Leishmania infantum clinical and veterinary isolates sensitivity to reference drugs: first steps to define the drug sensitivity landscape

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Introduction
Zoonotic visceral leishmaniasis is a perfect example of the “One Health” concept importance. This concept recognizes that the health of people is intertwined with the health of animals that share our environment and will benefit from a unified approach. Thus, it is essential that Health policies include clinical and veterinary perspectives with solid interdisciplinary partnerships.

Some species of Leishmania, like Leishmania infantum, are responsible not only for a human disease called Leishmaniasis, but also for a disease of high veterinary impact called Canine Leishmaniasis. The protozoan responsible for this disease is transmitted by Phlebotomine sandflies. These vectors are responsible for the active circulation of the protozoan parasite between animals and humans.

The disease has few therapeutic options and is often associated with relapses that produce the ideal conditions for the development of resistance to the used pharmaceuticals. This scenario constitutes a considerable risk to human health, as the few available drugs are for veterinary and clinical use. Therefore, it is essential to understand if circulating parasites isolated from infected dogs and humans have increased resistance to commonly used anti-leishmanial drugs.

Aim

➢ Setup the relevant assay to evaluate drug susceptibility using L. infantum promastigotes.
➢ Evaluate susceptibility to reference compounds in circulating L. infantum promastigotes recovered from infected humans and dogs.

Methodology

Fig. 2 – Schematic representation of methodology used for evaluation of L. infantum susceptibility to reference compounds.

Conclusion

• Parasite number and medium composition were shown to influence the IC50 value for some reference drugs.
• JPCMS is significantly more resistant to Amphotericin B (DRI>2) and susceptible to Miltefosine (DRI<0.5).
• HSJ001 was significantly more resistant to Miltefosine (DRI=5).

Fig. 3 – Schematic representation of molecular events and colour associated to the reduction of resazurin to resorufin, and reversible reduction of resorufin to dihydroresorufin.

Fig. 4 – Relation between starting number of parasites per well with absolute growth. Absolute L. infantum wild type and HSJ001 promastigotes growth after 72h, evaluated by resorufin fluorescence emitted at 590nm upon excitation at 544nm, in function of starting inoculum. Each individual point in the depicted curve represents the average and standard deviation of two independent assays performed in duplicate.

Table 1 - EC50 and 95% confidence interval for the five reference compounds in each L. Infantum strain. The represented values are obtained from the merged data from at least 4 independent assays. The EC50 determination is performed using nonlinear regression: log(inhibitor) vs. normalized response - Variable slope in Graph Pad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA.

Fig. 5 – Drug resistance index for each reference compounds. Drug resistance index for each of the reference compounds was calculated using the formula: EC50 of a strain / Median EC50 considering all the tested strains. The horizontal dashed lines represent the two-fold susceptibility or resistance threshold.

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