



دراسات جزيئية و فسيولوجية على الماعز المعداه تجريبياً
بالتريبانوسوما إيفانزى

رسالة كجزء مكمل للحصول على درجة الماجستير في العلوم
(علم الحيوان)

مقدمة من
تامر حلمى عبد العزيز سيد أحمد
بكالوريوس العلوم – شعبة الحيوان (1999)
كلية العلوم – جامعة عين شمس

تحت إشراف

أ.د/ نادية محمد عبد العزيز البيه
أستاذ الفسيولوجي – قسم علم الحيوان – كلية العلوم – جامعة عين شمس

أ.د/ طارق محمد أحمد المتناوى
أستاذ الطفيليات – قسم الطفيليات و أمراض الحيوان – شعبة البحوث البيطرية
المركز القومي للبحوث

د/ محمد محمود عبد العزيز
أستاذ مساعد الطفيليات – قسم الطفيليات و أمراض الحيوان – شعبة البحوث البيطرية
المركز القومي للبحوث

قسم علم الحيوان
كلية العلوم – جامعة عين شمس
2007 م



جامعة عين شمس

كلية العلوم

رسالة ماجستير

اسم الطالب: تامر حلمى عبد العزيز سيد أحمد

عنوان الرسالة:

دراسات جزيئية و فسيولوجية على الماعز المعداه تجريبياً
بالتريبانوسوما إيفانزى.

اسم الدرجة: ماجستير في العلوم

الاسم / نادية محمد البية	الوظيفة / أستاذ فسيولوجيا الحيوان
الاسم / طارق محمد المتناوى	الوظيفة / أستاذ الطفيليات
الاسم / محمد محمود عبد العزيز	الوظيفة / أستاذ باحث مساعد الطفيليات

تاريخ البحث: / / 2007م

الدراسات العليا

ختم الأجازة: أجازت الرسالة بتاريخ / / 2007م

موافقة مجلس الكلية موافقة مجلس الجامعة

/ / 2007م / / 2007م



جامعة عين شمس

كلية العلوم

صفحة العنوان

اسم الطالب: تامر حلمى عبد العزيز سيد أحمد

الدرجة العلمية: ماجستير في العلوم.

القسم التابع له: قسم علم الحيوان.

الجامعة: عين شمس.

سنة التخرج: 1999م.

سنة المنح: 2007م



جامعة عين شمس

شكر

أشكر السادة الأساتذة الذين قاموا بالأشراف و هم :

أ.د/ نادية محمد عبد العزيز البية
أ.د/ طارق محمد أحمد المتناوى
د/ محمد محمود عبد العزيز

كما أشكر السادة الأساتذة الأتي أسمائهم:

أ.د/ محمد صابر حسانين
د/ سعاد محمد نصر إبراهيم

Molecular and Physiological Studies on Goats Experimentally Infected with *Trypanosoma evansi*

A Thesis

Submitted in partial fulfillment of requirements for the award of
the degree of Master of Science

By

Tamer Helmi Abd El-Aziz

B.Sc.(Zoology)-1999-Faculty of science-Ain Shams University

Supervisors

Prof. Dr. Nadia Mohammed El-Beih

Prof. of Physiology, Zoology Department, Faculty of Science
Ain Shams University

Prof. Dr. Tarke Mohammed El-Metanawey

Prof. of Parasitology, Parasitology and Animal Disease Department,
Veterinary Research Division, National Research Centre

Dr. Mohammed Mahmoud Abd El-Aziz

Assist. Prof. of Parasitology, Parasitology and Animal Disease Department,
Veterinary Research Division, National Research Centre

Zoology Department

Faculty of Science

Ain Shams University

2007

Acknowledgment

*I would like to express my cordial gratitude, indebtedness, and sincere thanks to **Prof. Dr. Nadia El-Beih**, Prof. of Physiology, Department of Zoology, Faculty of Science, Ain Shams University, for her guidance, supervision with great enthusiasm the work undertaken, encouragement and for reading and criticizing the manuscript.*

*I wish to express my greatest gratitude, deep thanks and appreciation to **Prof. Dr. Tarke Mohammed El-Metanawey**, Prof. of Parasitology, Parasitology and Animal Disease Department,*

Veterinary Research Division, National Research Centre, for his supervision, reading and criticizing the manuscript.

