

Ain Shams University. Women's College. Zoology Department.

### Molecular and Histopathological Following up of Toxoplasmagondii Infection in Experimentally Infected New Zealand Rabbits

A Thesis submit for (Ph.D.) Degree in Zoology

#### By Mona Ahmed Mohamed Soliman Farah

Assist. Lecturer in Zoology Department Faculty of Women for Arts, Science and Education Ain-Shams University

#### **Supervisors**

Prof. Dr. Eglal A. Koura

Prof. of Protozoology & Parasitology Zoology Department, Faculty of Women for Arts, Science and Education Ain-Shams University

Prof. Dr. Adel S. Amin

Prof. Dr. Ashraf M. Barakat

ProfessorofMolecular Biology Biotechnology Research Unit Animal Reproduction Research Institute Head of Dept. of Zoonotic and Epidemiological Diseases National Research Centre (NRC)

Prof. Dr. Samah F. M. Ali

Dr. Hanan M. M. Ismail

ProfessorofMolecular Biology Biotechnology Research Unit Animal Reproduction Research Institute

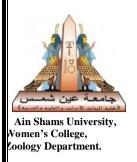
Lecturer, Zoology Department, Faculty of Women for Arts, Science and Education Ain Shams University

# بسم الله الرحمن الرحيم



## حدق الله العظيم

سورة التوبة 105



#### APPROVAL SHEET

Title: Molecular and Histopathological Following up of

Toxoplasmagondii Infection, in Experimentally Infected New Zealand

Rabbits

Name: Mona Ahmed Mohamed Soliman Farah Scientific Degree: Ph.D. In Zoology

#### **Supervisors**

Prof. Dr. Eglal A. Koura

Prof. of Protozoology & Parasitology Zoology Department, Faculty of Women for Arts, Science and Education Ain-Shams University

Prof. Dr. Adel S. Amin

Prof. Dr. Ashraf M. Barakat

ProfessorofMolecular Biology Biotechnology Research Unit Animal Reproduction Research Institute Head of Dept. of Zoonotic and Epidemiological Diseases National Research Centre (NRC)

Prof. Dr. Samah F. M. Ali

Dr. Hanan M. M. Ismail

ProfessorofMolecular Biology Biotechnology Research Unit Animal Reproduction Research Institute Lecturer, Zoology Department, Faculty of Women for Arts, Science and Education Ain Shams University

## **QUALIFICATION**

Name: Mona Ahmed Mohamed Soliman Farah

Scientific Degree: M.Sc.

**Department** : Zoology

College: Women College for Arts, Science and Education

University: Ain Shams University

M.Sc. Graduation Year: 2014

Email: Mona\_farah25@yahoo.com

Mona.farah@women.asu.edu.eg



Glad to dedicate this work to my kind family, special dedication to my husband and my children Arwa& Ziad

First and foremost, "Allhamdulillah" for his most Merciful and most Gracious who gave me the ability to carry out this work which. May peace and blessings be upon **Prophet**Mohammad (PBUH), his family and his companions.

#### **ACKNOWLEDGMENT**

I would like to express my deep thanks and sincere gratitude to my **supervisor:** 

**Prof. Dr. Eglal A. Koura**, Prof. of Protozoology & Parasitology, Zoology Department, Faculty of Women for Arts, Science & Education, Ain-Shams University for her great efforts, suggesting the topic and planning the scheme of the present study, fruitful guidance, enrichment my background, enlightening me with her vast experience during the course of this work and for the refinement of the thesis. Her valuable help during the course of this study is greatly appreciated. I pray to almighty (**ALLAH**) to reward her in this life and the hereafter as well as May **ALLAH** assist me to do her some of the favors she did for me. No words seen to be sufficient to describe, to her owe much.

I wish to express my great thanks and deep appreciation to **Prof. Dr. Adel S. Amin**, Professor of Molecular Biology, Biotechnology Research Unit Animal, Reproduction Research Institute, for getting me involved with this subject, suggesting the topic and planning the scheme of the present study and for his guidance over the years of work. His constant encouragement made a huge difference in my work and enormously expanded the dimensions of the thesis.

Thanks to **Prof. Dr. Ashraf M. Barakat**, Head of Department of Zoonotic and Epidemiological Diseases, National Research Centre (NRC) for his helpful, guiding, discussions and continuous support.

I would like to express heartily thankful and thanks with love to **Prof. Dr. Samah F. Darwish,** Professor of Molecular Biology, Biotechnology Research Unit, Animal Reproduction Research Institute, not only for suggesting, planning the point of research and valuable supervision, but also for her great help, guidance, appropriate choice of research topic and continuous encouragement through this work.

I would like to express heartily thankful and thanks with love to **Dr. Hanan M. M. Ismail,** Lecturer in Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University, for helping me, sincere advice and supporting me during all steps of this work.

I wish to express my great thanks and deep appreciation to **Prof. Dr. Walid S. El-Nattat**, Professor in Reproduction and Artificial Insemenation Dept, National Research Centre for helping me in samples collection, **Prof. Dr. Hany A. Amer**Professor in Pathology of Reproduction Department, Animal Reproduction Research Institute for helping in histophathological studies and **Dr.Tahani S. Behor**ResearcherinBiotechnology Research Unit, Animal Reproduction Research Institute for helping in the practical part of PCR assay.

