

**Miltefosine activity in vitro against isolates of *Leishmania (Leishmania) infantum* from dogs of the municipality of Embu-Guaçu**

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**Abstract**

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

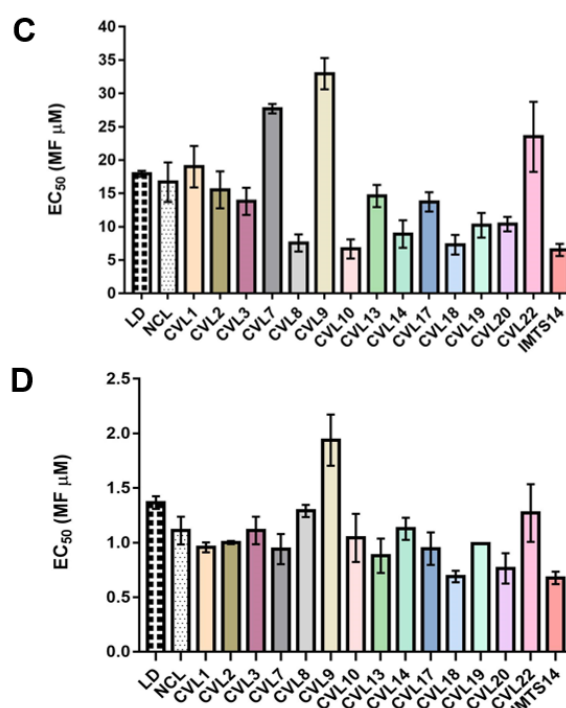
**Key words:** visceral leishmaniasis, *Leishmania infantum*, miltefosine, drug susceptibility.

**Introduction**

Visceral leishmaniasis is a parasitic disease caused by the protozoan parasite *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 MF, a drug already used in the treatment of VL in Southeast Asia. In this study, we aim to evaluate the susceptibility to MF *in vitro* of isolates of *L. (L.) infantum* from dogs of the municipality of Embu-Guaçu. Considering the potential of MF 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**

Isolates were initially typed by polymerase chain reaction (PCR) of *hsp70* gene followed by digestion with the restriction enzyme *HaeIII* as previously described<sup>1</sup> (Fig. 1A and 1B). The *in vitro* susceptibility of isolates to MF in promastigote and amastigote form were determined by calculating the EC<sub>50</sub> and EC<sub>90</sub> values. The EC<sub>50</sub> values of MF against promastigotes ranged from 6.5 to 34.14  $\mu$ M (Fig. 1C), while the EC<sub>50</sub> values of MF against the intracellular amastigote form varied from 0.6 to 2.07  $\mu$ M (Fig. 1D). These findings suggest a moderate variation in MF susceptibility of these isolates from dogs.



**Figure 1.** A. PCR amplification of the *hsp70* gene (1,286 bp amplified product). B. Digestion of the PCR product with the restriction enzyme *HaeIII*. Legend: 1- *L. (L.) infantum* (MHOM/BR/1972/LD); 2- *L. (L.) infantum* (MHOM/BR/2005/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). C. EC<sub>50</sub> values determined against promastigotes of isolates from dogs. D. EC<sub>50</sub> values determined against intracellular amastigotes 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.

**Acknowledgement**

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<sup>1</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.