*I wish to express my greatest gratitude, deep thanks and appreciation to **Dr. Mohammed Mahmoud Abed El-Aziz** Assist. Prof. of Parasitology, Parasitology and Animal Disease Department, Veterinary Research Division, National Research Centre, for his supervision, reading and criticizing the manuscript.*

*I wish to express my sincere appreciation to **Prof. Dr. Mohammed Saber Hassanane**, Prof. Dr. of Animal Genetics, Department of Cell Biology, Genetic Engineering and biotechnology Division, National Research Centre, for his faithful help in the part of molecular studies.*

*I am deeply grateful to **Dr. Soad Mohamed Nasr Ibrahim**, Assist. Prof. of Clinical Pathology, Department of Parasitology and Animal Disease, Veterinary Research Division, National Research Centre for her faithful help in the part of physiological studies and for reading and criticizing the manuscript.*

ABSTRACT

Tamer Helmi Sayed-Ahamed

M.Sc., Faculty of Science, Ain Shams Univ., Cairo-2007

Molecular and Physiological Studies on Goats Experimentally Infected with *Trypanosoma evansi*

Keywords: *Trypanosoma evansi*, goats, PCR, diagnosis, haemoglobin, proteins, lipids, kidney functions, electrolytes

The present experiment was conducted to compare between sensitivity of polymerase chain reaction (PCR) and different diagnostic methods; wet blood film (WBF), buffy coat technique (BCT), mouse inoculation test (MI) and card agglutination trypanosoma test (CATT) for the diagnosis of *Trypanosoma evansi*. The effect of *Trypanosoma evansi* on some blood parameters in experimentally infected goats during eight weeks was also studied. Two equal groups of adult dry Baladi goats were used. The first group was kept as control. The other group was infected with *Trypanosoma evansi* isolated from camels. The study revealed that the combined estimates of sensitivity (CE_{se}) of PCR proved to be highest (93.8%) followed by the CATT, MI, BCT and WBF 60%, 55.4%, 18.5% and 13.8% respectively. Results revealed significant decrease in haemoglobin content, glucose, albumin, albumin/globulin (A/G) ratio, total lipids, T. Chol, HDL-Chol, LDL-Chol, sodium, potassium and urea. Significant increase in total proteins, globulin, triglycerides and chloride were observed. There is no significant variation occurred in serum creatinine.

List of Tables

Table	Pages
1 Comparative study of six diagnostic methods in blood samples from horses experimentally infected with <i>T. evansi</i> .	7
2 Comparison of thin blood smear method and PCR for detection of <i>T. congolense</i> and <i>T. brucei</i> subspecies in cattle.	21
3 Primer sequence of TBR1/TBR2 and predicted amplification product size.	48
4 Comparative study of five diagnostic techniques in blood samples from five experimentally infected goats with <i>T. evansi</i> .	66
5 Sensitivity (%) of five diagnostic methods, calculated per week (Se _s) in detecting <i>T. evansi</i> in experimentally infected goats.	68
6 Relationship between CATT/ <i>T. evansi</i> and MI in detecting <i>T. evansi</i> in experimentally infected goats.	71
7 Relationship between PCR and MI in detecting <i>T. evansi</i> in experimentally infected goats.	71
8 Detection of trypanosomes, DNA and antibodies in blood samples five goats following infection with <i>T. evansi</i> .	72
9 Mean $\pm$ standard error of changes in Haemoglobin content (g/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	76

10	Mean $\pm$ standard error of changes in serum total protein level (g/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	79
11	Mean $\pm$ standard error of changes in serum albumin level (g/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	81
12	Mean $\pm$ standard error of changes in serum globulin level (g/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	84
13	Mean $\pm$ standard error of changes in A/G ratios of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	87
14	Mean $\pm$ standard error of changes in serum total lipid level (mg/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	89
15	Mean $\pm$ standard error of changes in serum total cholesterol level (mg/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	92
16	Mean $\pm$ standard error of changes in serum HDL-cholesterol (mg/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	95
17	Mean $\pm$ standard error of changes in LDL-cholesterol level (mg/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	97
18	Mean $\pm$ standard error of changes in serum triglyceride level (mg/dl) of goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	100

