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شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



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Ain Shams University



Faculty of Science

Molecular Studies of *Toxoplasma gondii* from Ruminants in Northern-West Egypt

**A thesis submitted for the award of PhD. degree
In Zoology (Parasitology)**

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قالوا

لسبب انك لا تعلم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

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*With gratitude to
My husband Nour Fleslam Hammad and my
children
(Nada, Ahmed, Mohammed & Fares)*

*I dedicate this work to my sisters and the soul of my
mother and my father*



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Abstract

Toxoplasma gondii is a coccidian parasite known for its heavy toll on people and livestock. It can cause abortion and a variety of congenital diseases. The current study aimed to examine some seroprevalence and molecular attributes of *T. gondii* obtained from ruminants in the North-West of Egypt. Specimens were random selected from five different locations in Alexandria and Matrouh governorates. A total of 483 blood samples, collected from 96 mixed flocks, were screened for anti-*T. gondii* IgG antibodies using enzymelinked immunosorbent assay (ELISA). The seropositive results were then confirmed using polymerase chain reaction (PCR) primers for the B1 and P30 genes. Specific PCR products were selected for sequencing and alignment against the GenBank, where phylogeny has been examined using the maximum likelihood, neighbor-joining, and maximum parsimony in MEGA6. ELISA confirmed the presence of *T. gondii* in 188 of the investigated samples (38.92%), indicating a higher prevalence in camels (64.51%) and sheep (43.75%) as compared to goats (27.93 %) and cattle (13.46%). PCR confirmed the presence of *T. gondii* specific sequences in 159 seropositive specimens, with homology between 98.3 and 100%. Four strains/clones were generated and recorded in GenBank database under accession numbers of OL960022 *T._gondii_GM1* from Alexandria, and *T._gondii_GM2* (OL960023), OL960024 *T._gondii_SM*, and *T._gondii_CM* (OL960025) from Matrouh. The genetic distances between the investigated variants ranged from 0.1 to 0.9, and 7 single nucleotide polymorphisms (SNPs), were identified in the examined *T. gondii* specimens. The camel *T. gondii* parasite, isolated from Matrouh, showed a 100% homology with the most dangerous reference strains of *T. gondii*-RH in GenBank database. Our results showed that B1 and P30-specific PCR could detect *T. gondii* in blood samples more accurately than ELISA. In addition, the statistical analysis of our data indicated that species, age, sex, and animal location were all risk factors for toxoplasmosis. These findings are likely to boost disease control and help contain the spread of *T. gondii* infections.

Keywords: *Toxoplasma gondii*, ELISA, B1 gene, P30 gene, Ruminants, Egypt

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List of Abbreviations

Abbrev.	Full-term
ABI	: Applied Biosystems
AG	: Agglutination Tests
AIDS	: Acquired Immunodeficiency Syndrome
B1 gene	: <i>Toxoplasma gondii</i> Gene
BLAST	: Basic Local Alignment Search Tool
CF	: Complement Fixation
DDW	: Double Distilled Water
DNA	: Deoxyribonucleic Acid
DT	: Sabin-Feldman Dye Test
EDTA	: Ethylene Diamine Tetraacetic Acid,
ELISA	: Enzyme-Linked Immunosorbent Assay
HRM	: High Resolution Melting Analysis
IFAT	: Indirect Fluorescent Antibody Test
IgA	: Immunoglobulin A
IgE	: Immunoglobulin E
IgG	: Immunoglobulin G
IgM	: Immunoglobulin M
ITS-1	: Internal Transcribed Spacer
LAT	: Latex Agglutination Test
MAG1	: Matrix Protein
MAT	: Modified Agglutination test
MIC2	: Microneme Proteins

MLE	: Multilocus Enzyme Electrophoresis
MLST	: Multilocus Sequence Typing
NC	: Negative Control
NCBI	: National Center for Biotechnology Information
n-PCR	: Nested PCR
OD	: Optical Density
PC	: Positive Control
PCR	: Polymerase Chain Reaction A Technique
PCR-RFLP	: Polymerase Chain Reaction-Restriction Fragment Length Polymorphis
qPCR	: Quantitative Real-Time PCR
ROP1	: Rhoptry Proteins
SAG1	: Surface Antigens Of <i>Toxoplasma gondii</i>
SNPs	: Single Nucleotide Polymorphisms
SPSS	: Statistical Package For Social Sciences
<i>T. gondii</i>	: <i>Toxoplasma gondii</i>
TBE	: Tris borate EDTA

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