

## ANALYSIS OF Toxoplasma gondii EXCRETORY SECRETORY ANTIGENS (ESA) AND IDENTIFICATION OF POTENTIAL MARKERS OF ACUTE INFECTION

by

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## Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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## DEDICATION

This thesis is dedicated to my beloved husband, Ahmad,

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## TABLE OF CONTENTS

Dedication	ii
Acknowledgment	iii
Table of Contents	V
List of Tables	XV
List of Figures	xvii
List of Abbreviations	XX
Abstrak	xxi
Abstract	xxiii

### CHAPTER ONE: INTRODUCTION

1.1	Overview of toxoplasmosis	1
1.2	Historical perspective of <i>Toxoplasma gondii</i>	2
1.3	Epidemiology of toxoplasma infection	3
1.4	Toxoplasma gondii and its life cycle	7
1.5	Immunology of toxoplasma infection	.14
1.6	Pathogenesis	15
1.7	Clinical Disease	.18
	1.7.1 Infection in immunocompetent individuals	.18
	1.7.2 Infection in immunocompromised patients	.19
	1.7.3 Congenital toxoplasmosis	.21
	1.7.4 Ocular infection	21
	1.7.5 Toxoplasma exposure and risk of schizophrenia	23
1.8	Diagnosis	23

	1.8.1	Animal and cell culture inoculation	24
	1.8.2	Histological diagnosis	24
	1.8.3	Sabin-Feldman dye test	25
	1.8.4	Complement fixation test	26
	1.8.5	Latex agglutination test/ Indirect agglutination test	27
	1.8.6	Indirect fluorescent assay	27
	1.8.7	Enzyme immunoassays	28
	1.8.8	Immunosorbent agglutination assay	.30
	1.8.9	Direct agglutination test	31
	1.8.10	IgG avidity test	32
	1.8.11	Western blot	33
	1.8.12	Polymerase chain reaction	35
	1.8.13	Recombinant antigens for serodiagnosis	38
1.9	Transmi	ission	41
	1.9.1	Carnivorism transmission	41
	1.9.2	Transplacental transmission	42
	1.9.3	Fecal- oral transmission	43
	1.9.4	Transmission by organ transplantation and blood transfusion	43
	1.9.5	Other routes of transmission	44
1.10	Prever	ntion	45
	1.10.1	Primary prevention	45
	1.10.2	Secondary prevention – screening	46
1.11	Treatmen	at	47
	1.11.1	Infection in the immunocompromised host	47
	1.11.2	Maternal and fetal infection	48

	1.11.3	Chorioretinitis	49
	1.11.4	Infection in the immunocompetent host	50
1.12	Animal	models and <i>Toxoplasma gondii</i>	50
1.13	In vitro	of Toxoplasma gondii	53
1.14	Proteomi	cs	54
	1.14.1	Protein isolation and identification	56
	1.14.2	Application of proteomics to the study of <i>Toxoplasma gondii</i>	58
	1.14.3	Excretory secretory antigens of <i>Toxoplasma gondii</i>	61
1.15	Statemen	t of the problem and rationale for the study	63
1.16	Objective	es of the study	64

### CHAPTER TWO: MATERIALS AND METHODS

2.1	Serum samples			
2.2	Classification of the serum samples			
2.3	Indirect '	Toxo IgG –Enzyme –Linked Immunosorbent Assay	67	
	2.3.1	Preparation of reagents for ELISA	68	
		2.3.1.1 Coating buffer	.68	
		2.3.1.2 Phosphate buffered saline (PBS)	68	
		2.3.1.3 Blocking stock solution 10%	.68	
		2.3.1.4 Phosphate buffered saline-Tween 20 (PBS-T)	69	
		2.3.1.5 <i>T. gondii</i> soluble antigen	.69	
		2.3.1.6 Secondary antibody solution	.69	
		2.3.1.7 Substrate solution	.69	

	2.3.2	Optimization of various parameters in Toxo-IgG ELISA70
		2.3.2.1 Concentration of <i>T. gondii</i> soluble antigen and dilution of primary antibody70
		2.3.2.2 Dilution of secondary antibodies
	2.3.3	Toxo-IgG ELISA71
2.4	Platelia®	<sup>9</sup> Toxo IgM TMB kit72
	2.4.1	Preparation of reagents for Platelia® Toxo IgM TMB kit72
		2.4.1.1 Reagents ready to use72
		2.4.1.2 Reagents to be reconstituted75
	2.4.2	Method of performing Platelia <sup>®</sup> Toxo IgM TMB kit75
2.5	Preparat	ion of <i>in vivo</i> ESA76
	2.5.1	<i>T. gondii</i> Parasites (RH strain)76
	2.5.2	Animal infection76
	2.5.3	Enumeration of the number of <i>in vivo</i> parasites77
	2.5.4	Calculating the percentage of viable parasites78
	2.5.5	<i>In vivo</i> excretory secretory antigens
	2.5.6	Concentration of in vivo excretory secretory antigens
	2.5.7	Control for <i>in vivo</i> antigen79
2.6	Preparat	ion of <i>in vitro</i> ESA80
	2.6.1	Preparation of reagents for cell culture
		2.6.1.1 Luria-Bertani broth (LB broth)
		2.6.1.2 Roswell Park Memorial Institute medium (RPMI)80
		2.6.1.3 Dulbecco's Modified Eagle's Medium (DMEM)80
		2.6.1.4 Phosphate-buffered saline stock solution
		2.6.1.5 PBS – working solution
		2.6.1.6 Heat-inactivated fetal bovine serum (FBS)

		2.6.1.7 Types of media
		2.6.1.8 Protease inhibitor working solution
	2.6.2	African green monkey kidney cells (VERO) cell line
	2.6.3	Culture procedure and conditions
	2.6.4	Thawing of VERO cells from frozen storage
	2.6.5	Sub-culturing of VERO cells
	2.6.6	Enumeration of the total number of VERO cells for use in the preparation of T. <i>gondii</i> ESA
	2.6.7	Calculating the percentage of viable cells
	2.6.8	Freezing down of VERO cells85
	2.6.9	Enumeration the total number of <i>in vitro</i> parasites85
	2.6.10	Optimization of of host cell confluence and tachyzoite seed number87
	2.6.11	Statistical analysis for <i>T. gondii</i> cell culture88
	2.6.12	Collection of supernatant containing <i>in vitro</i> ESA88
	2.6.13	Concentration of <i>in vitro</i> excretory secretory antigens
	2.6.14	Control for in vitro antigen91
2.7	Determin	nation of protein concentration91
	2.7.1	Preparation of reagents for determination of protein concentration91
		2.7.1.1 Bovine Serum Albumin stock solution (BSA)91
		2.7.1.2 Standard BSA solution92
		2.7.1.3 Protein assay working solution
	2.7.2	Method for determination of protein concentration92
2.8	Identific	ation of <i>T. gondii</i> ESA by one-dimension gel electrophoresis assay94
	2.8.1	Preparation of reagents for SDS-PAGE94
		2.8.1.1 Resolving gel buffer, pH 9.394
		2.8.1.2 Stacking gel buffer, pH 6.894

