# EXPERIMENTAL AND MOLECULAR BIOLOGICAL STUDIES ON THE ROLE OF FLESH FLIES IN THE TRANSMISSION OF TRICHINOSIS

A thesis Submitted for Ph. D degree in ZOOLOGY (Molecular biology)

By

#### **Nour Eldin Shams Eldin Aly**

B.Sc. in Zoology (1984) Collage of Science-Minya UniversityM. Sc. In Parasitology and Immunology, Faculty of Science Cairo University, (2002)

Under Supervision of:

#### Prof. Dr. Ameen A. Ashour

Professor of Parasitology Faculty of Science-Ain Shams University

#### Prof. Dr. Mohammed S. Salama

Professor of Molecular Biology Faculty of Science-Ain Shams University

#### Prof. Dr. Mohammed. M. El-Bahy

Professor of Parasitology Faculty of Veterinary Medicine-Cairo University

#### Prof. Dr. Kamelia A. M. Allam

Professor of Parasitology
The Research Institute of Medical Entomology,
General Organization for Teaching Hospitals and Institutes

Zoology Department-Faculty of Science Ain Shams University-2014 **Student Name:** Nour Eldin Shams Eldin Aly

**Thesis Title:** Experimental and Molecular biological studies on the role of Fleshflies in the transmission of trichinosis

**Degree:** Philosophy Doctor of Zoology Science (Molecular Biology)

This thesis has been supervised by:

#### Prof. Dr. Ameen A. Ashour

Professor of Parasitology, Faculty of Science-Ain Shams University

#### Prof. Dr. Mohammed S. Salama

Professor of Molecular Biology, Faculty of Science-Ain Shams University

#### Prof. Dr. Mohammed. M. El-Bahy

Professor of Parasitology, Faculty of Veterinary Medicine Cairo University

#### Prof. Dr. Kamelia A. M. Allam

Professor of Parasitology, The Research Institute of Medical Entomology General Organization for Teaching Hospitals and Institutes

#### **Approval Sheet**

**Ph. D Thesis Title** Experimental and molecular biological

studies on the role of flesh flies in the

transmission of trichinosis

Candidate name Nour Eldin Shams Eldin Aly

Submitted to degree Philosophy Doctor of Science in Zoology

(Molecular Biology)

**Prof. Dr. Ameen. A.** Professor of Parasitology

**Ashour** Faculty of Science-Ain Shams University

**Prof. Dr. Mohammed** Professor of Molecular Biology

S. Salama Faculty of Science-Ain Shams University

**Prof. Dr. Mohammed M.** Professor of Parasitology, Faculty of

**El-Bahy** Veterinary Medicine-Cairo University

**Prof. Dr. Kamelia A. M.** Professor of Parasitology, The Research **Allam** Institute of Medical Entomology, General

Organization for Teaching Hospitals and

Institutes

## TO

My Mother, My dear wife,

My sons and the soul of

My father

#### **Acknowledgment**

Thank God for giving me the effort to complete this work.

I would like to offer my sincere thanks to **Prof. Dr. Ameen A. Ashour**, Professor of Parasitology, Faculty of Science-Ain Shams University for his supporting this work, and fruitful advices, encouragement and revision of the manuscript.

Also I wish to express my deepest gratitude to: **Prof. Dr. Mohammed S. Salama**, Professor of Molecular Biology, Faculty of Science; Ain shams University for his keen supervision, powerful appreciations and kind observations.

Many thanks to **Prof. Dr. Mohammed M. El-Bahy**, Professor of Parasitology Faculty of Veterinary Medicine-Cairo University for suggesting the point of this work, deep concern, encouragement, continuous advices and supporting this work.

Great thanks to **Prof. Dr. Kamelia A. M. Allam**, Professor of medical Parasitology. The Research Institute of Medical Entomology-General Organization for Teaching Hospitals and Institutes, for her kind observations, precious guidance, and useful helps during different stages of this work.

Great thanks to **Dr/ Mohammed Ismail Soliman**, Professor of medical Parasitology and the dean of The Research Institute of Medical Entomology-The general Organization for Teaching Hospitals and Institutes, who provided me all equipment, chemicals and all facilities to finish this work.

