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# ABSTRACT BOOK

## "There will be epidemics..."


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eukaryotic organisms. Additional experiments were also performed to better characterize the aforementioned domain (ONC) as well as Lmj*YinP,* a novel molecule involved in pathogenesis and drug resistance. All our data reinforce the hypothesis that LmjYinP and ONC domain may be robust druggable targets against leishmaniasis.

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### COMPARISON OF WHOLE-GENOME SEQUENCING, SANGER SEQUENCING, AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS FOR *LEISHMANIA VIANNIA* MIXED AND HYBRID INFECTION SPECIES IDENTIFICATION

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The prognosis and treatment of leishmaniasis is largely dependent on the infecting species. Varying degrees of disease severity are present, in addition to mixed and hybrid infections, which pose diagnostic challenges. The causative species identification of these types of infections relies on standard techniques such as restriction fragment length polymorphism (RFLP) analysis, and Sanger sequencing (SS). Whole-genome sequencing (WGS) is a robust and increasingly cost-efficient alternative that can improve *Leishmania* species identification. Here, we validated and compared WGS as a potential alternative to RFLP-SS standards in a cohort of 3 ATCC strains (*L. V. braziliensis*, *L. V. guyanensis* and *L. V. panamensis*) and 5 clinical *Leishmania Viannia* isolates of potential hybrids or mixed infections. DNA extraction, followed by *internal transcribed spacer1* (*ITS1)* and heat shock protein 70 (*HSP70*) PCR-RFLP was carried out and SS was performed for the *ITS2*, cysteine proteinase b, and *HSP*70 loci. After *de novo* assembly, sequences were mapped, and homology compared to both ATCC strains and reference genomes at NCBI. All samples went through a diagnostic pipeline to identify species present by all three techniques. Concordant validation was assessed within each isolate, over a 6-week period, to determine if final identification was consistent with the initial. All ATCC isolates were confirmed to be single-species of either *L. V. braziliensis*, *L. V. guyanensis*, or *L. V. panamensis* by WGS; whereas RFLP-SS was unable to definitively speciate two of three isolates. Clinical isolates were identified as a combination of single-species, mixed, and hybrid infections of a variety of *Viannia* species by WGS; while RFLP-SS was largely unable to definitively speciate four of five isolates. We have utilized WGS to differentiate mixed and hybrid infections by species. Ambiguous infection samples, previously speciated by RFLP-SS, were more reliably discerned into single-species, mixed, and hybrid categories by WGS. WGS is a potentially useful alternative to RFLP-SS for the diagnosis and species identification of complex tegumentary *Leishmania* infections.

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### THE GP63 GENE CLUSTER IS HIGHLY POLYMORPHIC IN NATURAL *LEISHMANIA (VIANNIA) BRAZILIENSIS* POPULATIONS, BUT FUNCTIONAL SITES ARE CONSERVED

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GP63 or leishmanolysin is the major surface protease of *Leishmania* spp. involved in parasite virulence and host cell interaction. As such, GP63 is a potential target of eventual vaccines against these protozoa. In the current study we evaluate the polymorphism of gp63 in *Leishmania (Viannia) braziliensis* isolated from 41 American tegumentary leishmaniasis (ATL) cases from Corte de Pedra, Brazil. Parasites were obtained from lesions by needle aspiration and cultivation. Genomic DNA was extracted, and 405 bp fragments, including sequences encoding the putative macrophage interacting sites, were amplified from gp63 genes of all isolates. DNA amplicons were cloned into plasmid vectors and ten clones per *L. braziliensis* isolate were sequenced. Alignment of cloned sequences showed extensive polymorphism among gp63 genes within, and between parasite isolates. Overall, 45 different polymorphic alleles were detected. The predicted peptides showed overall conservation below 50%. In marked contrast, the conservation at segments with putative functional domains approached 90% (Fisher's exact test $p<0.0001$). Synthetic peptides based on these short, conserved sequences were capable of significantly inhibiting *in vitro* infection of monocyte derived macrophages by *L. braziliensis* in a dose dependent manner. These findings show that gp63 is very polymorphic even among parasites from a same endemic focus, but the functional domains interacting with the mammalian host environment are conserved.

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### EVALUATION OF REAL TIME PCR FOR DIAGNOSIS OF POST-KALA-AZAR DERMAL LEISHMANIASIS (PKDL) IN AN ENDEMIC *FOCI* OF BANGLADESH

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Post-kala-azar dermal leishmaniasis (PKDL) is a sequel of kala-azar or visceral leishmaniasis (VL) that is found in visceral leishmaniasis (VL) endemic countries including Bangladesh. Because of these enigmatic cases, the success of National Kala-azar elimination program (NKEP) is under threat. Unlike other endemic regions, the macular form of PKDL is the most common form in Bangladesh and diagnosis of these cases is more difficult than other forms. Until now, the diagnostic method for PKDL cases in endemic regions is limited to clinical examination and serology using the rK39 rapid test or microscopy. A suitable and accurate alternative method is necessary. In this study, we investigated the application of real time PCR as a potential method for diagnosis of PKDL in comparison with microscopy. For this study, 91 suspected macular PKDL cases from the Mymensingh district, Bangladesh were enrolled following diagnosis through clinical examination and the rk39 RDT. All cases were treated for PKDL and responded well after completion of the treatment. During enrollment, skin biopsy was collected from each patient and both microscopy and real time PCR were performed for detection and quantification of *leishmania donovani* body (LDB) and LD DNA respectively. Real time PCR detected 83 cases among all suspected PKDL patients with an encouraging sensitivity of 91.21% (83.41-96.13) whereas microscopy showed 50.55% (39.86-61.20) sensitivity only. These findings suggest that real time PCR is a promising tool for diagnosis of PKDL in endemic regions. In addition to diagnosis, the quantitative ability of this method could be exploited for after-treatment prognosis and cure assessment of PKDL cases.