- 19 Mean $\pm$ standard error of changes in serum glucose level (mg/dl) of goats experimentally infected with *T. evansi* in different periods for 8 weeks. 102
- 20 Mean $\pm$ standard error of changes in serum urea level (mg/dl) of goats experimentally infected with *T. evansi* in different periods for 8 weeks. 105
- 21 Mean $\pm$ standard error of changes in serum creatinine level (mg/dl) in goats experimentally infected with *T. evansi*. 108
- 22 Mean $\pm$ standard error of changes in serum sodium level (mEq/l) of goats experimentally infected with *T. evansi* in different periods for 8 weeks. 110
- 23 Mean $\pm$ standard error of changes in serum potassium level (mEq/l) goats experimentally infected with *T. evansi* in different periods for 8 weeks. 113
- 24 Mean $\pm$ standard error of changes in serum chloride level (mEq/l) of goats experimentally infected with *T. evansi* in different periods for 8 weeks. 116

List of Figures

Figures		Pages
1	Relative sensitivities of different diagnostic tests for detection of <i>T. evansi</i> in experimentally infected goats.	67
2	Sensitivity (%) of five diagnostic methods, calculated per week (Se_s) in detecting <i>T. evansi</i> in experimentally infected goats.	69
3	Percentage of positive results with WBF, BCT, MI, CATT and PCR for the five goats experimentally infected with <i>T. evansi</i> .	73
4	Electrophoresed amplification products are shown in an ethidium bromide-staining agarose gel.	74
5	Mean of haemoglobin values in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	77
6	Mean of total serum protein level in goats experimentally infected with <i>T. evansi</i> .	80
7	Mean of serum albumin level in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	82
8	Mean of serum globulin level in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	85

9	Mean of A/G ratios in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	88
10	Mean of serum glucose level in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	90
11	Mean of serum total lipid level in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	93
12	Mean of serum total cholesterol level in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	96
13	Mean of serum HDL-cholesterol in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	98
14	Mean of serum LDL-cholesterol in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	101
15	Mean of serum triglycerides level in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	103
16	Mean of serum urea level in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	106
17	Mean of serum creatinine level in goats experimentally infected with <i>T. evansi</i> in different periods for 8 weeks.	109

- 18 Mean of serum sodium level (mEq/l) of female goats experimentally infected with *T. evansi* in different periods for 8 weeks. 111
- 19 Mean of serum potassium level in goats experimentally infected with *T. evansi* in different periods for 8 weeks. 114
- 20 Mean of serum chloride level in goats experimentally infected with *T. evansi* in different periods for 8 weeks. 117

List of Abbreviations

A/G ratio	Albumin/Globulin ratio
Ab-ELISA	Antibody-Enzyme Linked Immunosorbent Assay
Ag-ELISA	Antigen-Enzyme Linked Immunosorbent Assay
BCT	Buffy Coat Technique
BUN	Blood Urea Nitrogen
CATT	Card Agglutination Trypanosoma Test
DA	Direct Agglutination test
ddH ₂ O	Double distilled water
DID	Double Immunodiffusion
EDTA	Ethylene Diamine Tetra acetic Acid
ELB	Erythrocyte Lysing Buffer
ELISA	Enzyme Linked Immunosorbent Assay
GSS	Giemsa-Stained Smear
Hb	Haemoglobin
HCT	Haematocrite Centrifugation Technique
HDL	High Density Lipoprotein
IFAT	Indirect Fluorescence Antibody Test
IFT	Indirect Fluorescence Test
ITS1	Internal Transcribed Spacer 1
LAT	Latex Agglutination Test
LDL	Low Density Lipoprotein
LLB	Leucocyte Lysing Buffer
m-AECT	mini-Anion-Exchange Centrifugation Technique
MHCT	Micro Haematocrite Centrifugation Technique
MI	Mouse Inoculation
O.D.	Optical Density
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
rDNA	ribosomal Deoxyribonucleic Acid
RoTat 1.2	Rod Trypanozoon Antigen Type 1.2
rpm	Rotate Per Minute
SCM	Strout's Concentration Method
SDS	Sodium Dodecyl Sulphate

STDM	Standard Trypanosoma Detection Method
<i>T.</i>	<i>Trypanosoma</i>
TBS	Thin Stained Blood Smear
T-Chol.	Total Cholesterol
TL	Immune Trypanolysis Test
VAT	Variable Antigen Type
VLDL	Very Low Density Lipoprotein
VSG	Variable Surface Glycoprotein
WBF	Wet Blood Film