DETECTION OF *Leishmania infantum* DNA DIRECTLY FROM CLINICAL SERUM SAMPLES VIA LAMP WITHOUT DNA EXTRACTION

FERNANDA DOS SANTOS SILVA[1]; RAPHAEL FRANÇA OLIVEIRA[1]; PAULO OTÁVIO LOURENÇO MOREIRA[1]; BRUNO MENDES ROATT[2]; RODRIGO DIAN DE OLIVEIRA AGUIAR[2]; ALEXANDRE BARBOSA REIS[2]; ALESSANDRA MARA DE SOUSA[1]; RUBENS LIMA DO MONTE-NETO[1].

[1]BIOTECNOLOGIA APLICADA AO ESTUDO DE PATÓGENOS (BAP) – INSTITUTO RENÉ RACHOU – FUNDAÇÃO OSWALDO CRUZ, BELO HORIZONTE, MINAS GERAIS, BRASIL.

[2]LABORATÓRIO DE IMUNOPATOLOGIA/NUPEB – UNIVERSIDADE FEDERAL DE OURO PRETO - UFOP.

Abstract

The implementation of precise, reliable, and efficient diagnostic tools is crucial for controlling leishmaniasis. The loop-mediated isothermal DNA amplification (LAMP) technique is compatible with the point-of-care scenario. It is fast and easy to perform, eliminating the need for expensive equipment, presenting a promising alternative for molecular diagnosis. Employing serum as a biological sample for the LAMP reaction simplifies the pre-analytical step, allowing for precise and minimally invasive detection of *Leishmania* DNA, which facilitates diagnosis in areas with limited infrastructure. In this study, we validated the use of serum from dogs naturally infected with *Leishmania infantum*, confirmed by DPP and ELISA, from an endemic area in Ouro Preto, Minas Gerais, Brazil. We utilized species-specific primers developed by our team, integrated into LeishID—a LAMP-based molecular diagnostic solution that demonstrated high sensitivity and specificity at clinically relevant concentrations. We analyzed 69 blood samples from dogs, including 23 symptomatic, 23 asymptomatic, and 23 uninfected dogs. For the LAMP assay, we employed a colorimetric detection using hydroxynaphthol blue (HNB), where a positive reaction is indicated by a blue color change, while negative samples remain purple. LAMP reactions were conducted at 65 °C for 40 min, and the results were validated by agarose gel electrophoresis. The colorimetric test correctly identified all positive (symptomatic) and negative samples, achieving 100% sensitivity and specificity. In positive asymptomatic dogs, electrophoresis identified 16 positive samples, resulting in a sensitivity of 76.67% (95% CI = 57–90%) and a specificity of 100% (95% CI = 85–100%). The use of DNA extraction-free crude serum, combined with the LeishID platform, enabled molecular detection of *Leishmania*, ensuring high sensitivity and specificity. This approach is particularly suitable for field applications in resource-limited endemic regions.

Keywords: *Leishmania;* LAMP; serum
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