	2.8.1.3 Ammonium persulfate (AP), 20% (w/v)95
	2.8.1.4 Preparation of solutions for resolving and stacking gel95
	2.8.1.5 Sample buffer (5X)95
	2.8.1.6 Running buffer
	2.8.1.7 Coomassie Blue Stain96
	2.8.1.8 Coomassie Destaining Solution
2.8.2	Casting SDS-PAGE Gel96
2.8.3	Running SDS-PAGE98
	ation of antigenic epitopes of <i>T. gondii</i> ESA n samples by Western Blotting98
2.9.1	Preparation of reagents for immunodetection
	2.9.1.1 Transfer buffer
	2.9.1.2 Washing Buffer (stock solution)
	2.9.1.3 Working washing solution
	2.9.1.4 Blocking Solution / Diluent
	2.9.1.5 Primary antibodies
	2.9.1.6 Secondary antibody-enzyme conjugated
	2.9.1.7 SuperSignal® West Pico chemiluminescent substrate100
	2.9.1.8 Kodak RPX-Omat developer and replenisher100
	2.9.1.9 Kodak RPX-Omat LO fixer & replenisher100
2.9.2	Methods for determination of antigenicity of ESA100
	2.9.2.1 Protein transfer onto nitrocellulose membrane100
	2.9.2.2 Immunoprobing using primary and secondary antibodies103
	2.9.2.3 Visualisation using chemiluminescence substrate105

2.10 Protein molecular weight marker105			
2.10.1	The Precision plus Protein <sup>™</sup> standards, kaleidoscope marker105		
2.10.2	Precision Plu	s Protein <sup>™</sup> standards, unstained marker106	
		s parameters to identify antigenic SA106	
2.11.1	Concentration	n of <i>T. gondii</i> ESA for SDS-PAGE107	
2.11.2	Dilution of p	rimary antibodies (serum dilution)107	
2.11.3	Dilution of se	econdary antibodies (conjugate dilution)107	
2.12 Glycopr	otein evaluation	n107	
2.12.1	Preparation o	f reagents for glycoprotein staining108	
2.12.2	Glycoprotein	staining method109	
2.13 Rheumatoid factors absorbent (Rf-Absorbent)110			
2.13.1	Preparation o	f serum samples with Rf-absorbent110	
2.14 Background remover			
2.15 Two-Dir	mensional elect	rophoresis111	
2.15.1	Preparation o	f reagents for protein fractionation114	
	2.15.1.1	Stock solutions	
	2.15.1.2	Protein IPG strips rehydration solution114	
	2.15.1.3	OFFGEL protein sample116	
2.15.2	Methods for j	protein fractionation116	
	2.15.2.1	First dimension of OFFGEL fractionation116	
	2.15.2.2	Second dimension of OFFGEL fractionation118	
2.16 Visualization of OFFGEL results			
2.16.1	General silve	r staining119	
	2.16.1.1	Preparation of reagents for general silver staining119	
	2.16.1.2	General silver staining method120	

2.17 Protein i	dentification using OFFGEL fractions122
2.17.1	Preparation of reagents for protein identification122
2.17.2	Methods for protein identification122
2.18 Silver sta	aining methods for mass spectrometry (MS)122
	2.18.1 Preparation of reagents for MS compatible silver staining122
	2.18.2 MS compatible silver staining procedure
2.19 Protein e	extraction and digestion from the silver stained gel125
2.19.1	Preparation of reagents for destaining of silver stained gel for in-gel digestion
2.19.2	Destaining method of silver stained gel for in-gel digestion125
2.19.3	In-gel digestion of silver stained gel126
2.19.4	Extraction of peptide126
2.20 Protein e	extraction and digestion from the Coomassie Blue stained gel127
2.20.1	Preparation of reagents for protein extraction from Coomassie Blue stained gel
2.20.2	Band preparation and de-staining129
2.20.3	Reduction and Alkylation129
2.20.4	Digestion130
	clean up using Zip Tips in preparation for MALDI-TOF TOF lysis
2.21.1	Preparation of reagents for sample clean up130
2.21.2	Zip Tips sample clean up procedure131
2.22 Mass spe	ectrometry analysis and bioinformatics

### CHAPTER THREE: RESULTS

3.1	Classification of <i>Toxoplasma</i> Serum samples134		
3.2	Determir	nation of positive cut-off optical density value (COV)	136
3.3	Optimiza	tion of various parameters in Toxo-IgG ELISA	136
3.4	Optimiza	tion of the number of tachyzoite for animal infection	138
3.5	Optimiza	tion of <i>Toxoplasma gondii</i> cultivation in VERO cell line	138
	3.5.1	RPMI-1640	138
	3.5.2	DMEM	141
	3.5.3	Statistical analysis for cell culture	143
3.6	In vivo ex	ccretory secretory antigens	146
3.7	-	tion of various parameters to identify antigenic epitopes of ESA	146
	3.7.1	Concentration of <i>T. gondii</i> ESA for SDS-PAGE	146
	3.7.2	Determination of ESA profile by SDS-PAGE	146
	3.7.3	Optimization of primary and secondary antibodies	149
	3.7.4	Rheumatoid factors	149
3.8	Determir	nation of protein concentration	154
3.9	Assessm	ent of presence of glycoprotein	154
3.1(		ation of antigenic epitopes of <i>T. gondii</i> ESA in serum samples by Blotting	156
	3.10.1	Identification of antigenic bands of <i>in vivo</i> ESA	156
	3.10.2	In vivo control antigen	161
	3.10.3	Identification of antigenic bands of <i>in vitro</i> ESA	166
	3.10.4	In vitro control antigens	170
3.11	l Two-Din	nensional electrophoresis	174

CHAPTER FOUR: DISCUSSION	
CHAPTER FIVE: SUMMARY AND CONCLUSIONS	218
REFERENCES	
APPENDIXES	
PUBLICATIONS	
PATENT FILED	
PAPER PRESENTATIONS	
MANUSCRIPTS IN PREPARATION	

## LIST OF TABLES

Table 2.1	Contents of Platelia <sup>®</sup> Toxo IgM TMB kit	73
Table 2.2	Interpretation of results by the Platelia <sup>®</sup> Toxo IgM TMB kit	
Table 2.3	Serial dilution for determination of standard protein concentration	
Table 2.4	Preparation of solutions for resolving and stacking gels	
Table 2.5	List of serum samples tested with in vivo ESA	
Table 2.6	List of serum samples tested with in vitro ESA	
Table 2.7	Preparation of rehydration solution, volume per IPG strip	
Table 2.8	OFFGEL protein sample preparation	
Table 2.9	Silver staining chemical components and incubation time	
Table 2.10	Silver staining methods for MS	
Table 3.1	Classification of the serum samples	135
Table 3.2	Optimization of the concentration of <i>T. gondii</i> soluble antigen and serum dilution for Toxo-IgG ELISA	137
Table 3.3	Optimization of secondary antibody dilution for Toxo-IgG ELISA	137
Table 3.4	Viability, yield and degree of host cell contamination of in <i>vitro</i> grown <i>T. gondii</i> using flasks with various confluences of VERO cells in RPMI-1640 medium	140
Table 3.5	Viability, yield and degree of host cell contamination of <i>in vitro</i> grown <i>T. gondii</i> with flasks with various confluences of VERO cells in DMEM medium	142
Table 3.6	Summary of the reactivities of the three antigenic bands (12, 20 and 30 kDa) from <i>in vivo</i> ESA from blots probed with anti-human IgM HRP and IgA-HRP	163
Table 3.7	Summary of the reactivities of various antigenic band combinations (12 and/or 20, 12 and/or 30 and 20 and/or 30 kDa) from <i>in vivo</i> ES from blots probed with anti-human IgM-HRP and IgA-HRP	

