

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

بسم الله الرحمن الرحيم





MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY



Cairo University Faculty of Veterinary Medicine Department of Clinical Pathology



Clinicopathological and Molecular Studies on Ovine Theileriosis in Egypt

Thesis presented by

Mahmoud Akram Ali Eliwa

(B.V.Sc., 2015, Cairo University)
Submitted for the degree of M.V.Sc.
(Clinical Pathology)

Under the supervision of

Prof. Dr. Mostafa Mahmoud Bashandy Omran
Professor of Clinical Pathology,
Faculty of Veterinary Medicine,
Cairo University

Dr. Khaled Mohamed Ahmed Mahran Assistant Professor of Clinical Pathology,

Faculty of Veterinary Medicine, Cairo University Prof. Dr. Waheed Mohamed Ali Mousa

Professor of Parasitology,
Faculty of Veterinary Medicine,
Cairo University

Supervision sheet

Clinicopathological and Molecular Studies on Ovine Theileriosis in Egypt

Master of science

In

Veterinary Medical Sciences

(Clinical Pathology)

By

Mahmoud Akram Ali Eliwa

B. V. Sc., 2015, Cairo University

Supervision committee

Prof. Dr. Mostafa Mahmoud Bashandy Omran
Professor of Clinical Pathology,
Faculty of Veterinary Medicine,
Cairo University

Dr. Khaled Mohamed Ahmed Mahran

Assistant Professor of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University Prof. Dr. Waheed Mohamed Ali Mousa

Professor of Parasitology,
Faculty of Veterinary Medicine,
Cairo University

Cairo University

Faculty of Veterinary Medicine

Department of Clinical Pathology

Name: Mahmoud Akram Ali Eliwa

Date of birth: 10/11/1992

Title of the thesis: Clinicopathological and Molecular Studies on Ovine

Theileriosis in Egypt

Prof. Dr. Mostafa Mahmoud Bashandy Omran

Professor of Clinical Pathology, Faculty of Veterinary Medicine,

Cairo University

Dr. Khaled Mohamed Ahmed Mahran

Assistant Professor of Clinical Pathology, Faculty of Veterinary Medicine,

Cairo University

Prof. Dr. Waheed Mohamed Ali Mousa

Professor of Parasitology, Faculty of Veterinary Medicine, Cairo University

Abstract

Ovine theileriosis is one of the hemoprotazoal disease that is transmitted by tick bites and causes severe economic loss. This study aimed to diagnose sheep theileriosis in 3 regions of Egypt (Cairo, Giza and Al Monofia) by using different clinicopathological, pathological and molecular tools. A total of 152 sheep blood samples were selected randomly from farms and veterinary clinics. The incidence of Theileria infection by microscopic examination of blood smears was 21%, while the incidence by PCR (using universal *Theileria* primer) was 36.8%. The use of speciesspecific primers showed a result of 53.6% single infection; *Theileria ovis*, and 46.4% mixed infection; T. ovis and T. lestoquardi. Depending on the PCR results, the samples were divided into three groups: Theileria negative group, T. ovis group and mixed T. ovis and T. lestoquardi group. The examination of all infected groups did not show any significant changes between them. The hemogram showed significant hypochromic anemia, leukopenia, neutropenia, macrocytic lymphopenia, monocytopenia, eosinopenia and thrombocytopenia in *Theileria* infected groups in comparison with *Theileria* negative group. Biochemical analysis showed significant hypoproteinemia, hypoalbuminemia, total and indirect hyperbilirubinemia with elevations of AST and GGT activities and azotemia which was characterized by increase of BUN and creatinine concentrations while non-significant changes were detected in A:G ratio and direct bilirubin concentration. The pathological examination showed lymphocytic depletion and necrosis with hemorrhages in lymph nodes and spleen. Sequence and phylogenetic analysis were performed by targeting the 18S rRNA gene of *Theileria* species (GenBank Accession Numbers MT002826 and MT002827). In conclusion, this is the first report of phylogeny of T. lestoquardi infected sheep in Egypt.



