

Entomology Department Faculty of Science Ain Shams University

SANDFLY VECTORS AND RODENT RESERVOIRS RESPONSIBLE FOR LEISHMANIASIS TRANSMISSION IN A REMOTE AREA OF NORTH-SINAI (EGYPT).

In partial fulfillment of the requirements of

Master of Science Degree (M.Sc) in Entomology

Ain Shams University

(2009)

By

Abdallah Mohammed Samy Esmail

(B.Sc. Entomology 2005)

Supervised by

Dr. Magdi Gebril Shehata

Dr. Adel Ramzy Fahmy

Professor of Medical Entomology Entomology Department Faculty of Science Ain Shams University Lecturer of Entomology Entomology Department Faculty of Science Ain Shams University

Dr. Said Abdallah Doha

Researcher Research and Training Center on Vectors of Diseases Ain Shams University

Examination Committee:

Prof. Fatma Kamel Adham

Professor of Medical Entomology, Entomology Department, Faculty of Science, Cairo University.

Prof. Mostafa Mostafa Kamel El Awdi

Professor of Biochemistry, and Head of Medical Biotechnology Department, National Research center.

Prof. Magdi Gebril Shehata

Professor of Medical Entomology, Entomology Department, Faculty of Science, Ain Shams University.

Supervisors:

Prof. Magdi Gebril Shehata

Professor of Medical Entomology, Entomology Department, Faculty of Science, Ain Shams University.

Dr. Adel Ramzy Fahmy

Lecturer o Entomology, Entomology Department, Faculty of Science, Ain Shams University.

Dr. Said Abdallah Doha

Researcher, Research and Training Center on Vectors of Diseases, Ain Shams University.

Biography

DATE AND PLACE OF BIRTH:

September 25, 1984/ Sharqiya governorate

DEGREE AWARDED:

B.Sc (Entomology), Entomology Department, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt (2005).

OCCUPATION:

Demonstrator of Entomology, Entomology Department, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt.

DATE OF REGISTRATION FOR THE DEGREE:

February, 2007

Dedication:

I am pleased to dedicate this work to my beloved, parents, brother, and all my friends.

ACKNOWLEDGEMENT

First of all, thanks to "**Allah**" to whom I relate the success in achieving any progress in my life.

I wish to express my deep thanks and sincere gratitude to Professor **Magdi G. Shehata**, Entomology Department, Faculty of Science, Ain Shams University, for suggesting the problem and the plan of work of this study. My thanks are also due to his kind guidance and encouragement throughout the study.

I am greatly thankful for **Dr. Adel R. Fahmy**, lecturer of Entomology, Entomology Department, Faculty of Science, Ain Shams University for his continuous supervision, helpful suggestions, and relentless reading of the manuscript as well as his supportive encouragement.

My sincere thanks to **Dr. Said A. Doha**, Researcher, Research and Training Center on Vectors of Diseases (RTC), Ain Shams University, for his thoughtful advices, direct supervision, training in the field trips and *in vitro* investigations of sandflies and *Leishmania* parasites.

I also wish to extend my gratitude to professor **Bahira M. El Sawaf** and **Dr. Mohammed A. Kenawy**, Entomology Department, Faculty of Science, Ain Shams University, for their helpful comments for improvement of the manuscript.

My thanks are also due to **Dr. Hany A. Kamal**, Researcher, Research and Training Center on Vectors of Diseases (RTC), Ain Shams University for his help in conducting my research and providing technical training in *Leishmania* maintenance. Also thanks go to **Dr. Jeffrey T. Villinski**, **Dr. Shabaan El Hosssary** and **Miss Rania Kaldas**, Vector Biology Research Program, U.S. Naval Medical Research Unit No. 3, Cairo, Egypt for providing facilities and technical training on real time PCR. Molecular analysis was supported by a grant from NAMRU.3.

Special thanks to **Dr. Awni F. Sallem**, El Barth local Hospital physician, Rafah, Northern Sinai for supporting *Leishmania* NNN-cultures cultivated from lesions, and description of CL lesions among cases attend clinics in El Barth hospital. *Leishmania* Parasite isolation was conducted by physician in accord with the international regulations and guidelines of the Declaration of Helsinki. Every patient signed a consent form.

