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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 2013

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Contents

page

Contents i

List of Tables vii

List of Figures viii

Abbreviations xi

Acknowledgements xii

ABSTRACT xv

1. Introduction 1

1.1 General Objectives: 9

1.2 Specific Objectives: 9

2. Literature Review 10

3. Materials and Methods 15

3.1 Isolation of visceral and cutaneous leishmaniasis causing strains in Sri Lanka 15

3.1.1. Culture of the VL strain and submission for strain identification 15

3.1.2 Culture of the CL strain 17

3.1.3 Multi Locus Enzyme Electrophoresis (MLEE) 17

3.1.4 Sequencing of 6-Phospho gluconate dehydrogenase gene (6PGDH) 18

3.2. A cross sectional study to assess the sero- prevalence and risk factors for leishmaniasis in an endemic region of Sri Lanka 19

3.2.1 Study area 19

3.2.2 Sampling method 20

3.2.2.1 Exclusion criteria 20

3.2.3 Rapid diagnostic test (RDT) 21

3.2.4 Slit Skin Smear 21

3.2.5 Socio-demographic data collection 22

3.2.6 Data on environmental risk factors 24

3.2.7 Social awareness programme 24

3.2.8 Management and follow up of newly diagnosed leishmaniasis patients 24

3.2.9 Statistical analysis 25

3.2.10 Ethical clearance 25

3.3 Detection of *Leishmania donovani* DNA using DNA based methods in Sri Lanka 26

3.3.1. Study samples 26

3.3.2 Sample number calculation 26

3.3.3 Exclusion criteria 27

3.3.4 Slit skin smear 27

3.3.5 Punch biopsy 27

3.3.6 Sample storage and transport 27

3.3.7 DNA extraction 28

3.3.8 Primers 28

3.3.8.1 Genus *Leishmania* specific Kinetoplast (kDNA) PCR 29

3.3.8.2 *Leishmania donovani* species specific PCR 30

3.3.8.3 Internal Transcribed Spacer I (ITS 1) PCR 31

3.3.9 Restriction Fragment Length Polymorphism (RFLP) 32

3.3.10 Positive controls 33

3.3.11 Negative controls 33

3.3.12 PCR product visualization and capture 34

3.3.13 Ethical clearance 34

3.4 Taxonomic identification of sand flies captured from diagnosed CL patients' habitats in Matale district; an active transmission area for CL 34

3.4.1 Study area 34

3.4.2 Collection of sand flies 35

3.4.3 Sand fly identification 35

3.4.4 Statistical analysis 36

3.4.4.1 Principal component analysis (PCA) 37

3.4.5 Ethical clearance 39

4. Results 40

4.1 First characterization of the strain causing autochthonous visceral leishmaniasis in Sri Lanka 40

4.1.1 Multi Locus Enzyme Electrophoresis (MLEE) of the VL strain 40

4.1.2 Visceral leishmaniasis causing strain identification by 6-Phopshogluconate dehydrogenase (6PGDH) partial gene sequence and CLUSTALW alignment of partial amino acid sequence 40

4.1.3 Cutaneous leishmaniasis strain identification 41

4.2 A cross sectional study to assess the sero- prevalence and risk factors for transmission of leishmaniasis in an endemic region of Sri Lanka 43

4.2.1 Socio-demographic characteristics and clinical aspects of CL cases 43

4.2.2 Environmental risk factors..... 44

4.2.3 Social awareness..... 45

4.3 Detection of *Leishmania* DNA in punch biopsy samples using DNA based methods 54

4.3.1 Spectrum of clinical presentations observed during sample collection..... 54

4.3.2 Slit skin smear 54

4.3.3 DNA quantification 55

4.3.4 Sensitivity assays of the selected three sets of primers 56

4.3.4.1 Genus *Leishmania* specific JW11/12 primers 56

4.3.4.2 *Leishmania donovani* species specific kDNA PCR (Ldf/Ldr) 57

4.3.4.3 Internal Transcribed Spacer I (ITS 1) PCR (LITSR/L5.8S)..... 57

4.3.5 Specificity assays..... 58

4.3.5.1 The presence of *M. leprae* DNA from a smear positive skin punch biopsy sample 58

4.3.5.2 Specificity assays of JW11/12 primers in the presence of *M. tuberculosis, M. leprae* and human DNA..... 60

4.3.5.3 Specificity assays of Ldf/Ldr (*L. donovani* specific) primers in the presence of human, *M. leprae* and *M. tuberculosis* DNA 61

4.3.5.4 Specificity assays of LITSR/L5.8S (ITS 1) primers in the presence of human, *M. leprae* and *M. tuberculosis* DNA..... 63

4.3.6 PCR of smear positive CL punch biopsy samples..... 65

4.3.6.1 JW11/12 kDNA PCR..... 65

4.3.6.2 Ldf/Ldr PCR for smear positive DNA samples 69

4.3.6.3 ITS 1 (LITSR/L5.8s) PCR 70

4.3.7 Restriction Fragment Length Polymorphism (RFLP) 72

4.4 Taxonomic identification of sand flies captured from diagnosed CL patients' habitats in Matale district. 75

4.4.1 Sand fly taxonomy 75

4.4.2 The male sand flies of the study population 77

4.4.3 Principal component analysis (PCA) 77

5. Discussion 86

6. Conclusions 97

6.1 Recommendations 98

7. References 100

8. Appendices 114

Appendix 1: List of publications and communications 114

Appendix 2: Questionnaires & Consent forms 118

2.1 Title of the study: Detection of *Leishmania* DNA from skin biopsy samples using DNA based techniques in Sri Lankan patients 118

