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Investigation of susceptibility to paromomycin in isolates from dogs of Leishmania (Leishmania) infantum

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Abstract
In Brazil, visceral leishmaniasis (VL) is a parasitic disease caused by the protozoan Leishmania (Leishmania) infantum. This disease is serious and may be lethal if not treated. The treatment of leishmaniasis in Brazil consists in the use of pentavalent antimonials and/or amphotericin B. These drugs are toxic, have several side effects and the effectiveness of treatment has decreased in the last years. Paromomycin is an alternative drug already used in the treatment of VL in Asia with effectiveness rate higher than 90%. In this project, we aimed to evaluate the susceptibility in vitro to paromomycin of isolates of L. (L.) infantum from dogs of the city of Embu-Guaçu, State of São Paulo.

Key words: *visceral leishmaniasis, Leishmania infantum, paromomycin, drug susceptibility*

Introduction

Visceral leishmaniasis is a disease caused by the protozoan parasite *L. (L.) infantum* in South America and Europe. The disease may be lethal if the patient is not treated. In Brazil, about 3,000 new cases of the disease have been reported annually, with an increasing number of cases in urban and periurban areas. VL is zoonotic in Brazil and 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. Paromomycin is an aminoglycoside antibiotic extracted from cultures of *Streptomyces riomosus var.* This drug is highly effective against *L. (L.) donovani*, the parasite responsible for VL in Asia. It is urgent to investigate the potential of this drug against *L. (L.) infantum*, the species responsible for VL in the Mediterranean and Latin America. In this study, we aim to evaluate the susceptibility to paromomycin *in vitro* of isolates of *L. (L.) infantum* from dogs of the municipality of Embu-Guaçu, State of São Paulo, Brazil.

Results and Discussion

Isolates of *L. (L.) infantum* from dogs of the city of Embu-Guaçu were previously typed according to the protocol described by Cupolillo *et al*., 1994 [1] and confirmed by the polymerase chain reaction (PCR) of the *hsp70* gene followed by digestion with the restriction enzyme *Hae*III (Fig. 1). A total of 14 isolates were confirmed as *L. (L.) infantum* by this molecular typing method. As control, genomic DNA of a reference strain of *L. (L.) infantum* (LD) was used (Fig. 1).

Paromomycin susceptibility *in vitro* in promastigote form was determined by the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. The EC_{50} values of the isolates demonstrated a moderate variation in susceptibility to paromomycin, ranging from 70.68 µM to 125.6 µM. Our next goal is to determine the activity of paromomycin against the intracellular amastigote form of these isolates.

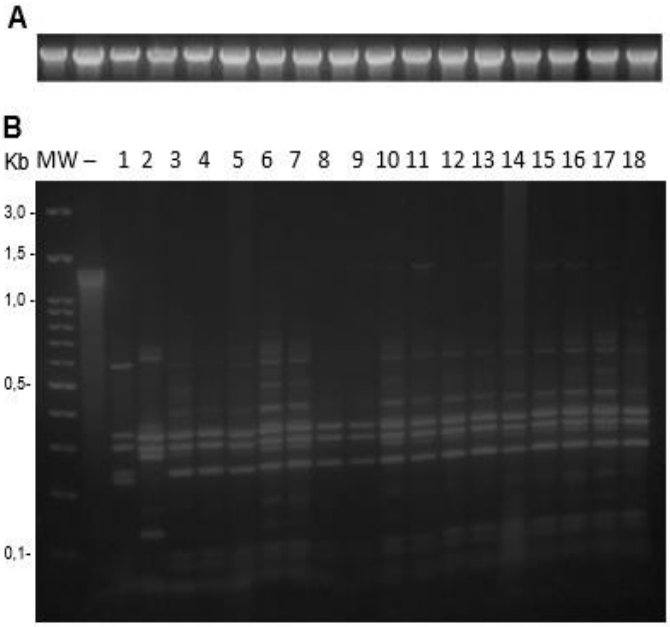

Figure 1. A. PCR amplification of the *hsp70* gene (1,286bp). B. Digestion of the PCR amplified product with the restriction enzyme *Hae*III. Legend: 1- *L. (L.) amazonensis* (M2269); 2- *L. (V.) braziliensis* (M2903); 3- *L. (L.) infantum* (LD); 4- CVL1; 5- CVL2; 6- CVL3; 7- CVL7; 8- CVL8; 9- CVL9; 10- CVL10, 11- CVL13; 12- CVL14; 13- CVL17; 14- CVL18; 15- CVL19; 16- CVL20; 17- CVL22; 18-IMTS14.

Conclusions

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

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[1] Cupolillo, E., G. Grimaldi, Jr., et al. (1994). "A general classification of New World Leishmania using numerical zymotaxonomy." Am J Trop Med Hyg 50(3): 296-311.