Table 3.8	Summary of the reactivities of the combinations of all 3 antigenic bands (12, 20 and 30 kDa) bands from <i>in vivo</i> ESA from blots probed with anti-human IgM-HRP and IgA- HRP	165
Table 3.9	Summary of the results of the <i>in vivo</i> ESA bands when incubated with serum samples from a subgroup of Group I which were positiv for circulating antigen, and probed with anti-human IgM & IgA	e 165
Table 3.10	Summary of the reactivity of the 10 kDa antigenic band from <i>in vitro</i> ESA when probed with anti-human IgM- HRP	172
Table 3.11	Summary of the reactivity of the 10 kDa antigenic band from <i>in vitro</i> ESA when probed with anti-human IgA- HRP	172
Table 3.12	Summary of the reactivities of the 10 kDa antigenic band from <i>in vitro</i> ESA from blots probed with anti-human IgM-HRP and/or IgA- HRP	173
Table 3.13	Summary of the reactivities of combination 10 kDa (in-vitro ESA) and 12kDa (in-vivo ESA) from blots probed with anti-human IgM-HRP, IgA-HRP and IgM-HRP and/or IgA-HRP	173
Table 3.14	The pI ranges of the antigenic bands from OFFGEL factions	181
Table 3.15	Summary of mass spectrometric results of the ESA antigenic proteins	194

### LIST OF FIGURES

Figure 1.1	Distribution of human toxoplasmosis	4
Figure 1.2	Pathways for Toxoplasma gondii infection	
Figure1.3.a	T. gondii oocysts in a fecal floatation	
Figure 1.3.b	T. gondii cyst in brain tissue stained with hematoxylin and eosin	
Figure 1.3.c	T. gondii tachyzoites, stained with Giemsa, smear of peritoneal	
Figure 1.4	Schematic representation of T. gondii tachyzoite	
Figure 1.5	Summary of overall methodology in this study	
Figure 2.1.a	T. gondii tachyzoites arranged in rosette within the VERO cell	86
Figure 2.1.b	Schematic representation of eight parasites	86
Figure 2.2	T. gondii tachyzoite after the VERO cell ruptured	
Figure 2.3	Stirred ultrafiltration cell	90
Figure 2.4	An example of a graph of standard BSA concentrations	93
Figure 2.5	Trans-Blot® SD Semi-Dry electrophoretic transfer cell	102
Figure 2.6	The arrangement of filter papers, gel and nitrocellulose membrane for Western Blot	102
Figure 2.7	Fractionation principle of the Agilent 3100 OFFGEL Fractionator	112
Figure2.8	Agilent, OFFGEL fractionator, 3100	113
Figure 2.9	OFFGEL Electrodes, trays and frames	113
Figure 3.1	Multivariate analysis of <i>T. gondii</i> tachyzoite yields at different cell confluence and tachyzoite inoculum in RPMI-1640	144
Figure 3.2	Multivariate analysis of <i>T. gondii</i> tachyzoite yields at different cell confluence and tachyzoite inoculum in DMEM	145
Figure 3.3	Gel profile of different protein amounts of ESA antigens in 12% SDS-PAGE, Coomassie blue staining	147

Figure 3.4	Gel profile of ESA antigens in different percentage of SDS-PAGE, Coomassie blue staining	148
Figure 3.5	Western blot pattern using different dilutions of the serum sample from a probable acute patient when probed with peroxidase conjugate anti- human IgM antibody	150
Figure 3.6	Western blot profile of serum sample from a probable acute patient when probed with different dilutions of peroxidase conjugate anti- human IgM antibody	151
Figure 3.7	Western blot profile of serum sample from a probable acute patient when probed with different dilutions of peroxidase conjugate anti- human IgA antibody	152
Figure 3.8	Western blot profiles of serum samples with or without RF absorbent when probed with peroxidase conjugate anti- human IgM and IgA antibodies	153
Figure 3.9	Glycoprotein staining on SDS-PAGE of in vivo ESA	155
Figure 3.10	Representative Western blot pattern of <i>in vivo</i> grown <i>T. gondii</i> ESA recognized by different groups of serum samples when probed with peroxidase conjugate anti- human IgM antibody	159
Figure 3.11	Representative Western blot pattern of <i>in vivo</i> grown <i>T. gondii</i> ESA recognized by different groups of serum samples when probed with peroxidase conjugate anti- human IgA antibody	160
Figure 3.12	Western blot pattern of in <i>vivo</i> control antigen incubated with probable acute serum sample and probed with anti- human IgM-HRP and IgA- HRP	162
Figure 3.13	Representative Western blot pattern of <i>in vitro</i> grown <i>T. gondii</i> ESA recognized by different groups of serum samples when probed with peroxidase conjugate anti- human IgM antibody	168
Figure 3.14	Representative Western blot pattern of <i>in vitro</i> grown <i>T. gondii</i> ESA recognized by different groups of serum samples when probed with peroxidase conjugate anti- human IgA antibody	169
Figure 3.15	Western blot pattern of in <i>vitro</i> control antigen when it was incubated with probable acute serum sample and probed with anti- human IgM and IgA- HRP	171
Figure 3.16	Plotted graph of run parameters during ESA fractionation, using the 3100 OFFGEL High Res Kit, pH 3-10	175

Figure 3.17	Gel profile (12% SDS-PAGE) of OFFGEL fractions of <i>in vivo</i> grown <i>T. gondii</i> ESA using the 3100 OFFGEL High Res Kit, pH 3-10, (Silver staining)	176
Figure 3.18	Gel profile (12% SDS-PAGE) of OFFGEL fractions of <i>in vitro</i> grown <i>T. gondii</i> ESA using the 3100 OFFGEL High Res Kit, pH 3-10, (Silver staining)	177
Figure 3.19	Western blot results on OFFGEL fractions (in vivo ESA)	178
Figure 3.20	Western blot results on OFF GEL fractions (in vitro ESA)	
Figure 3.21	Mascot search results for 10 kDa antigenic protein	183
Figure 3.22	Amino acid and DNA sequences of the best two candidates protein identifications for 10 kDa	184
Figure 3.23	Mascot search results for 20 kDa antigenic protein	186
Figure 3.24	Amino acid and DNA sequences of the best two candidates protein identifications for 20 kDa	187
Figure 3.25	Mascot search results for 30 kDa antigenic protein	189
Figure 3.26	Amino acid and DNA sequences of the best two candidates protein identifications for 30 kDa	190
Figure 3.27	Identification of amino acid and DNA sequences of 12 kDa	192