2.2 Title of the study: Determination of sero-prevalence in a community with active transmission of cutaneous leishmaniasis 125

2.3 Title of the study: Taxonomic identification of sand flies captured from diagnosed CL patients' habitats in Matale district 145

Appendix 3: Raw data 149

3.1. Title of the study: Detection of *Leishmania* DNA from skin biopsy samples using DNA based techniques in Sri Lankan patients..... 149

3.2 Title of the study: Taxonomic identification of sand flies captured from diagnosed CL patients habitats in Matale district..... 162

List of Tables page

Table i Distribution of the population by socio demographic characteristics and rK-39 anti-*Leishmania* antibody detection test (RDT) status 48

Table ii Clinical assessment of confirmed CL patients (n=31) 50

Table iii Environmental risk factors associated with CL 51

Table iv Summary of logistic regression analysis using CL status as the dependent variable 52

Table v Social awareness on leishmaniasis and the sand fly vector 53

Table vi RFLP cleavage positions 72

Table vii Summary of patients reported from different locations in the Matale district and number of identified sand flies from each location 79

Table viii Comparison of morphometric characteristics (Mean ± SD and range) for sand flies collected at active transmission locations in the Matale district, Sri Lanka, in this study, with published data for *P. argentipes s. l.* 80

Table ix Component loadings for four Varimax-rotated components extracted from 11 morphological characteristics of *P. annandalei* (n=10; Ilango 2010), *P. argentipes s.s.* (n=9; Ilango 2010), *P. glaucus* (n=10; Ilango 2010) and study sand fly population (n=60; Matale district). 81

List of Figures page

Figure i Autochthonous 3rd VL patient (A) and a Giemsa stained slide of the first successful *in-vitro* culture of the endogenous VL causing *Leishmania* strain (B). 16

Figure ii CL patient with a plaque like lesion. 17

Figure iii Patient with a smear positive CL lesion (A), a doctor dressing up after a slit skin smear has been obtained (B) 22

Figure iv Social awareness programme and data collection at Thalawa MOH area..... 25

Figure v Partial amino acid sequence alignment of the 6-phosphogluconate dehydrogenase (6PGDH) isoenzyme from different *Leishmania* species.. 42

Figure vi RDT results from Thalawa. 45

Figure vii RDT results from the positive control. 46

Figure viii Land use patterns of the Thalawa Medical Officer of Health (MOH) area showing the distribution of persons screened. 47

Figure ix Spectrum of clinical presentations seen among CL patients in Sri Lanka. 54

Figure x Sample 8 with very few amastigote like forms in the slit skin smear. 55

Figure xi Macrophages filled with amastigotes.. 55

Figure xii Serial dilutions of CL DNA amplified with JW11/12 primers (Rodgers *et al.* 1990). 56

Figure xiii Serial dilutions of CL DNA amplified with *L. donovani* specific primers (Salotra *et al.* 2001). 57

Figure xiv Serial dilutions of CL DNA amplified with ITS 1 primers (El Tai *et al.* 2001).. 58

Figure xv One of the smear positive lepromatous leprosy samples in acid fast stain:..... 59

Figure xvi *Mycobacerium leprae* DNA amplified (n=3/5 samples) with *M. leprae* specific primers. 59

Figure xvii *M. tuberculosis* DNA amplification with JW11/12 kDNA primers..... 60

Figure xviii Specificity of JW11/12 Primers in the presence of human, *M. leprae* and *M. tuberculosis* DNA_ 61

Figure xix Specificity of *L. donovani* Ldf/Ldr primers in the presence of human *M. leprae* and *M. tuberculosis* DNA. 62

Figure xx *M. tuberculosis* DNA amplification with *L. donovani* specific kDNA primers Ldf/Ldr (Salotra *et al.* 2001).. 63

Figure xxi Specificity test of ITS 1 Primers (LITSR/L5.8S) in the presence of *M. tuberculosis, M. leprae* and human DNA..... 64

Figure xxii DNA extracted from punch biopsy samples of smear positive cutaneous lesions amplified with JW11/12 primers.. 66

Figure xxiii Samples that became positive with slit skin smear and negative with PCR. 66

Figure xxiv Spotting. 67

Figure xxv Spiking of PCR negative CL samples with *L. donovni* culture DNA (1pg/µl)..68

Figure xxvi DNA extracted from punch biopsy samples of smear positive cutaneous lesions amplified with Ldf/Ldr primers. 69

Figure xxvii Nucleotide sequence from 1 to 316 of ITS 1 region. 70

Figure xxviii Testing of samples with ITS 1 (LITSR/L5.8S) primers..... 71

Figure xxix *Leishmania* species identification by ITS 1 PCR-RFLP.. 73

Figure xxx *Leishmania* species identification by ITS 1 PCR-RFLP. 74

Figure xxxi Matale study area. 82

Figure xxxii Light microscope images of antennal flagellomeres, mouthparts and genitalia of male and female sand flies collected in the Matale district of Sri Lanka.. 83

Figure xxxiii *Sergentomyia babu* female sand fly. 84

Figure xxxiv Principle component analysis regression scores for *P. annandalei* (n=10; Ilango 2010), *P. argentipes s.s.* (n=9; Ilango 2010), *P. glaucus* (n=10; Ilango 2010) and study sand fly population (n=60; Matale district).. 85

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 leishmanaisis

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 lll 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 lll 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