DEVELOPMENT OF REAL-TIME PCR TO MONITOR PARASITE LOAD IN *LUTZOMYIA* SP AND ITS USE TO DISCRIMINATE BETWEEN *LEISHMANIA (V) BRAZILIENSIS* AND *LEISHMANIA (L) CHAGASI* INFECTIONS

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Previously we demonstrated the use of multiplex-PCR and non-radioactive hybridization to identify natural infections by *Leishmania* sp in sandfly vectors from endemic areas of Rio de Janeiro, where an infection rate of 2% was inferred. At present, we are optimizing a real-time PCR (SYBR-green) targeting the kDNA minicircles conserved region from *Leishmania*, in order to diagnose and estimate parasite load in wild phlebotomines from distinct areas of Brazil, presenting different endemic levels for both cutaneous and visceral leishmanioses. Groups of ten male *Lutzomyia* from a same species were spiked with serial dilutions of kDNA, total DNA, or *L. braziliensis* and *L. chagasi* promastigotes, consisting in reconstituted samples to generate the standards for absolute quantification. The assay was able to detect DNA concentration corresponding to 10^{-2} parasite/µL in five independent runs. Interestingly, we observed that the dissociation curve analysis of the amplified products was able to distinguish the two tested *Leishmania* species. The amplicons sequencing is currently underway to determine the basis for the temperature melting distinction between both species. This result suggests the potential use of the SYBR-green methodology to discriminate *Leishmania* species circulating in distinct endemic areas from Brazil.
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