IVD_20 - Identifying Linear B-Cell Epitopes in Leishmania infantum Recombinant Proteins Using Microarray Technology for Enhanced Serodiagnosis of Visceral Leishmaniasis

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Introduction: Visceral leishmaniasis (VL), caused by *Leishmania infantum*, exhibits concerning expansion in urban areas. In Brazil, serodiagnosis is the mainstay of diagnosis and consists of a rapid test (DPP) and a confirmatory ELISA (EIE-LVC) for dogs (CVL) or immunofluorescence for humans. However, these methods exhibit limitations in accuracy and cross-reactivity with other *Trypanosomatidae* species. Recombinant *L. infantum* proteins (rLci1, rLci2, rLci5, rLci12) demonstrated superior accuracy (84%-92%) in ELISA compared to tests recommended by the Ministry of Health. However, residual cross-reactivity with *Trypanosoma spp.* persisted.

Objectives: This study aimed to identify more immunogenic and specific epitopes to improve diagnostic sensitivity and specificity.

Methodology: To enhance specificity, *in silico* (BLAST-p, Clustal Omega) and microarray analysis identified conserved epitopes amongst *L. infantum* and *T. cruzi*. Epitopes specific to *Leishmania spp.* were targeted for further analysis. Furthermore, the microarray assay was conducted using sera from VL patients (n=10), healthy controls (n=5), and individuals infected with *Trypanosoma cruzi* (n=5) to assess cross-reactivity. Additionally, recombinant proteins rK28 and rK39 were included for comparison.

Results: *In silico* evaluation revealed high similarity (>80%) with *T. cruzi* only for rLci1 and 12, while rLci5 and 2 exhibited higher similarity only to *Leishmania* proteins. The mean IgG fluorescence of VL patients sera significantly differed from healthy controls and Chagas disease patients sera. Microarray assays revealed distinct fluorescence patterns for different patient groups, suggesting potential for differentiation. Notably, all proteins displayed significantly higher mean fluorescence against VL patient sera compared to controls and those infected with other pathogens ($p < 0.05$). To refine these findings, a combination of *in silico* prediction (Bepi-pred 2.0) and further microarray analysis led to the construction of three novel chimeric proteins incorporating the mapped, highly immunoreactive linear B-cell epitopes.

Conclusion: These findings identify specific candidate epitopes displaying promising potential for the development of novel, highly accurate VL diagnostic tests based on chimeric proteins. Further validation and comparison with established assays are necessary.

Keywords: Leishmania; Recombinant proteins; Epitope mapping