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EVALUATION OF HOMOGENEITY OF *L. INFANTUM* AND *L. DONOVANI* INFECTION IN THE HAMSTER BY REAL-TIME DNA qPCR AND GIEMSA-STAINED IMPRINTS

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Evaluation of drug efficacy and treatment outcome in *Leishmania*-infected animals and patients is generally based on post-treatment viable parasite burdens in the various target organs. While microscopic examination of Giemsa-stained imprints is still considered as the golden standard for quantification of parasite load, DNA qPCR can be considered as an attractive alternative due to its higher sensitivity and specificity, together with its high accuracy and reproducibility. Both methods depart from a very small piece randomly taken from the target organ (liver or spleen) and total organ burdens are then extrapolated based on the assumption that the infection is homogeneously distributed throughout the organ. To our knowledge, the aspect of homogeneity using microscopy and qPCR has not yet been addressed in-depth.

Taking into account some inherent biological variation, the infection in liver and spleen was highly homogeneous in hamsters that had been infected with 10^7 spleen-derived amastigotes of *L. infantum* and *L. donovani*. In animals treated with miltefosine, the low burdens of parasites were also equally distributed. For both techniques, organs and treatment groups, over 90% of the measurements lie within the acceptable range of 70% variation and over 70% (liver) and 85% (spleen) lie within the more stringent range of 50% variation. Related to its higher sensitivity, burdens determined by DNA qPCR show slightly more variation than by microscopy. The inter-individual variation between hamsters of a same group was found non-significant, illustrating the high reproducibility of the hamster model for both *Leishmania* species.

To focus on viable post-treatment burdens, RNA qPCR is currently being explored, as is the determination of the lowest detection level of infection using the three methods (microscopy, DNA qPCR and RNA qPCR).