### ABBREVATIONS

2-DE	Two-Dimensional Electrophoresis
CA	Circulating Antigen
COV	Cut Of Value
DMEM	Dulbecco's Modified Eagle's Medium
ELISA	Enzyme-Linked Immunosorbent Assay
ESA	Excretory Secretory Antigen
ESI	Electro-Spray Ionization
EST	Expressed Sequence Tag
FBS	Fetal Bovine Serum
FGE-OM	Functional Genomics Experiment Object Model
Ig	Immunoglobulin
LC	Liquid Chromatography
MALDI	Matrix-Assisted Laser Desorption/Ionization
MS	Mass Spectrometric
PBS	Phosphate Buffered Saline
p <i>I</i>	Isoelectric Point
PMF	Peptide Mass Fingerprinting
PTM	Post Translational Modification
RPMI	Roswell Park Memorial Institute medium
RPMI-PS	RPMI-1640 containing penicillin/ streptomycin
SDS	Sodium Dodecyl Sulphate
PAGE	Polyacrylamide Gel Electrophoresis
T. gondii	Toxoplasma gondii

## ANALISIS ANTIGEN REMBESAN PERKUMUHAN Toxoplasma gondii DAN MENGENALPASTI PENANDA POTENSI INFEKSI AKUT

#### ABSTRAK

Toxoplasma gondii merupakan parasit protozoa obligat intrasellular. Infeksi oleh organisma ini tersebar luas dan penting kepada manusia, khususnya wanita hamil dan pesakit yang sistem imunnya terkompromi. Melainkan jika dirawat, toksoplasmosis boleh menyebabkan morbiditi yang teruk bahkan kematian. Dengan itu ujian makmal yang sesuai adalah sangat penting untuk mengenalpasti kes toksoplasmosis akut atau teraktif semula. Diagnosis toksoplasmosis akut masih mempunyai banyak cabaran, di mana satu panel ujian diperlukan untuk pengesahan penyakit ini. Antigen rembesan perkumuhan (ESA) toksoplasma sangat imunogenik dalam infeksi, baik pada manusia mahu pun infeksi eksperimental. Sehubungan dengan itu, ESA merupakan antigen yang sesuai untuk penyelidikan penanda infeksi baru, terutama untuk mengesan kes toksoplasmosis akut. Dalam kajian ini, ESA dihasilkan melalui dua pendekatan iaitu: 1) daripada takizoit T.gondii strain RH melalui infeksi intraperitoneum pada mencit Swiss albino, 2) daripada kultur in vitro T.gondii. Dalam penghasilan ESA *in vitro*, optimisasi kaedah kultur sel untuk propagasi T. *gondii* dilakukan untuk mendapatkan sumber parasit yang konsisten dengan hasil dan viabiliti yang maksimum, disamping pencemaran sel perumah yang minimum. Sampel serum diperolehi daripada pelbagai kategori individu : pesakit yang disyaki terjangkit dan positif untuk antibodi anti- *Toxoplasma* IgM dan negatif / positif untuk anti- *Toxoplasma* IgG; individu yang mengidap jangkitan kronik dan positif untuk anti- Toxoplasma IgG tetapi negatif untuk anti- Toxoplasma IgM, dan daripada

pesakit yang mengidap jangkitan lain, serta daripada individu sihat sebagai kumpulan kawalan. Analisis SDS-PAGE dijalankan ke atas ESA, diikuti dengan analisis Western blot menggunakan serum di atas dan di prob dengan antibodi anti-manusia IgM dan IgA yang terkonjugat dengan enzim peroxidase (HRP). Substrat kemiluminesen digunakan untuk mengesan reaktivi Western blot. Jalur antigenik dengan anggaran berat molekul 30, 20, dan 12 kDa daripada ESA in vivo dan 10 kDa daripada ESA in vitro menunjukkan potensi yang baik sebagai penanda infeksi akut. Sensitiviti maksimum bagi jalur daripada ESA in vivo adalah 98.7% dengan kombinasi blot IgM dan IgA, manakala spesifisiti adalah 84% dan 70% apabila di uji dengan serum daripada jangkitan lain dan individu sihat, masing-masing untuk blot IgM dan IgA. Manakala untuk ESA in vitro, sensitiviti adalah 80% dan spesifisitinya adalah 96.7% dengan kombinasi blot IgM dan IgA. Elektroforesis gel dua dimensi dilakukan melalui kaedah fraksinasi OFF GEL untuk menentukan titik isoelektrik protein, diikuti dengan analisis SDS-PAGE. Hal ini membolehkan jalur protein dipisahkan dengan baik untuk dipotong dan dikenal pasti dengan mass spektrometri (analisis MALDI TOF /TOF). Dua daripada tiga protein yang telah dikenal pasti pada setiap penanda infeksi tersebut adalah seperti berikut; ubiquitin (protein ribosomal CEP52 protein cantuman) dan polyubiquitin untuk protein bersaiz 10 kDa; protein microneme 10 dan protein dense granul 7 untuk protein bersaiz 20 kDa; dan phosphoglycerate mutase 1 dan phosphoglycerate mutase untuk protein bersaiz 30 kDa. Bagi protein 12 kDa, ia dikenalpasti sebagai thioredoxin. Kajian ini telah beraya mengenalpasti komponen ESA dengan nilai diagnostik yang berpotensi untuk mengesan antibodi anti-Toxoplasma IgA dan IgM dalam infeksi toksoplasmosis akut.

## ANALYSIS OF Toxoplasma gondii EXCRETORY SECRETORY ANTIGENS (ESA) AND IDENTIFICATION OF POTENTIAL MARKERS OF ACUTE INFECTION

#### ABSTRACT

Toxoplasma gondii is an obligate intracellular protozoan parasite. Infection with this organism is widespread and important in humans, especially pregnant women and immunosuppressed patients. Unless treated, it can result in severe morbidity and even death. These severe sequelae thus emphasize the importance of appropriate laboratory investigations in potential cases of acute/reactivated Toxoplasma infection. Diagnosis of acute toxoplasmosis still poses a lot of challenges, and a panel of tests is required for confirmation of toxoplasmosis. Excretory secretory antigens (ESA) are highly immunogenic during both human and experimental infections. Thus they are good candidates for investigation into new infection markers, especially for detection of probable cases of acute toxoplasmosis. In this study, ESA was prepared using two approaches namely: 1) from tachyzoites of RH strain of T. gondii by intraperitoneal infection of Swiss albino mice, 2) from in vitro culture of *T. gondii*. For use in production of the *in vitro* ESA, optimizations of cell culture method for T. gondii propagation was performed to obtain a consistent source of parasites with maximum yield and viability, but minimum host cell contamination. Serum samples were obtained from various categories of individuals: probable cases of acute toxoplasmosis from patients suspected of toxoplasmosis who were positive for anti-Toxoplasma IgM and negative/positive for IgG; chronically infected patients who were positive for anti-Toxoplasma IgG but negative for IgM; and from patients with other infections, as well as from healthy controls. The ESA was subjected to SDS-PAGE, followed by Western blot analysis using the above sera and probed with peroxidase conjugated anti-human IgM and IgA antibodies. The blots were then developed using chemiluminescence substrate. Antigenic bands of approximate molecular weights of 30, 20, and 12 kDa from in vivo ESA and 10 kDa from in vitro ESA when probed with anti-human IgM-HRP and IgA-HRP showed good potential as acute infection markers. The maximum sensitivity of bands from in vivo ESA was 98.7% with combination of IgM and IgA blots; while the specificities were 84% and 70% when probed with serum from other infections and healthy controls in IgM blots and IgA blots respectively. Meanwhile for in vitro ESA, 80% sensitivity and 96.7% specificity were obtained with combination of IgM and IgA blots. Two-dimensional gel electrophoresis was performed by OFF GEL fractionation to determine the isoelectric points of the proteins, followed by SDS-PAGE analysis. This enabled the well-separated bands to be excised for identification by mass spectrometry (MALDI TOF/TOF analysis). The two top identifications for three of the proteins were as follows; ubiquitin (ribosomal protein CEP52 fusion protein) and polyubiquitin for 10 kDa; microneme protein 10 and dense granule protein 7 for 20 kDa; and phosphoglycerate mutase 1 and phosphoglycerate mutase for 30 kDa protein. The protein indentified for 12 kDa was thioredoxin. In this study ESA components with potential diagnostic value for detection of anti- Toxoplasma IgA and IgM during the acute toxoplasmosis were successfully identified.

