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 parasites, 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 described1 (Fig. 1A and 1B). The in vitro susceptibility of isolates to MF in promastigote and amastigote form were determined by calculating the EC50 and EC90 values. The EC50 values of MF against promastigotes ranged from 6.5 to 34.14 μM (Fig. 1C), while the EC90 values of MF against the intracellular amastigote form varied from 0.6 to 2.07 μM (Fig. 1D). These findings suggest a moderate variation in MF susceptibility of these 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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