My grateful thanks to **Assist-Prof.** / Yousrea Abdel Hamid, the head of Entomology and Molecular Biology department, the Research Institute of Medical Entomology-General Organization for Teaching Hospitals and Institutes, for sharing in the laboratory work, her guides, appreciations and her helpful advices through the work stages.

#### **List of abbreviations**

Am. American
Ab. Antibody
Abs. Biochem. Biochemistry

C° Degree centeer

C° Degree centegrate

Company
C<sup>T</sup> Company
Thermal cycle

**DNA** Deoxyribo-nucleic acid dpi. Day post infection

**Ed.** Edition

**ELISA** Enzyme-linked Immunosorbent assay

**Epidem.** Epidemiology

Fig. Figure
Hist. Histology
Hyg. Hygiene

IgMImmunoglobulin- MIgAImmunoglobulin- AIgGImmunoglobulin- GIgG 1Immunoglobulin- G 1IgG 2Immunoglobulin- G 2IgEImmunoglobulin- EIgDImmunoglobulin- D

Immuno. Immunology Infect. International

J. Journal
M Mole
Med. Medical
Min. Minute
μl Micro liter
nM Nano mole

Rn normalized reporter NTC No template control

**Path.** Pathology

PBS Phosphate Buffer Saline PCR Polymerase chain reaction

P.G. Pico gramParasitol. Parasitology

**Rn**+ The Rn value of all reactants

**Rn-** The Rn value of an un-reacted sample

 $\Delta$ Rn. delta Rn The magnitude of the signal generated by

the given set of PCR

**RNA** Ribo-nucleic acid

Soc.SocietyScien.ScienceSpp.SpeciesTrop.Tropical

**T. spiralis** Trichinella spiralis

Vet. Veterinary Vol. Volume

## **List of Tables**

Table	Discretion	Page
No.		No.
1	Rat groups used during the study	<b>71</b>
2	The primers and the probe design for <i>T. spiralis</i> detection by using real-time PCR	<b>79</b>
3	The percentage of the <i>T. spiralis</i> larvae in the infected pig diaphragm muscle samples.	86
4	Development of <i>Trichinella spiralis</i> larvae (pig origin) in the experimental rats	89
5	Development of <i>T. spiralis</i> infection in <i>Sarcopha</i> , adults and larval stages (the 2 <sup>nd</sup> generation) after exposure to infected rat muscles, along <i>Trichinel</i> infection period and the fly life cycle.	97
6	Development of <i>T. spiralis</i> infection in <i>Sarcopha</i> , larval instars (from the 3 <sup>rd</sup> generation), after exposure to infected rat muscles, along <i>Trichineli</i> infection period and the rest of fly life cycle.	98
7	Infection of rats by <i>T. spiralis</i> from infected <i>Sarcophaga</i> maggots (the 3 <sup>rd</sup> larval instars), 35 dpi.	105
8	The Ct values of the amplificated DNA copies of tissues samples in the real-time PCR device	107
9	The extracted results by the real-time PCR device	1091
10	The $C_T$ values of the amplification DNA copies of tissue samples in the real-time PCR device.	110
11	The C <sub>T</sub> values of the amplification DNA copies of tissues samples in the real-time PCR device	114
12	The $C_T$ values of the amplification DNA copies of tissues samples in the real-time PCR device.	118

## **List of figures**

Fig.	Figures descriptions	Page
no.		no.
1	The wooden cadge for rearing of fleshflies	74
2	The steps of PCR reaction in the real-time PCR device	85
3	Cross section in the diaphragm muscles of infected pig, showing the encysted <i>Trichinella</i> larvae.	87
4	Individual Fresh compressed sample from the infected diaphragm muscle of infected pig.	87
5	Fresh compressed sample from the infected diaphragm muscle of infected pig, by the trichinoscope.	88
6	Dissected infected rat.	90
7	Cross section in the skeletal muscles of the infected rats.	90
8	Fresh compressed sample slide, in the skeletal muscles of the infected rats.	91
9	Adult male of <i>T. spiralis</i> from the intestine content of the infected rats.	93
10	The posterior end of the adult male of the <i>T</i> . <i>spiralis</i> extracted from the intestine lumen of the infected rats.	93
11	Adult female of <i>T. spiralis</i> from the intestine contents of the infected rats	94
12	Fresh compressed sample slide in the skeletal muscles of the infected rats.	94
13	Photographic picture of the adult <i>Sarcophaga argyrostoma</i> from the 1 <sup>st</sup> generation.	96