#### CHAPTER ONE

#### **INTRODUCTION**

#### 1.1 Overview of toxoplasmosis

Toxoplasmosis, caused by intracellular protozoan parasite, *Toxoplasma gondii*, is widely spread throughout the world (Jackson & Hutchison, 1989). In recent years, the significance of congenital toxoplasmosis has been increasingly recognized (Bhopale, 2003a) and it is documented that over half a billion of the world's human population has serum antibodies to *T. gondii* (Dubey, 1997). The incidence of toxoplasmosis, especially toxoplasmic encephalitis, has risen dramatically within the increasing population of patients with AIDS (Luft & Remington, 1992), whereas patients with a variety of neoplastic diseases as well as patients receiving immunosuppressive therapy are at risk of reactivation of *T. gondii* infection (Israelski & Remington, 1993).

The term 'toxoplasmosis' describes the clinical or pathological disease caused by *Toxoplasma gondii* i.e. symptomatic course of infection. *T. gondii* infection is reserved to describe the asymptomatic primary infection or persistence of the parasite in tissues (chronic or latent infection) observed in the majority of infected immunocompetent individuals (Remington *et al.*, 2001). In clinical aspects, toxoplasmosis can be divided into five categories, namely those (1) acquired by immunocompetent patients, (2) acquired during pregnancy, (3) acquired congenitally, (4) acquired by or reactivated in immunodeficient patients, and (5) ocular infections. In any of the above categories, clinical presentations are not

specific and a wide differential diagnosis must be considered since methods of diagnosis and their interpretations may vary for each clinical category (Montoya, 2002). In view of the above facts, it emphasizes the importance of understanding the overall aspects of toxoplasmosis and *T. gondii* infection for a better prevention, diagnosis and treatment of the disease.

#### 1.2 Historical perspective of *Toxoplasma gondii*

T. gondii protozoan parasite was first discovered in 1908 by Nicolle and Manceaux (1908 cited in Louis & Kami, 2007) and named a year later based on its morphology. The name Toxoplasma was derived from Greek word "toxo," meaning arc or bow, which resembled the curved shape of the trophozoites and "plasma," referring to life (Nicolle & Manceaux, 1909 cited in Louis & Kami, 2007). Its medical importance remained unknown until 1939 when T. gondii was identified in tissues of a congenitally infected infant (Wolf et al., 1939). Veterinary importance became known when epidemic toxoplasmosis abortions were recognized in sheep (Hartley & Marshall, 1957). The discovery of a T. gondii specific antibody test, the Sabin Feldman dye test, in 1948 led to the recognition that T. gondii is a common parasite of warm-blooded hosts with a worldwide distribution (Sabin & Feldman, 1948). Frenkel et al. discovered T. gondii life cycle in 1970 when they found that felids are its definitive host and an environmentally resistant stage (oocyst) is excreted in feces of infected cats (Frenkel et al., 1970). The discovery of its widespread infection in certain marine wildlife (sea otters) indicates contamination of our seas with T. gondii oocysts washed from land (Cole et al., 2000). Since there is still no vaccine to prevent toxoplasmosis in humans, hygiene remains the best preventive measure (Dubey, 2008).

#### 1.3 Epidemiology of *Toxoplasma* infection

Toxoplasmosis is a zoonosis distributed worldwide. In humans, the prevalence of the infection rises with age, and does not vary greatly between sexes. *Toxoplasma* infection shows different incidence in different climate, with lower incidence in cold regions, hot and arid areas, or at high elevations (Montoya & Liesenfeld, 2004). In general, there are considerable geographic differences in toxoplasmosis prevalence rates (Figure 1.1). For instance 10% in Palo Alto, California; 15% in Boston, Massachusetts; 30% in Birmingham, Alabama; 36% in Strasbourg, France; 81% in the Central African Republic (Remington *et al.*, 2001). The difference of the incidence of toxoplasmosis infection with the population group within the same environment may be explained by differences in exposure to sources of the infection.

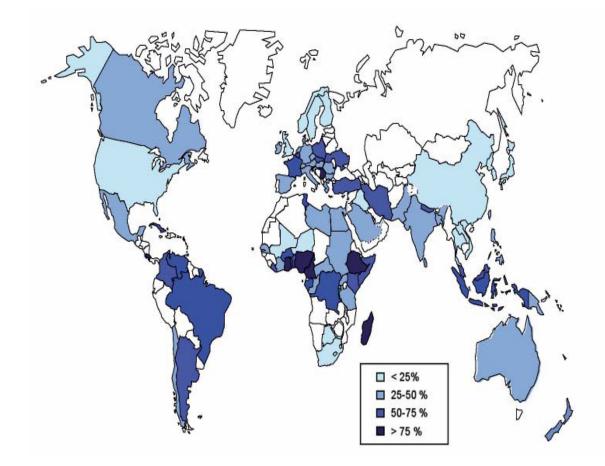


Figure 1.1 Distribution of human toxoplasmosis (Tenter et al., 2000)

In a National Health and Examination Survey in the USA from 1999- 2000, 4234 sera samples were collected from persons aged 12 to 49. Based on this survey 84.2% of women of childbearing age in the United States were found to be seronegative, and thereby are at risk of acquiring *T. gondii* infection during gestation. *T. gondii* antibody prevalence was higher among non-Hispanic black persons than among non-Hispanic white persons, and increases with age (Jones, *et al.*, 2003). Lopez *et al.*, in the year 2000 studied the rate of congenital toxoplasmosis in the United States, and estimated that between 1- 10 per 10,000 live births and 400 to 4000 infants would be born each year with congenital toxoplasmosis.

There are three factors that directly correlate with the incidence of congenital toxoplasmosis in newborns, namely (1) the incidence of primary infection among women during pregnancy; (2) the gestational age at which a pregnant woman acquires the infection; and (3) the public health programs instituted for prevention, detection, and treatment of the infection during pregnancy. Even though in some countries the screening for *Toxoplasma* infection is compulsory during pregnancy such as Austria and France, in many countries routine serological screening is still not performed (Montoya & Rosso, 2005).