DEDICATION

I dedicate this work to **my family** (my parents, my wife, my brothers, my sister and my son) for their patience, love, support and encouragement to complete this thesis.

ACKNOWLEDGMENT

First of All, I would like to express my all-embracing gratitude and praise to **Allah**, glorified is He, for his unmitigated support and graceful benevolence in carrying out this thesis.

I would like to thank my advisors, **Prof. Dr. Mostafa**M. Bashandy and Dr. Khaled M. A. Mahran, Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt, and **Prof. Dr. Waheed M. Mousa**, Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Egypt for their help and encouragement during the period of this thesis. They have been a continuous source of help and gave enormous support in my search.

I would like to thank the veterinarians and my wife who helped me in samples collection from veterinary clinics, sheep farms and slaughterhouse.

I would like to acknowledge all staff of Genome Research Unit and Clinical Pathology unit, Animal Health Research Institute (AHRI) and the Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo university who provided me an opportunity to join their team as intern, and gave access to the laboratory and research facilities, withsout their precious support it would not be possible to conduct this research.

CONTENTS

		page
INTR	RODUCTION	2
REVI	IEW OF LITERATURE	5
1.	Definition	5
2.	Etiology	5
3.	Distribution of the disease	5
4.	Economic significance	6
5.	Incidence of <i>Theileria</i> spp. infection in sheep	7
6.	Life cycle	8
7.	Transmission and pathogenesis of Theileria	9
8.	Diagnostic approach of theileriosis	10
8.1.	Clinical signs	10
8.2.	Microscopic examination of Giemsa-stained blood film	10
8.3.	Clinicopathological changes during Theileria infection in she	ep. 12
8.4.	Histopathological effect of <i>Theileria</i> infection in sheep	15
8.5.	Serological assays	17
8.6.	Molecular investigations of ovine theileriosis	18
8.6.1	Polymerase chain reaction (PCR)	18
8.6.2.	Sequence and phylogenetic analysis	22
9.	Prevention and Treatment	24
PUBI	LISHED PAPER	25
DISC	USSION	58
CON	CLUSION AND RECOMMENDATIONS	63
SUM	MARY	64
A PPE	ENDIX	67

REFERENCES	85
الملخص العربي	

LIST OF TABLES

Table	Title	Page
1	Numbers of examined sheep according to different	29
	geographical localities.	-
2	Primers design of different <i>Theileria</i> primers sets.	31
3	The incidence of clinical signs of theileriosis in sheep.	34
4	The prevalence of ovine theileriosis in the different	34
4	localities.	34
5	Prevalence of <i>Theileria</i> infections with different species.	37
6	Hematological parameters of <i>Theileria</i> infected and control	40
	groups.	40
7	Biochemical parameters of <i>Theileria</i> infected and control	11
,	groups.	71

LIST OF FIGURES

Figure	Title	Page
1	Microphotograph showing intra-erythrocytic merozoits of sheep blood smears (Field stain, x100).	35
2	Microphotograph showing intra-erythrocytic merozoits of sheep blood smears (Field stain, x100).	36
3	Microphotograph showing lymphocytic microschizont of sheep blood smears (Field stain, x100).	36
4	Microphotograph showing lymphocytic macroschizonts (Koch's blue bodies) of lymph nodes smears (Field stain, x100).	37
5	Ethidium bromide-stained gel electrophoresis showing specific bands from PCR amplified products by using Theileria spp. universal primer in lane 3,6 and 10. Lane M: marker 100 bp.	38
6	Ethidium bromide-stained gel electrophoresis showing specific bands from PCR amplified products by using <i>T. ovis</i> specific primer in lane 1, 2, 3, 4, 5, 6, 7, 8, 10 and 11. Lane M: marker 100 bp.	38
7	Ethidium bromide-stained gel electrophoresis showing specific bands from PCR amplified products by using <i>T. lestoquardi</i> specific primer in lane 6, 8, 9 and 10. Lane M: marker 100 bp.	39
8	Macrophotograph of lymph node with enlargement and sub-capsular hemorrhages.	42
9	Macrophotograph of spleen with enlargement and subcapsular hemorrhages.	42
10	Microphotograph of sheep spleen showing (A) diffuse hemorrhage with deposition of brownish hemosiderin pigment in addition to necrotic foci diffuse depletion of the lymphoid tissue (H and E, x200) and (B) diffuse deposition of bluish hemosiderin pigment (Prussian blue, x200).	43
11	Microphotograph of sheep lymph node showing depletion and necrosis of multiple 503 lymphoid follicles (arrows) (H and E, x200).	44
12	Phylogenetic tree of <i>T. lestoquardi</i> (MT002826) revealed 100% homology with <i>T. lestoquardi</i> from (KF771185),	45

