

**Molecular detection and sero-prevalence of
Leishmania donovani & risk factor analysis
in selected areas in Sri Lanka.**

by

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**Thesis submitted to the University of Sri Jayewardenepura for the
award of the Degree of Doctor of Philosophy on 15th of August
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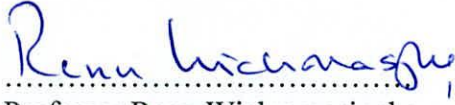
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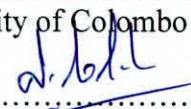
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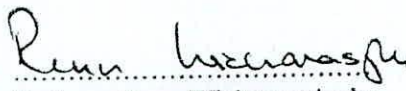


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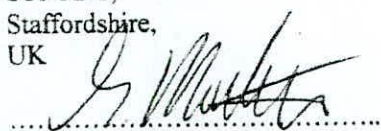
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Abbreviations

CL	= Cutaneous leishmaniasis
EUMHNSL	= Epidemiology Unit Ministry of Healthcare and Nutrition, Sri Lanka
GPS	= Global Positioning System
ITS	= Internal Transcribed Spacer
kDNA	= Kinetoplast DNA
MCL	= Mucocutaneous leishmaniasis
ML	= Mucosal leishmaniasis
MLEE	= Multi locus enzyme electrophoresis
MOH	= Medical officer of health
PCA	= Principal component analysis
PCR	= Polymerase chain reaction
6PGDH	= 6-Phosphogluconate dehydrogenase
PHM	= Public health midwife
RDT	= Rapid diagnostic test
UNOCHAS	= United Nations Office for the Coordination of Humanitarian Affairs Sri Lanka
VL	= Visceral leishmaniasis

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ABSTRACT

Introduction: Leishmaniasis is a newly emerged and established disease in Sri Lanka. This study describes the application of a highly sensitive and specific *Leishmania* DNA based assay to skin punch biopsy samples, determination of sero-prevalence of anti-*Leishmania* antibodies, risk factor analysis for transmission of leishmaniasis in an 'at risk' community and sand fly identification by traditional methods in diagnosed patients' habitats.

Methodology: The *Leishmania* strain causing visceral leishmaniasis (VL) in Sri Lanka was characterized by Multi Locus Enzyme Electrophoresis and VL and cutaneous leishmaniasis (CL) causing strains were confirmed by sequencing the 6-phosphogluconate dehydrogenase (6PGDH) gene. Three sets of previously described primers (kDNA: JW11/12, Ldf/Ldr and ITS 1:LITSR/L5.8S) were used to detect *Leishmania* DNA in skin punch biopsy samples. Digestion of the ITS 1 region with Hae III and Restriction Fragment Length Polymorphism (RFLP) was used for parasite identification.

A cross sectional study of 1000 individuals, was carried out in a high CL prevalent area, {estimated population~61,674, Thalawa Medical Officer of Health (MOH) area}, to assess presence of the rK39 anti-*Leishmania* antibody in sera, (with the rK39 rapid diagnostic

test, RDT); CL prevalence with microscopy of slit skin smears, and to analyze risk factors for transmission of leishmaniasis using a pre-tested, interviewer administered, questionnaire and global positioning system. Randomly selected sand flies (n=96) from habitats close to diagnosed CL patients houses were captured using the cattle bait from Matale district, and taxonomically identified using taxonomic keys. Morphometric measurements were analyzed and compared with previously described data using the student's *t*-test and principal component analysis (PCA).

Results: The endogenous VL causing strain in Sri Lanka was identified as *Leishmania donovani* MON-37 by MLEE. The CL strain isolated was also characterized as *Leishmania donovani* MON-37 by sequencing the 6PGDH gene. The sensitivity of JW and ITS 1 primers in detecting *Leishmania* DNA by PCR was 92% (n=35/38). Sensitivity assay using Ld primers by PCR was 71% (n=27/38). The specificity of JW and ITS 1 primers were 100% while Ld primers showed some cross-amplification with *Mycobacterium tuberculosis* DNA. All amplified ITS 1 fragments following digestion with Hae III RFLP gave a pattern characteristic of *L. donovani*. The prevalence of CL was 3.2% (31/954) and sero-positivity to anti-*Leishmania* antibodies was 0.1% in Thalawa MOH area. Living in close proximity to paddy fields has shown to be the only risk factor (p=0.01) for the transmission of leishmaniasis in Thalawa. Out of the 96 sand flies identified 95 were *P. argentipes* and one was genus *Sergentomyia*. PCA analysis showed that *P. argentipes* identified from Sri Lanka formed a separate cluster when compared with the recently described *P. argentipes*, *P. annadalei* and *P. glaucus* sibling species indicating existence of a morphologically distinct of *P. argentipes* population in Sri Lanka.

Conclusions and recommendation: *L. donovani* MON-37 causes both CL and VL in Sri Lanka. JW11/12 and ITS 1 primers could be recommended to detect *Leishmania* DNA in skin biopsy samples from Sri Lankan patients. High prevalence of CL was detected in the study area and *P. argentipes* in Sri Lanka form a separate morphological cluster. These results would indicate the need for comparison studies to detect phenotypic and genotypic differences in CL and VL causing Sri Lankan *L. donovani* MON 37 strains, need for active case detection studies, improve surveillance of CL, early treatment to reduce the human reservoir, studies on sand fly biology and implementation of control measures to control and combat the spread of leishmaniasis in the country