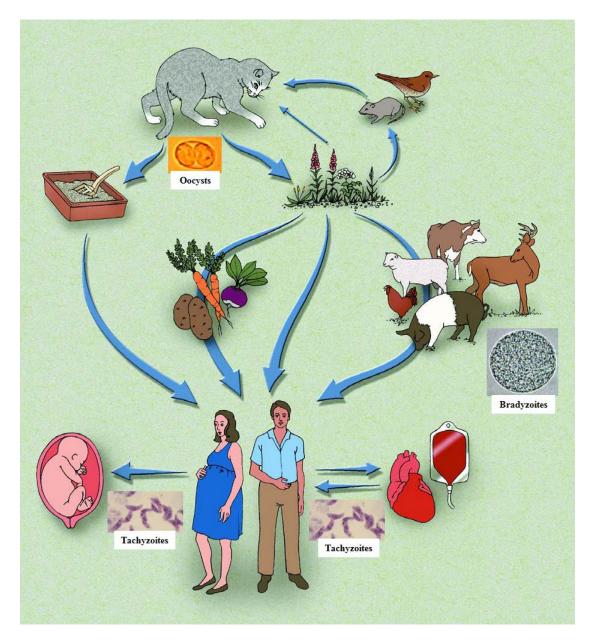
*T. gondii* seroprevalence in women at child-bearing age was reported by Tenter *et al.*, (2000), nation-wide data collection. The rates of positive seroprevalence were 58% in Central European countries, 51–72% in several Latin-American countries, and 54–77% in West African countries. Low seroprevalence, 4–39%, was reported in

southwest Asia, China and Korea as well as in cold climate areas such as Scandinavian countries (11–28%) (Tenter *et al.*, 2000).

In Malaysia the seroprevalence of toxoplasmosis have been reported in several Nissapatorn and Khairul, (2004) reported that the seroprevalence of studies. toxoplasmosis among 505 HIV/AIDS patients who were admitted at the Hospital Kuala Lumpur during January 2001- December 2002 was 226 (44.8 %). This seroprevalence was much higher than in other similar studies, e.g. 15-37% in France (Leport & Remington, 1992), 21% in Malaysia (Nissapatorn et al., 2002), 22.4% in Thailand (Nissapatorn et al., 2001) and 10-40% in USA (Luft & Remington, 1988). The majority of patients were male (75.7%), Chinese (53%), married (51.3%), unemployed (51.3%) and heterosexual who engaged commercial sex workers (59.3%) who are at risk of HIV infection (Nissapatorn et al., 2004). The seroprevalence of toxoplasmosis in Malaysian pregnant women was found to be 49% (Azmi et al., 2003). In one study, congenital toxoplasmosis (IgM positive), was found only in 12 (0.35%) of 3420 infants whom were clinically suspected of acquiring congenital infection (Osman et al., 1992). It was also reported that there was a significant difference (p<0.05) in *Toxoplasma* seroprevalence rates among races; the highest rate was in the Malays (55.7%), followed by the Indian (55.3%) and the Chinese (19.4%) (Azmi et al., 2003).

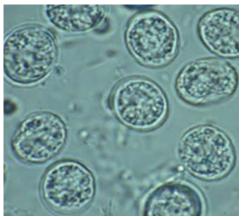
#### 1.4 *Toxoplasma gondii* and its life cycle

The life cycle of *T. gondii* is illustrated in Figure1.2. *T. gondii* is an obligate intracellular protozoan that exists in nature in three forms namely oocyst, tissue cyst and tachyzoites. The oocyst (Figure 1.3.a) releases sporozoites and can spread in the environment and contaminate water, soil, fruits, vegetables and herbivores following consumption of infected plant material (Coutinho *et al.*, 1982). Oocytes have been found to be very stable, particularly in warm and humid environments, and resistant to many disinfecting agents (Dumetre & Darde, 2003), but survive poorly in arid, cold climates (Jones *et al.*, 2001). The tissue cyst (Figure 1.3.b) harbours bradyzoites, is the encysted stage which contains relatively slowly multiplying organisms (brady=slow, zoite=organism). Bradyzoites are also called cystozoites. Tissue cysts persist in the body as long as the host lives (Frenkel, 1973a). Tachyzoites (tachy=fast, Figure 1.3.c) has also been called a trophozoite, the proliferative form, the feeding form, and the endozoite. It divides into two by a specialized process called endodyogeny (Goldman *et al.*, 1958).



#### Figure 1.2 Pathways for Toxoplasma gondii infection

The feline intestinal tract is the only source for the production of *T. gondii* oocysts. Transmission to humans usually occurs through the ingestion of oocysts from contaminated sources (e.g., soil, cat litter, garden vegetables, and water) or the ingestion of tissue cysts in undercooked meat from infected animals. Although fetal infection most often occurs after acute *T. gondii* infection in a pregnant woman, it also can occur after the reactivation of latent infection in an immunocompromised pregnant woman (Jones, *et al.*, 2003).



**Figure1.3.a.** *T. gondii* oocysts in a fecal floatation www.dpd.cdc.gov/.../body\_Toxoplasmosis\_il6.htm

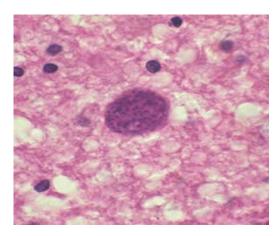
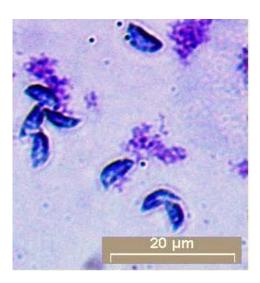


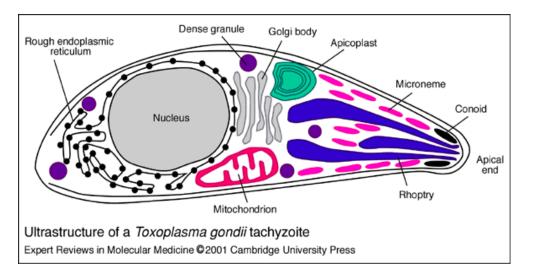
Figure 1.3.b. *T. gondii* cyst in brain tissue stained with hematoxylin and eosin www.dpd.cdc.gov/DPDx/HTML/.../body\_Toxoplasmosis\_mic1.htm



**Figure 1.3.c.** *T. gondii* tachyzoites, stained with Giemsa, from a smear of peritoneal fluid obtained from a laboratory-inoculated mouse (author)

Tachyzoite is crescent or oval-shaped (approximately 2 by 7  $\mu$ m) (Figure 1.4), comprised of a unique cytoskeleton (subpellicular microtubules, conoid), endosymbiontic derived organelles (mitochondrion, apicoplast) and secretory organelles (rhoptries, micronemes, dense granules) other than the eukaryotic universal organelles. The conoid is a concave truncated cone consisting of fibers wound into a spiral, like a compressed spring. It is made of tubulin organized in a unique fashion, very different from typical microtubules (Hu *et al.*, 2002). Apicoplast is a typical plastid placed above the Golgi, limited by multiple membranes. In the infectious stage it is relatively uniform in shape, bounded by possibly four membranes, and filled with granular and filamentous content (Kohler, *et al.*, 1997).

