شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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Cairo University
Faculty of Veterinary Medicine
Department of Medicine and Infectious Diseases

Advanced studies on Toxoplasmosis in small ruminants with reproductive disorders

A thesis presented by

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B.V.Sc. Faculty of Veterinary Medicine- Kafrelsheikh University 2016

For the degree of M.V.Sc

(Infectious Diseases)

Under the supervision of

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Abstract

Toxoplasmosis is an infectious disease caused by protozoan *T. gondii* which causes reproductive failure in small ruminants. Newly developed methods as Genotyping is essential to explain the virulence, epidemiology, and effective treatment and control in human and animals. This study was conducted to assess the seroprevalence of *T. gondii* in sheep and goats in Egypt especially those with a history of reproductive disorders including abortion and molecular identification of *T. gondii* followed by studying the genetic variety of *T. gondii* isolates rotating in Egypt. Blood samples were collected from 138 and 212 live ewes and she-goats from five Governorates of Egypt. Also, the blood and its corresponding tissue samples were collected From 180 ewes and 206 she-goats from (El-Moneib, El-Warak and El-Basatin abattoirs) at Cairo, Giza governorates of Egypt. All samples were serologically examined using ELISA and the tissue samples of the seropositive animals were digested and microscopically examined then bio-assayed in mice. All the *T. gondii* isolates were subjected to molecular identification using PCR and genotyped using nested PCR (n-PCR) combined with restriction enzyme analysis (RFLP) of the *SAG2* gene. The total seropositivity of live ewes and she-goats was 26.8% and 21.2%, respectively, while in slaughtered ewes and she-goats was 16.6% and 33%, respectively. 66.6% and 53.3% of slaughtered ewes, 26.4%, and 17.6% of slaughtered she-goats were positive using microscopic examination and mice viability test, respectively. *Toxoplasma gondii* tissue cysts were detected with associated characteristic histopathological changes of different organs. Twenty-eight *T. gondii* isolates were confirmed using PCR.

Among 24 milk samples from seropositive live ewes and she-goats, only 12.5% and 6.25%, were positive using PCR respectively. Genotyping confirmed 26 isolates (92.8%) as type II, 2 (7.1%) as type III. Here, Type II and III were the genotypes mostly circling among small ruminants in Egypt and this is the most significant for public health in Egypt.

Keywords: *Toxoplasma gondii*; small ruminants; abortion; ELISA; Histopathology; PCR; Genotyping.

DEDICATION

I dedicate this work to my parents, my husband & my daughters whom I am indebted to them for happiness in my life

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In the name of Allah, the Merciful and Most Gracious

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LIST OF ABBREVIATIONS

Bp	Base pair
°C	Celsius Degrees
CSF	Cerebrospinal fluid
DDW	Deionized Distilled Water
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assays
gm	Gram
H&E	Hematoxylin and Eosin
HCl	Hydrochloride
HRP	Horseradish Peroxidase
hrs(s)	Hour(s)
IFAT	Indirect Fluorescent Antibody Test
IHAT	Indirect Hemagglutination Test
LAT	Latex Agglutination Test
M	Molar
MAT	Modified Agglutination Test
ml	Milliliter
mM	Millimolar
µl	Microliter
nm	Nanometer
nPCR	Nested Polymerase Chain Reaction
OD	Optical Density
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
rpm	Rotation per minute
rRNA	Ribosomal ribonucleic acid
SFDT	Sabin Feldman Dye Test
T. gondii	*Toxoplasma gondii*
TE	Toxoplasmic encephalitis
Tris	Tris aminomethane

Chapter (1)

Introduction