I am also grateful to Professor Akila M. El Shafei, Head of Entomology Department, Faculty of Science, Ain Shams University and all my colleagues for their continuous support throughout this work.

Contents:

Title	page
Dedication	i
Acknowledgements	ii
ABBREVIATIONS	iv
LIST OF TABLES	vi
LIST OF FIGURES	viii
Abstract	1
Introduction	2
Chapter 1: Literature reviews	5
1. Sandfly species composition and their relation to <i>Leishmania</i>	5
transmission	
2. Sandfly sex ratio	10
3. Sandfly activity rhythm	11
4. <i>Leishmania</i> isolation from wild caught sandflies	13
5. Experimental infection of sandfly	16
6. Leishmaniasis reservoir hosts	23
7. Leishmania typing and Identification	28
Chapter 2: Materials and Methods	38
1. Study area	38
1.1. Location	38
1.2. Climatic conditions and microhabitat	38
2. Sandfly collection and processing	41
2.1. Sticky paper traps	41
2.2. CDC light traps	41
2.3. Nocturnal activity of sandflies	45
2.4. Handling techniques for collected sandflies	45
2.5. Sandflies identification	45
2.6. Colonization of sandflies	46
2.7. Sandflies dissection	47
3. Rodents collection and identification	48
4. <i>Leishmania</i> isolates	48
4.1. Human <i>Leishmania</i> isolates	48
4.2. Rodents <i>Leishmania</i> isolates	51
4.3. Nomenclature of <i>Leishmania</i> isolates	51
5. <i>Leishmania</i> culture media	51
5.1. The solid medium (NNN medium)	52
5.2. Schneider's culture medium	53
6. Identification and typing of <i>Leishmania</i> isolates	53

	6.1. 6.2. 6.3. a)	Preparation of <i>Leishmania</i> isolates for identification Reference strains PCR identification DNA extraction and quantification	53 54 54 54
	b)	Real time PCR	54
	c)	Conventional PCR, Sequencing and Sequence Analysis	56
	d)	Bioinformatics softwares used for genetic data	56
		manipulation	00
	e)	Restriction fragment length polymorphism (RFLP)	57
		analysis.	
7.	Susceptibility	of rodents to L. tropica	57
8.	Sandfly Exper	rimental infection	58
9.	Experimental by the bite of	transmission of <i>L. major</i> and <i>L. tropica</i> to BALB/C mice <i>P. papatasi</i> and <i>P. sergenti</i>	60
10.	Statistical Ana	alysis	60
Ch	apter3: Result	S	61
1.	Sandfly specie	s composition	61
2.	Sandfly sex rat	io	61
3.	Sandfly activity	/ rhythm	63
4.	Detection of L	eishmania parasite from sandflies	65
5.	Rodent Specie	s Composition	68
6.	Detection of L	eishmania parasite in the collected rodents	69
7.	Clinical sample	es	72
8.	Confirmation of	of Leishmania species identity:	74
	a. P	arasite Culturing	74
	b. R	eal time PCR	75
	c. S	equencing of ribosomal internal transcribed spacer 1	75
	L) b b	ene Tree Analysis	84
	e A	nalysis of restriction fragment length polymorphisms	86
	с. н (]	RFLPs)	00
9.	Visceralization	of L. tropica from naturally infected Gerbillus	90
	pyramidum flo	weri	- 0
10.	Susceptibility of	of different rodent species to <i>Leishmania tropica</i> .	90
11.	Experimental	infection of <i>P. papatasi</i> and <i>P. sergenti</i> with <i>L. major</i>	91
12.	Experimental i	nfection of <i>P. papatasi</i> and <i>P. sergenti</i> with <i>L. tropica</i>	93
13.	Experimental t	ransmission of <i>L. major</i> and <i>L. tropica</i> to BALB/C mice	95
	by the bite of <i>I</i>	P. papatasi and P. sergenti	