There are three dissimilar secretory organelles that are essential for *T. gondii* invasion. First are micronemes which are small and rod-shaped structures, located in the most apical area of the parasite, behind the conoid. Second are the rhoptries, organized as a group of elongated, club-shaped organelles that extend from within the conoid toward the nucleus. The third type are dense granules, with spherical-shaped, found throughout the cell but mostly in the posterior part of the parasite. With the advantages of antibody development to specific proteins, it has been possible to begin to identify proteins specifically located in the different organelles (Louis & Kami, 2007).



**Figure 1.4** Schematic representation of *T. gondii* tachyzoite <u>http://journals.cambridge.org/fulltext\_content/ERM/ERM3\_01/S1462399401002204</u> <u>sup002.htm</u>

Tachyzoites require an intracellular habitat to survive and multiply, they reside and multiply within vacuoles in their host's cells, and can infect most phagocytic and nonphagocytic cell types, including placental cells. The presence of tachyzoites in human fluids or tissues is the hallmark of acute infection or reactivation of a latent infection (Singh, 2003). Ultrastructural studies revealed that tissue cysts develop and remain intracellular and bradyzoites differ from tachyzoites with respect to location of the nucleus that is central in tachyzoites and terminal in bradyzoites; amylopectin granule that is numerous in bradyzoites but absent or few in tachyzoites, and contents of rhoptries which is honeycomb in tachyzoites, but electron dense in older bradyzoites (Wanko *et al.*, 1962; Ferguson & Hutchison, 1987; Dubey *et al.*, 1998).

The life cycle of *T. gondii* consists of sexual and asexual stage that is divided between feline and non-feline infections, respectively (Figure1.2). The sexual stage takes place in the intestine of the definitive host, predominantly domestic cats which belong to the feline family. When bradyzoites or oocytes are ingested by a feline, formation of oocytes proceeds in the epithelium of the small intestine. Several million unsporulated oocytes may be released in the feces of a single cat over a period of 3–18 days, depending on the stage of the parasite ingested (Dubey *et al.*, 1998). In mild environmental conditions, oocytes may sporulate within a 3-week period (Dubey *et al.*, 1970), then infect humans and other intermediate hosts. Thus far the investigation of outbreaks of toxoplasmosis has led to recovery of oocytes from soil (Coutinho *et al.*, 1982) but not from water (Benenson *et al.*, 1982; Bowie *et al.*, 1997; Bahia-Oliveira *et al.*, 2003).

The asexual cycle consists of two distinct stages of growth depending on whether the infection is in the acute or chronic phase. The tachyzoite stage defines the rapidly growing form of the parasite found during the acute phase of toxoplasmosis. Tachyzoites are approximately 5 mm long and 2 mm wide (Smith, 1995). Usually after 64 to 128 parasites have accumulated per cell they undergo replication with a generation time of 6 to 8 hours (in vitro) until they exit the cell to infect neighbouring cells (Radke & White, 1998). In infected animals, tachyzoites differentiate into bradyzoites and form tissue cysts that first appear 7 to 10 days postinfection. These cysts are found predominantly in the central nervous system and muscle tissue, where they may reside as long as the host lives. The chronic stage of the asexual cycle occurs when there is a development of tissue cysts throughout the body. As they pass through the digestive tract, cysts that are ingested through eating infected tissue are ruptured, therefore causing bradyzoite release. These bradyzoites can then infect the epithelium of the intestinal lumen, and finally complete the asexual cycle by differentiating back to the rapidly dividing tachyzoite stage for dissemination throughout the body (Black & Boothroyd, 2000).

Under some conditions within the host, there is apparently a low rate of spontaneous reactivation whereby bradyzoites differentiate back to tachyzoites. In general, the immune response can efficiently prevent the dissemination of these tachyzoites but in immunocompromised hosts, however, such reactivation may be unchecked and/or become more frequent. This leads to the suggestion that the parasites might actively detect a lowered immunity against them (Gross & Pohl, 1996). In either case, the consequence can be a massive and potentially fatal recrudescence (Black *et al.*, 2000)

The transmission and sources of human infection are further elaborated in section 1.9.

#### 1.5 Immunology of *Toxoplasma* infection

T. gondii stimulates the production of IgG, IgM, IgA and IgE antibodies against both membrane and excretory secretory antigens (Schreiber & Feldman, 1980). Additionally IgA antibody may interfere with the initial interaction of the parasite with the host cell at mucus membrane. Human platelets are cytotoxic to tachyzoites in the absence of antibody (Yong et al., 1991). In a normal immune host, both cellular and the humoral immune responses control the infection, and it depends on the strain of T. gondii. Macrophages are not the only effector cells but are closely involved in regulation of cellular immunity through their production of immunological mediators. Tachyzoites stimulate macrophages to produce interleukin (IL-12) (Gazzinelli, et al., 1993; Sher & Reis, 1998). IL-12, in turn activates natural killer (NK) cells and T cells to produce interferon-  $\gamma$  (IFN-  $\gamma$ ). Production of IFN-  $\gamma$  is crucial for resistance to the infection (Gazzinelli et al., 1994; Daubener et al., 1996). The synergistic act of IFN- $\gamma$  and tumour necrosis factor (TNF) results in greatly enhanced production of free radicals and nitric oxide, both of which can mediate killing of tachyzoites by macrophages (Sibley et al., 1991; Langermans et al., 1992; Sher et al., 1993).

CD8+ T cells are considered to be the major effector cells among the T cell population. They are responsible for protection against *T. gondii* with CD4+ T cells playing a synergistic role (Parker *et al.*, 1991; Denkers *et al.*, 1996). T helper (Th-1) CD4+ T cells produce IL-2 (Khan *et al.*, 1994). IL-2 induces lymphokine activated killer cells at either NK cells or T cell phenotype which are cytotoxic for target cells infected with *T. gondii*. T helper cells (Th-2) are responsible for down regulation of protective cell mediated immune response cells by producing IL-4, IL-5 and IL-10 (Mosmann & Moore, 1991). In brain which is the most commonly affected site of latent *Toxoplasma* infection, CD4+ and CD8+ cells were reported to infiltrate into the CNS of mice (Hunter, *et al.*, 1994). T cell encounters parasite antigen that are likely presented by Glial cells in the context of major histocompatibility complex. This may result in production of cytokines such as INF-  $\gamma$  which can activate microglia to inhibit parasite replication and induction of cytotoxic T cells to lyse infected cells (Hunter & Remington, 1994).

#### 1.6 Pathogenesis

Humans are generally infected by *T. gondii* through ingestion of oocysts released in cat feces or by consuming uncooked/ undercooked meat from infected flock animals containing the long lived tissue cysts (Jackson & Hutchison, 1989). The ingestion of oocyst is an important source of infection. After ingestion, the outer walls of cysts or oocysts are ruptured by enzymatic degradation and bradyzoites and sporozoites are released into the intestinal lumen. Subsequently they invade and multiply within their surrounding cells and become tachyzoites inside the cells. Following that, the tachyzoites circulate *via* blood or lymphatic system to most of the organs of human

body (Frenkel, 1971). Thus, the host cells would be infected by the tachyzoites, replicate and invade the adjoining cell. In this phase, the acute features of the infection develop i.e. cell death and focal necrosis surrounded by an acute inflammatory response (Bhopale, 2003b).

