



Clinical and Laboratory Diagnosis of Some Blood Parasites in Dairy Cattle in Qena Governorate

PH.D. Thesis

Presented by

Arwa Sameh Ahmed Mohamed

(M.SC., Fac. of Vet. Med., South Valley University 2015)

In Veterinary Medical Science, Animal Medicine (Clinical Laboratory Diagnosis)

Under the supervision of

Prof. Dr. Adel El-Sayed Ahmed Mohamed

Prof. of Animal Medicine, Department of Animal Medicine

Faculty of Veterinary Medicine, South Valley University, Qena

Prof. Dr. Alsagher Omran Ahmed

Prof. of Infectious Diseases, Department of Animal Medicine

Faculty of Veterinary Medicine, South Valley University, Qena

Prof. Dr. Abu El-Magd Mahmoud Mohamed

Chief researcher, Department of Parasitology, Animal Health Research Institute, Dokki, Giza

Submitted to

Department of Animal Medicine

Faculty of Veterinary medicine

South Valley University

2022 / 1443H

Subjects	Page
List of abbreviations	iv
List of Tables	viii
List of Figures	ix
List of Photos	X
Introduction	1
Aims of the work	3
Literature	4
1. Theileriosis	6
1.1. Etiology	6
1.2. Classification	7
1.3. Life cycle of <i>Theileria</i> species	7
1.3.1. Life cycle of <i>Theileria</i> in vertebrate host	7
1.3.2. Life cycle in the tick vector	7
1.4 Transmission	8
1.5. Epidemiology	8
1.6. Pathogenesis	9
1.7. Clinical findings	10
1.8. Haematological findings	11
1.9. Biochemical findings	11
1.10. Diagnosis	12
1.11. Antigenic Variation	13
1.12. Immune response and immunity	14
1.13. Treatment and vaccination	15
2. Babesiosis:	16
2.1. Etiology	17

2.2. Classification	17
2.3. Life cycle of babesiosis	17
2.4. Transmission	18
2.5. Epidemiology	18
2.6. Pathogenesis	18
2.7. Clinical findings	19
2.8. Haematological findings	20
2.9. Biochemical findings	20
2.10. Diagnosis	20
2.11. Antigenic Variation	21
2.12. Immune response and immunity	22
2.13. Treatment and vaccination	22
3. Anaplasmosis:	23
3.1. Etiology	23
3.2. Classification	24
3.3. Life cycle of anaplasmosis	24
3.4. Transmission	25
3.5. Epidemiology	25
3.6. Pathogenesis	26
3.7. Clinical findings	26
3.8. Haematological findings	27
3.9. Biochemical findings	28
3.10. Diagnosis	28
3.11. Antigenic Variation	29
3.12. Immune Response and Immunity	30
3.13. Treatment and vaccination	30
4. Prevention and Control of Tick borne hemoparasitic diseases	31
4.1. Control Methods	31
4.1.1. Management	31

4.1.2. Control of ticks in cattle:	32
4.1.3. Selection of Tick Resistant Cattle Breeds:	34
4.1.4. Vaccination	34
Materials and Methods	35
I- Materials	35
A- Animal population	35
B- Sampling	35
II -Adopted methods	35
A- Clinical Examination	35
B- Blood films examination	36
C- Polymerase chain reaction	36
D- Hematological Examinations	40
E- Biochemical examinations	41
F- Statistical Analysis	41
Results	42
Discussion	58
Conclusion and Recommendation	67
Summary	71
References	73
Arabic Summary	١

LIST OF ABBREVIATIONS

Abbreviations	Explanation
ALT	Alanine transaminase
A/G ratio	Albumin/Globulin ratio
ALP	Alkaline Phosphatase
A. cohaerens	Ambylomma cohaerens
A. haebraeum	Ambylomma haebraeum,
A. haebreum	Ambylomma haebreum
A. lepidu	Ambylomma lepidu
A	Anaplasma
AST	Aspartate aminotransferase
AST	Aspartate transaminase
<i>B</i>	Babesia
bp	Base pair
BUN	Blood urea nitrogen
B. indicus	Bos indicus
B. taurus	Bos taurus
САТ	Card agglutination test
°C	Celsius
CFSPH	Center for Food Security and Public Health
cm	Centimeter
CSESP	Committee on Systematic and Evolution of the Society of Protozoologists
cELISA	competitive Enzyme-linked immunosorbent assay
cyt b	cytochrome b
dl	Deciliter
DNA	Deoxyribonucleic acid
D.B	Direct bilirubin

