

Study of Factors Affecting Growth and Development of *Leishmania* in Cultures

Thesis
Submitted in Partial Fulfillment of
M.D Degree In Parasitology



By
Anhar Abdel Aziz Mohamed

Supervisors

56451

518-9364

Prof. Dr. Magda El-Sayed Azab
Professor & Head of Parasitology Department

Prof. Dr. Sawsan Abdel Hamid Bishara
Professor of Parasitology

Prof. Dr. Mona Mahmoud Abdel Mawla
Professor of Parasitology

Dr. Elham Ahmed El-Sherif
Assistant Professor of Parasitology

Dr. Elen Mina Mikhail
Head of Parasitology Section, Central Lab, NMRU-3

Faculty of Medicine
Ain Shams University
1998







قال رب اشرح لي صدري وبيسر لي أمري
واحلل عقدة من لساني يفقهوا قولي
صدق الله العظيم
سورة طه
آية ٢٥-٢٨

ACKNOWLEDGEMENT

I am greatly indebted to **Prof. Dr. MAGDA EL-SAYED AZAB**, Head of Department of Parasitology, Faculty of Medicine, Ain Shams University. Words can not do to express my deep gratitude and sincere appreciation to her respectful personality. She furnished valuable suggestions for which I am grateful. Her instructions, remarks and fine touches are impressed on all pages of this dissertation.

I would like to express my sincere gratitude and indebtedness to **Prof. Dr. SAWSAN ABDEL HAMID BISHARA**, Professor of Parasitology, Faculty of Medicine, Ain Shams University, for her valuable advice, continuous guidance and support through this work.

Many thanks to **Dr. MONA ABDEL MAWLA**, Professor of Parasitology, Faculty of Medicine, Ain Shams University, for her careful supervision, valuable remarks and constructive criticism.

My deepest gratitude to **Dr. ILHAM EL-SHEREEF**, Assistant Professor of Parasitology, Faculty of Medicine, Ain Shams University, for her great help in teaching me the technical course and for her cooperation and encouragement.

I am also indebted to **Dr. ELEN MENA**, Head of Parasitology section, Central Lab. NAMRU-3, for the many facilities she provided and for her kind, continuous help and invaluable advice.

Thanks are due to all staff members of Parasitology Department, Faculty of Medicine, Ain Shams University, for their collaboration.

Acknowledgement

Study of Factors Affecting Growth and Development of *Leishmania* in Cultures

By

Anhar Abdel Aziz Mohamed

Faculty of Medicine- Ain Shams University

ABSTRACT

The aim of the present work was to study "in-vitro" the rate of growth and morphological forms of both *L.major* and *L.infantum* in El-ON's culture media supplemented with human, dog, rat, avian as well as rabbit blood which was used as a control. The effect of culture with these types of blood on the infectivity of both *Leishmania* strains to albino mice was also studied. In addition, the effect of both *Leishmania* strains inoculated into the chorio-allantoic membrane of chick embryo was also evaluated.

The results of the present study showed that good yield of both *L.major* and *L.infantum* parasites can be obtained in culture by using avian blood as substitutes for rabbit blood in El-ON's medium. In addition, rat blood gives good results with *L.infantum*. The morphological forms of *L.major* and *L.infantum* on all types of blood supplemented media: elongated promastigotes, spindle promastigotes, paramastigotes and amastigotes were present all through the culture period with variable percentages. Paramastigotes were seen in a dividing state only with *L.infantum* cultured on rat blood supplemented medium.

Out of the two *Leishmania* strains used, *L.major* is infective for the chick embryo. Also, infectivity to experimental animals is not affected by culture of both *Leishmania* strains on rabbit, human, rat, dog as well as avian blood supplemented media.

Abstract

CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	5
• Historical Review	5
• Taxonomy	8
➤ Classification of the genus <i>Leishmania</i>	8
➤ Biochemical and isoenzyme characterization of <i>Leishmania</i>	13
• Life cycle and Morphology of Leishmania Parasite	16
• Clinical forms of Leishmaniases	34
➤ Visceral Leishmaniases	34
➤ Cutaneous and Mucosal <i>Leishmaniases</i>	39
• In vitro cultivation of Leishmania Parasites	46
• Leishmania in the chick embryo	58
• Animal susceptibility to Leishmania	62
AIM AND PLAN OF THE WORK	68
MATERIALS AND METHODS	70
RESULTS	85
DISCUSSION	149
SUMMARY AND CONCLUSION	170
REFERENCES	175
ARABIC SUMMARY	

LIST OF ABBREVIATIONS

CAF:	Chorio-allantoic fluid.
CAM:	Chorio-allantoic membrane.
CL:	Cutaneous leishmaniasis.
DAT:	Direct agglutination test.
DNA:	Deoxyribonucleic acid.
ELISA:	Enzyme linked immunosorbent assay.
EM:	Electron microscopy.
HIV:	Human immunodeficiency virus.
IFAT:	Indirect immunofluorescent antibody test.
IHAT:	Indirect haemagglutination test.
VL:	Visceral leishmaniasis.
YS:	Yolk sac.

LIST OF FIGURES

- Fig. (1):(21)**
Diagrammatic representation of the principal ultrastructure of amastigote form of *Leishmania*.
- Fig. (2):(22)**
Diagrammatic representation of the principal ultrastructure of promastigote form of *Leishmania*.
- Fig. (3):(90)**
Growth curves of *L. major* strain cultured on different blood supplemented preparations of El-ON's medium.
- Fig. (4):(95)**
Growth curves of *L. infantum* strain cultured on different blood supplemented preparations of El-ON's medium.
- Fig. (5a):(98)**
Growth curves of both *L. major* and *L. infantum* strains cultured on rabbit blood supplemented medium.
- Fig. (5b):(99)**
Growth curves of both *L. major* and *L. infantum* strains cultured on human blood supplemented medium.
- Fig. (5c):(100)**
Growth curves of both *L. major* and *L. infantum* strains cultured on rat blood supplemented medium.

Fig. (5d):(101)

Growth curves of both *L. major* and *L. infantum* strains cultured on dog blood supplemented medium.

Fig. (5e):(102)

Growth curves of both *L. major* and *L. infantum* strains cultured on avian blood supplemented medium.

Fig. (6):(104)

Micrograph from Giemsa stained smear (magnification X1000) showing *L. major* elongated promastigotes from El-ON's medium supplemented with rabbit blood.

Fig. (7):(105)

Micrograph from Giemsa stained smear (magnification X1000) showing *L. major* spindle formed promastigote from El-ON's medium supplemented with rabbit blood.

Fig. (8):(106)

Micrograph from Giemsa stained smear (magnification X1000) showing *L. major* paramastigote form El-ON's medium supplemented with rabbit blood.

Fig. (9):(107)

Micrograph from Giemsa stained smear (magnification X1000) showing *L. major* amastigotes from El-ON's medium supplemented with rabbit blood.