*T. gondii* is able to invade any nucleated cell, the preliminary step of the parasite invasion process is recognition and attachment to the target cell. Upon encountering the host cell, the parasite attach to the cell membrane in an appropriate point that is recognized by the apical pole (Kasper & Mineo, 1994). Rhoptries and micronemes are two types of the organelles that appear to be involved at the anterior end of the parasite. ROP1, a rhoptry protein is secreted at the time of invasion and is associated with membrane of parasitophorous vacuoles (Kasper & Mineo, 1994). Two microneme proteins MIC-1 and MIC-2 contain thrombspondin like domains that may function in adhesion following their release on the surface of the parasite (Fourmaux *et al.*, 1996; Wan *et al.*, 1997).

An annular junction is formed between parasite and host cell membrane once the parasite attaches to its target cell, through which the parasite forcibly penetrates the host cell while apparently pulling the cell membrane around itself. Finally, the parasite is enclosed within a parasitophorous vacuole. Extracellular tachyzoites are highly susceptible to oxygen intermediates, changes in pH and osmotic variation and are killed by specific antibody in the presence of complement (Bhopale, 2003a and 2003b). The formation of tissue cysts under certain circumstances is an important

aspect of the pathogenesis of toxoplasmosis. It has been observed that inhibitors of mitochondrial function promote the transformation of tachyzoites to bradyzoites suggesting that the reliance of the parasite on mitochondrial function might be different in tachyzoites and bradyzoites (Bohne *et al.*, 1994; Tomavo & Boothroyd, 1995).

In general, pathogenesis of *T. gondii* infection can be divided into three different phases: first, the acute phase when tachyzoites multiply actively, disseminating through the lymphatic system to the different organs, and undergo intracellular growth causing cell necrosis. During this time parasites can be excreted through different biological fluids (faeces, urine, etc.), but these tachyzoites are very unstable and are easily destroyed (Montoya & Liesenfeld, 2004). Second is sub-acute phase that is characterized by the appearance of IgA antibodies specific for *T. gondii* enteroepithelial stages, which eliminate tachyzoite replication in the intestinal phase but not those localized in the nervous system. Third is the chronic phase that starts with tachyzoites beginning to disappear from visceral tissues and is characterized by persistence of bradyzoites within cysts. This phase is associated with a systemic immune response, which inhibits tachyzoites proliferation in blood and tissues such as liver, spleen, lungs, etc (Montoya & Liesenfeld, 2004).

#### **1.7** Clinical Disease

*Toxoplasma gondii* infection is one of the most common human zoonosis. Toxoplasmosis is a systemic disease, clinically, infection with *T. gondii* can go unnoticed or could cause signs and symptoms depending on the immune status of the patient and the clinical setting e.g. immunocompetent, immunocompromised, congenital toxoplasmosis or ocular disease (Biesiada *et al.*, 2006).

#### **1.7.1** Infection in immunocompetent individuals

Acute acquired infection with *T. gondii* in immunocompetent individuals most often is asymptomatic. Sign and symptom of infection present only in minority of individuals that are acutely infected with *T. gondii* (Remington, 1974). *T. gondii* infection usually causes self limiting and unspecific illness, therefore no treatment is needed. The most common clinical presentation is cervical lymphadenopathy that is present in 5% of clinically significant lymphadenopathy cases (McCabe *et al.*, 1987). Fever, headache, sore throat, cough, myalgia, malaise and night sweats are some of the general symptoms of toxoplasmosis in immunocompetent individuals. Rarely, myocarditis, polymyositis, pneumonitis, hepatitis and encephalitis can arise in healthy individuals (Montoya & Liesenfeld, 2004). Acute *T. gondii* infection in pregnant women is similar to *T. gondii* infection in other immunocompetent individuals. Most commonly the infection manifest as asymptomatic diseases, although cervical lymphadenopathy may occur. Acute infection may be transmitted to the fetus but, since it is asymptomatic, the infection often goes unnoticed (Louis & Kami, 2007).

#### **1.7.2** Infection in immunocompromised patients

*Toxoplasma gondii* is one of the most frequent protozoan causing opportunistic infections in immunocompromised individuals. Host immune function plays an important role in the pathogenicity of toxoplasmosis (Ferreira & Borges, 2002). Patients who are immunocompromised often develop life-threatening toxoplasmosis (Liesenfeld *et al.*, 1999).

Toxoplasmosis occurs as a consequence of reactivation of a latent infection in patients infected with AIDS (Luft *et al.*, 1992). It is believed that in patients with AIDS, the most common non-viral infection of the brain is toxoplasmosis. In HIV-infected patients, ocular toxoplasmosis can occur before the development of AIDS (Cristina & Rubens, 2007). Encephalitis is the clinically important manifestation of toxoplasmosis in AIDS patients and it is one of the most common cause of death among these patients (Dubey, 2004). However the risk of toxoplasmosis has decreased after introduction of primary prophylaxis against *T. gondii* and effective antiretroviral therapy (Jones *et al.*, 1999).

The clinical presentation of toxoplasmosis in immunocompromised patients ranges from asymptomatic reactivation, commonly observed in previously infected organ recipients and demonstrable by increased post-transplant antibody titers, to severe disseminated disease (Ferreira *et al.*, 2002). Toxoplasmosis encephalitis has always been recognized as the most common opportunistic infection of the central nervous system (CNS). Clinical appearance of toxoplasmic encephalitis varies from a subacute gradual process evolving over weeks, to an acute confusional state, with or without focal neurological deficit, evolving over days (Yeo *et al.*, 1983). Clinical manifestations include mental status changes, seizures, focal motor deficits, cranial nerve disturbances, sensory abnormalities, cerebellar signs, movement disorders and neuropsychiatric findings. Meningeal signs are rare. Constitutional symptoms and signs such as fever and malaise can vary. The most typical focal neurological findings are hemiparesis and speech abnormalities (Luft *et al.*, 1993). *Toxoplasma* pneumonia seems to be more frequent in recipients of bone-marrow transplants and

in patients with AIDS (Montoya & Liesenfeld, 2004). Elderly patients who acquire toxoplasmosis are at risk of developing a severe retinochoroiditis, likely secondary to the weakening of cellular immune function that occurs with aging (Lihteh, 2007).

Individuals under immunosuppressive therapy such as patients with malignant diseases or organ transplant recipients, who had been previously infected with *T. gondii*, might show an altered serological profile of this protozoan compatible with reactivation, such as increased IgG antibody titers or, less frequently, increased titers of acute phase antibodies, i.e. IgM, 4 to 13 weeks after the beginning of immunosuppression, and the presence or absence of clinical manifestations, a fact not observed in patients with AIDS. Alternatively, seronegative patients receiving organs from seropositive donors may show seroconversion 4 to 6 weeks after transplantation, in general accompanied by disseminated infection whose clinical manifestation usually happen at the same time with the occurrence of antibodies, although late manifestations, (about 10 months) after immunosuppressive therapy, have been reported (Luft *et al.*, 1983).