Chapter 4: Discussion	96
1. Sandfly species composition and sex ratio in El Barth village	96
2. Sandfly activity rhythm	98
3. Detection of <i>Leishmania</i> in collected sandflies	100
4. Rodent Species composition and sex ratio	101
5. Detection of <i>Leishmania</i> parasite in the collected rodents	102
6. Confirmation of Leishmania species identity	104
7. Visceralization of <i>L. tropica</i> from naturally infected <i>Gerbillus pyramidum floweri</i>	107
8. Susceptibility of different rodent species to <i>Leishmania tropica</i>	109
9. Experimental infection of sandflies	110
10. English summary	113
11. Conclusion	119
12. Appendices	121
13. References	123

ABBREVIATIONS:

ACL	American cutaneous leishmaniasis
6-FAM	6-carboxy-fluorescein
TAMRA	6-carboxy-tetramethyl-rhodamine
6-PGDH	6-phospho-D-gluconate Dehydrogenase
AMG	Anterior midgut
BLAST	Basic Local Alignment Search Tool
CAE	cellulose acetate electrophoresis
CDC	Center for Disease Control and Prevention
CL	cutaneous leishmaniasis
Ct	Cycler threshold
DNA	Deoxyribonucleic acid
ELIZA	Enzyme-linked immunosorbent assay
EF	Excreted factor
G.	Gerbillus
GPI	glucose phosphate isomerase
G-6-PDH	Glucose-6-phosphate Dehydrogenase
GOT	Glutamic oxaloacetic transaminase
ITS-1	Internal transcribed spacer-1
IDH	Isocitrate Dehydrogenase
kDNA	Kinetoplastid deoxyribonucleic acid
L.	Leishmania
LST	Leishmanin skin test
LCL	Localized cutaneous leishmanisis
LON	London
Lu.	Lutzomyia
ME	Malic enzyme
MDH	Mannitol Dehydrogenase
М.	Meriones
MON	Montpellier
MFO	Multinational Force and Observers
HEPES	N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid
NNN	Novy-MacNeal-Nicolle
PHAR	Pharynx
Р.	Phlebotomus

PBS	Phosphate buffer solution
PGM	Phosphoglucomutase
PGI	phosphoglucose Isomerase
PCR	Polymerase chain reaction
PMG	Posterior midgut
PROB	Proboscis
QT-NASBA	quantitative nucleic acid sequence based amplification
RFLPs	Restriction fragment length polymorphisms
rDNA	Ribosomal deoxyribonucleic acid
rRNA	ribosomal ribonucleic acid
RPMI	Roswell Park Memorial Institute
S.	Sergentomyia
SSU rRNA	Small- subunit ribosomal ribonucleic acid
SV	Stomodeal valve
SYBR	Synergy Brands
VL	visceral leishmaniasis
ZCL	zoonotic cutaneous leishmaniasis

List of tables:

Number	Title	Page
Table 1	The number of Phlebotomine sandflies collected and identified from each of the four sectors of the El Barth community of North-Sinai, Egypt. The proportion of sandflies of each species/ trap type in the total catch is represented as percentages.	62
Table 2	Sex ratio (Female: total) of Phlebotomine sandflies collected using sticky paper traps and CDC light traps from El Barth community.	64
Table 3	Infection rate of <i>Leishmania</i> -like flagellates in the sandflies collected from El-Barth community.	66
Table 4	Description of the seven naturally infected females <i>P</i> . <i>papatasi</i> collected from the study area	67
Table 5	The number of rodents collected and identified from each of the four sectors of the El Barth community of North-Sinai, Egypt. The proportion of each species in the total catch is represented as percentages.	68
Table 6	Description of nineteen rodent's isolates obtained from biopsy samples of <i>G. andersoni</i> and <i>G. pyramidum</i> <i>floweri</i> collected from the study area during the years 2006 and 2007	73
Table 7	Real time PCR, RFLP and sequence analysis results. Twenty-eight samples total were cultured from four sources. Real time PCR cycle threshold values (Ct) are reported for experiments using the LEIS and <i>L. major</i> primer-probe sets. RFLP analysis was performed on DNA from seven samples and sequencing DNA conducted on four samples. The <i>Leishmania</i> species identification assigned to each sample is recorded as the Conclusion. ND indicates where target DNA was not detected; experiments not preformed are indicated by a	88

dashed line.