14	A lateral view for the 3 <sup>rd</sup> larval instars of infected <i>Sarcophaga argyrostoma</i> .	100
15	A lateral view of the infected <i>Sarcophaga</i> 3 <sup>rd</sup> larval instars.	100
16	Posterior view of the infected 3 <sup>rd</sup> larval instars of <i>Sarcophaga argyrostoma</i> .	101
17	The <i>Sarcophaga</i> 3 <sup>rd</sup> larval instars feeding on the infected rats skeletal muscles, group- B	101
18	Whole dorsal view for the infected <i>Sarcophaga</i> 3 <sup>rd</sup> larval instars after exposure to infected rats skeletal muscles of group-B.	102
19	The compressed infected <i>Sarcophaga</i> 3 <sup>rd</sup> larval instars.	102
20	A fresh compressed sample of the free <i>T</i> . <i>spiralis</i> larva extracted from the infected <i>Sarcophaga</i> 3 <sup>rd</sup> larval instars.	103
21	A fresh compressed sample of the encysted <i>T</i> . <i>spiralis</i> larva extracted from the infected skeletal muscles of the rats of group-C.	104
22	Plate Sample Values -Quantitative PCR, 12 Hr 01 Min. mxp.	112
23	Curve-1, of the real-time PCR amplifications	113
24	Plate Sample Values of Quantitative PCR, 10Hr 44Min.mxp	116
25	Curve 2, of the real-time PCR amplifications	117
26	Plate Sample Values of Quantitative PCR, 12Hr 38Min.mxp.	120
27	Curve 3, the real time amplifications.	121

## **Index**

Subject	Page
	no.
Introduction	1
Aim of work	3
Review of Literature	4
Materials and methods	69
Results	86
Discussion	122
Conclusion	135
Recommendations	136
References	137
Summary	168
Arabic summary	-

## **Contents**

Subject	
	No.
Acknowledgement	-
List of abbreviations	-
List of tables	-
List of figures	-
Introduction	1
Review of Literature:	4
Historical review	4
Geographical Distribution of <i>Trichinella species</i> in the	6
world	
Distribution of <i>Trichinella spiralis</i> in Egypt	9
Taxonomy of the parasite	12
Morphological features of <i>T. spiralis</i>	13
Morphology of the adult male	13
Morphology of the adult female	14
Morphology of the infective first stage (encysted) larva	14
The life cycle of the parasite	15
The Enteral phase	18
The migratory phase	19

The Parenteral phase	
Epidemiology and prevalence of the parasite	22
Trichinosis in animals	24
Trichinosis in unusual hosts	32
The generalized life cycle of the flesh flies	36
Classification of the insect	37
Biological behavior of the flesh fly maggots	38
Trichinosis in humans	39
Clinical picture of the disease	41
Detection and diagnosis of the parasite	43
The direct method	43
The compressorium technique	43
The artificial digestion technique	45
Histological technique	47
The indirect methods	48
Immunodiagnosis method	48
Precipitation (Agglutination) technique	48
Serological methods	49
The principles of the PCR technique	49
PCR Primer and Probe Design	53
Real-time (R-T PCR)	54
The principles of the technique	54
TaqMan assay	60

TaqMan Magnetic Glass Particles (MGB) Probes		
Material and methods:		
1-Diagnosis of <i>T. spiralis</i> in naturally infected pig muscle samples		
		The compressorium technique
2-Preparation of the experimental animals	70	
3-Preparation of the infection positive control	70	
4-Selection and rearing of Flesh fly	72	
The rearing method	72	
a-The preparation of <i>Sarcophaga</i> the 1 <sup>st</sup> generation	72	
colony	12	
b-The Preparation of <i>Sarcophaga</i> the 2nd. generation	74	
colony		
c- The Preparation of the 3 <sup>rd</sup> generation colony	75	
d- Inducing of <i>Trichinella spiralis</i> infection in		
Sarcophaga 3 <sup>rd</sup> instar larvae	76	
5- Identification of the <i>T. spiralis</i> by using real-time PCR		
		Materials
Base of the technique	78	
a-Preparation of reagents	79	
b-Primer design	79	
c-DNA extraction	80	