>. The EC<sub>50</sub> values of miltefosine against promastigotes ranged from 6,5 ± 0,9 to 34,14 ± 1,56 µM (Fig. 1C). These findings suggest a moderate variation in miltefosine susceptibility of these isolates from dogs.

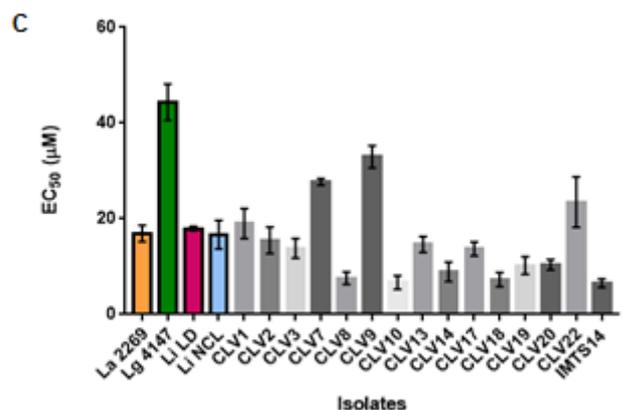


Figure 1. **A.** PCR amplification of the *hsp70* gene (1,286 bp). **B.** Digestion of the PCR amplified product with the restriction enzyme *Hae* III. Legend: 1- *L. (L.) infantum* LD; 2- *L. (L.) infantum* NCL; 3- CVL1; 4- CVL2; 5- CVL3; 6- CVL7; 7- CVL8; 8- CVL9; 9- CVL10; 10- CVL13; 11- CVL14; 12- CVL17; 13- CVL18; 14- CVL19; 15- CVL20; 16- CVL22; 17- IMTS 14; 18- *L. (L.) amazonensis* (MHOM/BR/1973/M2269); 19- *L. (V.) braziliensis* (MHOM/BR/1975/M2903); 20- *L. (V.) guyanensis* (MHOM/BR/1975/M4147). **C.** EC<sub>50</sub> values determined against promastigotes of isolates from dogs.

### Conclusions

The results obtained in this study will contribute to evaluate the potential of miltefosine against isolates of *L. (L.) infantum* from domestic dogs, the most important reservoir of VL in urban areas in Brazil.

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<sup>1</sup> Cupolillo, E.; Grimaldi, G.Jr.; Momen, H. Am J. A general classification of New World *Leishmania* using numerical zymotaxonomy. *Trop Med Hyg.* **1994**, 50(3):296-311.

<sup>2</sup> Montalvo, A.M.J.; Fraga, et al. Three new sensitive and specific heat-shock protein 70 PCRs for global *Leishmania* species identification. *Eur J Clin Microbiol Infect Dis.* **2012**, 31(7): 1453-61.

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