- Table 8 Susceptibility of wild and experimental animal to Leishmania tropica originally isolated from El Barth 91 community.
- Table 9 Infection rate, and location of L. major infection in P. papatasi examined 1, 3, 5, and 7 days post-feeding on infected blood meal (PMG: Posterior midgut, AMG: anterior midgut, SV: stomodeal valve, PHAR: pharynx, PROB: proboscis).
- Table 10 Infection rate, and location of *L. tropica* infection in *P*. papatasi and P. sergenti examined 1, 3, 5, and 7 days post-feeding on infected blood meal (PMG: Posterior midgut, AMG: anterior midgut, SV: stomodeal valve, PHAR: pharynx, PROB: proboscis).

94

92

List of figures:

-

_

Number	Title	Page
Figure 1	The study area showing the construction of living places, sandfly and rodent habitats.	39
Figure 2	Sandfly collection methods; CDC light trap	43
Figure 3	Wire box rodent trap placed near a rodent burrow.	49
Figure 4	Mean number of sandflies <i>P. papatasi</i> and <i>P. sergenti</i> collected from the study area each two hours throughout ten consecutive nights.	63
Figure 5	Illustrated photographs of infected greater gerbil (Demsy); <i>G. pyramidum floweri</i> . The arrow with black ink color refers to suspected <i>Leishmania</i> lesions in the footpad and tail regions.	70
Figure 6	Illustrated photographs showing different clinical appearance of <i>Leishmania</i> lesions. Physician differentiated the lesions into two categories; dry and wet according to their appearance. Dry lesions are presented in the right column and wet lesions on the left column.	76
Figure 7	Nucleotide sequence of ITS-1 DNA fragment from viable <i>Leishmania</i> culture; MHOM/EG/06/RTC-64 (accession FJ460456) originally collected from El Barth community.	79
Figure 8	Nucleotide sequence of ITS-1 DNA fragment from viable <i>Leishmania</i> culture; MHOM/EG/06/RTC-66 (accession FJ460457) originally collected from El Barth community.	79
Figure 9	Nucleotide sequence of ITS-1 DNA fragment from viable <i>Leishmania</i> culture; MHOM/EG/06/RTC-67 (accession FJ460459) originally collected from El Barth	80

community.

- Figure 10Nucleotide sequence of ITS-1 DNA fragment from
viable Leishmania culture; MGER/EG/06/RTC-73
(accession FJ460458) originally collected from El Barth
community.80
- Figure 11 Aligned sequences from the ribosomal internal transcribed spacer 1 (ITS1) of MHOM/EG/06/RTC-64. MHOM/EG/06/RTC-66. MHOM/EG/06 /RTC-67. MGER/EG/06/RTC-73 Leishmania isolates collected from El Barth community. Numbers correspond to the nucleotide sequence starting from the first nucleotide of 82 ITS-1. Dashed area means gaps and rectangle around nitrogen bases corresponds to difference in nucleotide sequence of isolate RTC-67, isolates RTC-66, and isolates RTC-73
- Figure 12 Dendrogram constructed using *Leishmania major*, *L. tropica* and *L. donovani* ITS1sequences. 310--338 bp of the ITS1--5.8S ribosomal RNA region from representative isolates were aligned and a gene tree constructed using maximum parsimony. Bootstrap analysis was performed with 500 replicates and bootstrap values reported at nodes.
- Figure 13RFLP analyses of the ITS1 amplicon amplified from
viable cultures and control DNA and digested with *Hae*
III. Numbers (2--8) represent the sample source (Table 87
8).
- Figure 14Spleen of greater gerbil (Demsy) Gerbillus pyramidum
floweri (N) normal spleen, (I) Infected spleen.
- Figure 15 Comparison of infection rate in sandflies *P. papatasi* and *P. Sergenti* dissected 1, 3, 5, 7, 9, and 11 days postfeeding on rabbit blood mixed with *L. major* 93 amastigotes (1:1).
- Figure 16Comparison of infection rate in sandflies P. papatasi
and P. sergenti dissected 1, 3, 5, and 7 days post-94

85

90