

B17104

**PREPARATION OF A VACCINE TO
CONTROL THE DEVELOPMENT OF
FASCIOLA GIGANTICA IN SHEEP**

A THESIS

Submitted to the Faculty of Science, Cairo University in partial
fulfillment of the requirements for the degree of Master of Science
(M. Sc.) In Zoology
"Immunology and Parasitology"

By
Noha Ahmed Mahana Osman
(B. Sc.)

**DEPARTMENT OF ZOOLOGY
FACULTY OF SCIENCE
CAIRO UNIVERSITY**

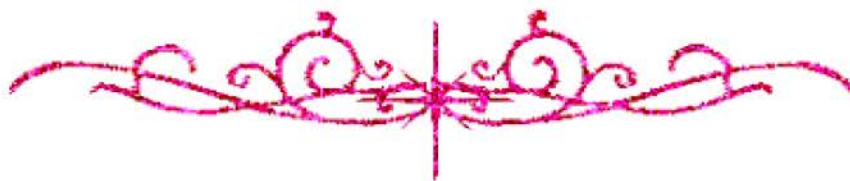
2002

بسم الله الرحمن الرحيم





شبكة المعلومات الجامعية التوثيق الالكتروني والميكرو فيلم



جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

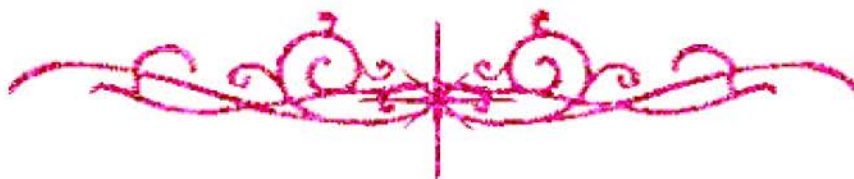
قسم

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



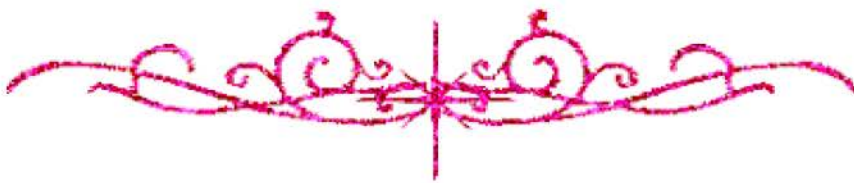
يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



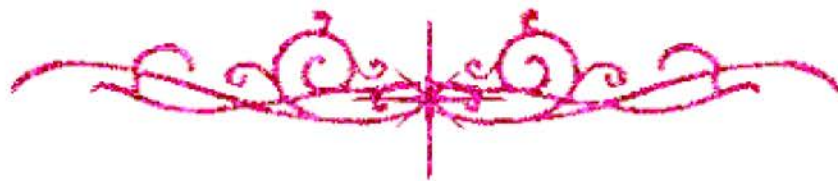


بعض الوثائق الأصلية تالفة





بالرسالة صفحات
لم ترد بالأصل



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APPROVAL SHEET

Title of the M. Sc. Thesis

**PREPARATION OF A VACCINE TO
CONTROL THE DEVELOPMENT OF
FASCIOLA GIGANTICA IN SHEEP**

Name of the Candidate

Noha Ahmed Mahana Osman

Submitted to the Faculty of Science

Cairo University

Zoology Department

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Cairo University
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TO WHOME IT MAY CONCERN

This is to certify that **Noha Ahmed Mahana** has attended and passed successfully the following post graduate courses as a partial fulfillment of the requirements of the degree of master of science (Immunology and Parasitology, 1999).

- 1- Advanced Immunology
- 2- Immunochemistry
- 3- Immunoparasitology
- 4- Applied Immunology
- 5- Developmental Comparative Immunology
- 6- Immunodiagnosis
- 7- Molecular Biology
- 8- Molecular Genetics
- 9- Physiology of Nervous System
- 10- Fine structure of Protozoa
- 11- Biochemistry of Parasites
- 12- Nematology
- 13- Phylogeny of Invertebrate
- 14- Biology of Parasites
- 15- Biostatistics
- 16- Computer Science
- 17- Deutsch
- 18- Structure and Function of Parasites
- 19- Microtechniques and Electron Microscopy
- 20- Radiobiology
- 21- Environmental Pollution

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Noha A. Mahana

ABSTRACT

Antigens of molecular mass 60, 32 and 28 kDa obtained from 10% SDS-PAGE gel of Sup.2 adult *F. gigantica* worm antigen by continuous elution method were evaluated for use as a vaccine against infection of sheep with *Fasciola gigantica*.

Sixteen sheep were divided into four group, control (unimmunized group) and D60, G32 and G28 groups immunized with 60, 32 and 28 kDa protein fractions, respectively. Blood samples were taken after the fifth immunization for determination of humoral responses.

Vaccination with the protein fractions induced high antibody titers to the immunogen and to Sup. 2 as judged by enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunoelectrotransblot (EITB) techniques. Anti-60 sheep sera were highly reactive, followed by anti-32 and anti-28 sera, respectively. The characters of protein fractions recognized by periodate oxidation using both ELISA and EITB and demonstrated that the targets of the tested antibody are of protein nature.

Metacercariae of *F. gigantica* were excysted based on the stimuli determined by Dixon (1966) with some modification of Tielense *et al.* (1980). Reproducibly higher levels of excystment with taurocholic acid (70-80%) than with bile (60-70%) were obtained. The involvement of antibodies in *in vitro* antibody dependent complement mediated cytotoxicity, ADCC and IFA assays against NEJs was studied.

After vaccination, all sheep were challenged where each animal in one group was orally infected with 150 metacercariae of *F. gigantica*. All sheep were slaughtered 12 weeks after challenge. The highest fluke burden reduction was observed in the group vaccinated with 60 kDa (41.3%, $P < 0.05$) this reduction was statistically significant in comparison with the control group.

The data thus suggest that the D-60 species contain molecule(s), which can be used as a vaccine for sheep fascioliasis.

LIST OF ABBREVIATIONS

ABTS	: 2, 2' Azino bis [3-ethylbenzothiazoline-6-sulfonic acid].
ADCC	: Antibody dependent-cell mediated cytotoxicity (killing).
BPB	: Bromophenol blue.
BSA	: Bovine serum albumin.
CatL	: Cathepsin L.
cDNA	: complementary DNA.
CFA or FCA	: Complete Freund's adjuvant.
EITB	: Enzyme-linked immunoelectrotransfer blot.
ELISA	: Enzyme-linked immunosorbent assay.
ES material	: Excretory/ secretory material.
FABP	: Fatty acid binding protein.
<i>F. hepatica</i>	: <i>Fasciola hepatica</i> .
Fh12	: <i>F. hepatica</i> 12 kDa.
Fhb	: Hemoglobin isolated from <i>F. hepatica</i> .
Fh SmIII(M)	: <i>Fasciola</i> / <i>Schistosoma</i> cross reactive antigen.
<i>F. gigantica</i>	: <i>Fasciola gigantica</i> .
Fg CatL	: Cathepsin L isolated from <i>F. gigantica</i> .
FgGST	: Glutathione S-transferase isolated from <i>F. gigantica</i> .
Fc	: Fraction crystalline portion of antibody.
G/gp	: Group.
GST	: Glutathione S-transferase.
H+L	: Heavy and light chain.
HCl	: Hydrochloric acid.
HEPES	: (N-[2-hydroxyethyl)piperazine-N'-[2-ethane sulfonic acid])).
hr	: Hour(s).
HRP	: Horseradish peroxidase.