Figure	Title	Page
	(KM117212 and KJ458988), (AF081135) and (KC778786). This isolate showed 99% homology with <i>T. annulata</i> from (KF429799), (AY508463), (MG599090) and (DQ287944).	
13	Phylogenetic tree of <i>T. ovis</i> (MT002827) revealed 98% homology with <i>T. ovis</i> from (MG203885), (EU622911), (KT851436), (FJ603460), (MG725961) and (MH819508).	45

FIGURES OF APPENDIX

Figure	Title	Page
1	Enlargement of parotid lymph node in infected sheep.	67
2	Tick bite wound on infected sheep	67
3	Nasal discharge of infected sheep.	68
4	Intra-erythrocytic merozoits of sheep blood smears (Field stain, x1000).	68
5	Lymphocytic macroschizont of sheep blood smears (Field stain, x1000).	69
6	Lymphocytic schizont of splenic impression smears of sheep (Field stain, x1000).	69
7	Intra-erythrocytic merozoits of splenic impression smears of sheep (Field stain, x1000).	70
8	RBCs count in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	71
9	Hematocrit in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	71
10	Hemoglobin in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	72
11	MCV in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	72
12	MCHC in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	73
13	Total leukocytic count in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	73
14	Neutrophil in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	74
15	Lymphocyte in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	74

Figure	Title	Page
	Monocyte in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	
16	infected group (GII) and mixed Theileria infected group	75
	(GIII).	
	Eosinophil in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	
17	infected group (GII) and mixed <i>Theileria</i> infected group	75
	(GIII).	
18	Platelets in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected	76
	group (GII) and mixed <i>Theileria</i> infected group (GIII).	7.0
40	Total proteins in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	
19	infected group (GII) and mixed <i>Theileria</i> infected group	76
	(GIII).	
20	Albumin in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected	77
	group (GII) and mixed <i>Theileria</i> infected group (GIII).	
21	Globulins in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	77
41	infected group (GII) and mixed <i>Theileria</i> infected group (GIII).	//
	A:G ratio in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	
22	infected group (GII) and mixed <i>Theileria</i> infected group	78
	(GIII).	/6
	AST in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected	
23	group (GII) and mixed <i>Theileria</i> infected group (GIII).	78
2.4	GGT in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected	=0
24	group (GII) and mixed <i>Theileria</i> infected group (GIII).	79
25	Urea in <i>Theileria</i> negative group (GI), <i>T. ovis</i> infected	70
25	group (GII) and mixed <i>Theileria</i> infected group (GIII).	79
	Creatinine in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	
26	infected group (GII) and mixed Theileria infected group	80
	(GIII).	
	Total bilirubin in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	
27	infected group (GII) and mixed Theileria infected group	80
	(GIII).	
	Direct bilirubin in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	
28	infected group (GII) and mixed <i>Theileria</i> infected group	81
	(GIII).	
	Indirect bilirubin in <i>Theileria</i> negative group (GI), <i>T. ovis</i>	
29	infected group (GII) and mixed <i>Theileria</i> infected group	81
	(GIII).	