With great pleasure, I would like to express my sincere gratitude to the staff members of Zoology Department, Faculty of Women, Ain Shams University. I also like to express my thanks with love to my friends for their support throughout this work.

There are no words are sufficient to express my gratitude thanks to my late **father**, my dear **mother**, my **husband**, my children **Arwa&Ziad**and all members of my family for their continuous help, support and encouragement extended to me during the progress of this work.

## TABLE OF CONTENTS

<u>Title</u>	Page
Table of contents	I
List of tables	$\mathbf{V}$
List of figures	VI
List of abbriviations	XI
Abstract	XIII
CHAPTER I: INTRODUCTION	1
Aim OfThe Work	6
CHAPTER II: REVIEW OF LITERATURE	7
1. The etiological agent of toxoplasmosis	7
2. General background on T. gondii	8
3. Life cycle of parasite	9
4. Source of infection and mode of transmission	18
5. Toxoplasma gondii in Egypt	30
6. Toxoplasma gondii and male reproductive system	35
7. Pathogenicity	37
7.1. Toxoplasmosis in the final host (cat)	37
7.2. Toxoplasmosis in human	39
7.3. Toxoplasmosis in intermediate host animals	41
8. Diagnosis	44
8.1. Classic methods	44
8.2. Molecular diagnosis	50
8.3. Histopathological effects of <i>T. gondii</i>	55

#### **CHAPTER III: MATERIALS AND METHODS**

* The parasite	58
* Experimental animals	58
<b>Experimental design</b>	
ExperimentI	59
*Doses and the route of administration	59
1- High dose infection	59
2- Low dose infection	60
*Samples collection and storages of experiment I	60
	60
Blood samples	60
Semen samples Tissue specimens	61
Tissue specimens	61
*Samples collection and startages of experimental H	62
*Samples collection and storages of experimental II	62
Blood samples	_
Tissue specimens	62
* Samples processing:	62
1. Histopathological Examination	62
2. DNA extraction from different samples types	63
A. DNA extraction from blood	63
B. DNA extraction from tissues	63
C. DNA extraction from semen	64
• DNA amplification by SYBR Green Real-Time PCR	64
assay	
<ul> <li>Optimization of Real time PCR conditions</li> </ul>	65
• Amplification programs:	66
<ul> <li>Standard curve and detection limit</li> </ul>	66
• Real time PCR analysis	67
<ul> <li>Laboratory condition for PCR</li> </ul>	69
• Equipments	70
*Assays used for detection of T. gondii by Real time PCR	•
	71
CHAPTER IV: RESULTS	
	<b>72</b>

1. Experiment I	72
1.1. High dose infection (group 1)	72
1.1.1. Clinical symptoms and post mortem examination	
	72
1.1.2. Semen analysis:	73
1.1.3. Detection of <i>Toxoplasma gondii</i> in blood samples by	75
Real Time PCR	13
1.1.4. Detection of <i>Toxoplasma gondii</i> in semen samples	77
by Real Time PCR	, ,
1.1.5. Histopathological finding of group (1)	79
1.2. Low dose infection:	83
1.2.1. Clinical symptoms and post mortem examination	83
1.2.2. Immune-competent group	84
1.2.2.1. Semen analysis	84
1.2.2.2. Detection of <i>Toxoplasma gondii</i> in blood samples	85
by Real Time PCR	
1.2.2.3. Detection of <i>Toxoplasma gondii</i> in semen samples	85
by Real Time PCR	
1.2.2.4. Detection of <i>Toxoplasma gondii</i> in tissue samples	87
by Real Time PCR 2.2.5 Historythological findings of immune computant	
.2.2.5. Histopathological findings of immune-competent	89
group 1.2.3. Immune-suppressive group	98
1.2.3.1. Semen analysis	98
1.2.3.2. Detection of <i>Toxoplasma gondii</i> in blood samples	
by Real Time PCR	99
1.2.3.3. Detection of <i>Toxoplasma gondii</i> in semen	99
1.2.3.4. Detection of <i>Toxoplasma gondii</i> in	
tissuesamples by Real Time PC	100
1.2.3.5. Histopathological findings of immune-	100
suppressive group	102
Comparison between different groups of experiment I	111
• Semen characteristics	111
<ul> <li>Following up the course of infection in semen of</li> </ul>	112
experimentally infected rabbits	114

2.Experiment II:	114	
2.1. Detection of <i>T. gondii</i> DNA in female rabbits mated	115	
with infected bucks		
2.2. Histopathological findings in female		
2.3. Detection of <i>T. gondii</i> in tissues of fetuses	120	
2.4. Histopathological finding of tissues collected from		
fetuses	121	
CHAPTER V: DISCUSSION	124	
CHAPTER VI: SUMMARY AND CONCLUSIONS	135	
CHAPTED VIII DEFEDENCES	120	
CHAPTER VII: REFERENCES	139	
CHAPTER VIII: ARABIC SUMMARY	180	

## LIST OF TABLES

No.	Title	
NO.		
Table (1)	Sequence primer pairs used in Real -Time	65
	PCR.	
Table (2)	Real time PCR components for 20µl reaction	65
	volume in each PCR tube.	
Table (3)	Real time amplification profile for B1	66
	primer.	
Table (4)	Semen analysis after infection with	112
	Toxoplasmagondii for different infection	
	periods (Mean ± Std. err.)	