MONITORING OF MILTEFORAN® TREATED *Leishmania infantum* NATURALLY INFECTED DOGS THROUGH THE LAMP-BASED LeishID TEST

RAPHAEL FRANÇA OLIVEIRA[1]; FERNANDA DOS SANTOS SILVA[1]; STEPHANIE MEGALE FERREIRA[1]; AMÁBILLI DE SOUZA ROSAR[2]; ALESSANDRA MARA DE SOUSA[1]; PATRÍCIA FLÁVIA QUARESMA[2]; RUBENS LIMA DO MONTE-NETO[1]

[1]GRUPO DE PESQUISAS EM BIOTECNOLOGIA APLICADA AO ESTUDO DE PATÓGENOS - BAP, INSTITUTO RENÉ RACHOU, FUNDAÇÃO OSWALDO CRUZ – FIOCRUZ MINAS, BELO HORIZONTE, MINAS GERAIS, BRASIL. [2]LABORATÓRIO DE PROTOZOOLOGIA - DEPARTAMENTO DE MICROBIOLOGIA, IMUNOLOGIA E PARASITOLOGIA – LABORATÓRIO DE PROTOZOOLOGIA, CENTRO DE CIÊNCIAS BIOLÓGICAS – UNIVERSIDADE FEDERAL DE SANTA CATARINA – UFSC, FLORIANÓPOLIS, SANTA CATARINA, BRASIL

Abstract

Dogs are the main urban reservoirs of *Leishmania infantum*, a pathogen commonly associated with canine visceral leishmaniasis (CVL). Given the lack of a definitive cure for this disease, the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA), in partnership with the Ministry of Health, approved the use of Milteforan® (miltefosine) to treat CVL. MAPA emphasizes that the decision to use the medication is the responsibility of the animal's tutor and highlights the need for parasitological monitoring of the treated dogs. Here we use LeishID - a LAMP (loop-mediated isothermal DNA amplification)-based method - for the molecular detection of *L. infantum* in clinical samples from naturally infected and treated dogs. These dogs lived with their tutors in Florianópolis, Santa Catarina, in southern Brazil. The DNA was extracted from skin samples of 11 dogs. The samples were collected at different time points: T0 (before treatment), T1 (one month after the start of treatment), T2 (six months after the end of treatment), and T3 (twelve months after the end of treatment). The LAMP reaction was performed using the WarmStart colorimetric master mix (NEB) and the amplified products were also resolved in agarose gel electrophoresis to confirm specific amplification. At time points T0, T2, and T3, 11 samples were identified as positive, while at time point T1, 10 samples tested positive. At T2, LAMP was able to detect *L. infantum* DNA in 100% of samples (11/11) while 54.5% (6/11) of samples were considered reagents by RT-qPCR. These findings indicate that LAMP demonstrates higher sensitivity in this context compared with RT-qPCR. The detection of *L. infantum* was observed at all points of all time, despite the administered treatment. Therefore, LeishID is a valuable tool for monitoring dogs undergoing treatment for visceral leishmaniasis, as it successfully detects the presence of the parasite's DNA in the animal's tissue even 12 months after the completion of treatment.

Keywords: *Leishmania infantum*; molecular diagnostics, LAMP, milteforan
Financial Support: Fapemig (RED-0032-22; RED-00196-23), CAPES, CNPq (312353/2023-5), Fiocruz