DDW	Double distilled water
ECF	East coast fever
EGP	Egyptian Pound
ELISA	Enzyme-linked immunosorbent assay
EDTA	Ethylenediaminetetraacetic acid
e.g.	Exempli gratia
fl	Femtoliter
FAO	Food and Agriculture Organization
GGT	Gamma-Glutamyl Transferase
GSTBS	Giemsa stained thin blood smear
gp	Glycoprotein
g	Gram
Hb	Haemoglobin
Н.	Hyalomma
ICT	Immunochromatographic test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
iELISA	Indirect Enzyme-linked immunosorbent assay
IFAT	Indirect fluorescent antibody test
ITM	Infection and treatment method
INF-γ	interferon-γ
kb	Kilobase
kDa	Kilodalton
Kg	Kilogram
kg	Kilogram
L	Liter
LAMP	Loop-mediated isothermal amplification
MSP	Major surface protein
МСН	Mean corpuscular haemoglobin

МСНС	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MPSA	merozoite / piroplasm surface antigen
MSAs	Merozoite surface antigens
μg	Microgram
μl	Microliter
μm	Micrometer
mg	Milligram
ml	Milliliter
mm	Millimeter
MALR	Ministry of Agriculture and Land Reclamation
min	Minute
NK	Natural killer
NO	Nitric oxide
NS	Non Significant
N	North
No.	Number
n	Number
OIE	Office of International Des Epizooties
PCV	Packed cell volume
pg	Picograms
PCR	Polymerase chain reaction
P value	Probability value
RT-PCR	Real-time Polymerase chain reaction
RDW	Red blood cell distribution width
RBCs	Red blood cells
<i>R</i> .	Rhipicephalus
RAPs	Rhoptry-associated proteins
RNA	Ribonucleic acid

rpm	Round per minute
sec.	Second
S	South
spp.	species
SBPs	Spherical body proteins
Т.	Theileria
TBDs	Tick-borne diseases
TBE	Tris borate EDTA
TNF-α	Tumor necrosis factor alpha
UV	Ultra Violet
VMSA	variable merozoite surface antigens
WBCs	White blood cells
WHO	World Health organization

LIST OF TABELS

Table No.	Title	Page
1	Theileria species	8
2	Babesia species	18
3	Anaplasma species	25
4	Oligonucleotide primers sequences	37
5	Preparation of PCR Master Mix	39
6	Cycling conditions of the primers during cPCR	40
7	Main clinical findings in clinically healthy cows and diseased ones	42
8	Results of percent of blood parasites in stained blood films	43
	examination	
9	Results of polymerase reaction technique	44
10	Results of polymerase reaction technique	46
11	Hematological parameters of Theileria infected cows	50
12	Hematological parameters of Babesia infected cows	51
13	Hematological parameters of Anaplasma infected cows	52
14	Hematological parameters of mixed infected cows	53
15	Biochemical parameters of Theileria infected cows	54
16	Biochemical parameters of Babesia infected cows	55
17	Biochemical parameters of Anaplasma infected cows	56
18	Biochemical parameters of mixed infected cows	57

LIST OF FIGURES

Figure No.	Title	Page
1	Results of percent of blood parasites in stained blood films	44
	examination	
2	Results of PCR technique	45

LIST OF PHOTOS

Photo No.	Title	Page
1	Morphological results of recovered blood parasites	47
2	Anaplasma marginale PCR photo details	47
3	Anaplasma marginale PCR photo details	47
4	Babesia spp. PCR photo details	48
5	Babesia spp. PCR photo details	48
6	Theileria annulata PCR photo details	49
7	Theileria annulata PCR photo details	49

Summary

Bovine theileriosis, babesiosis and anaplasmosis are a tick-borne hemoparasitic diseases and are responsible for huge economic losses in livestock sector in Egypt. *Theileria annulata* is a protozoan parasite that causes bovine theileriosis, *Babesia bigemina* and *Babesia bovis* are protozoan parasites that cause bovine babesiosis while *Anaplasma marginalis* is a rickettsial pathogen that causes bovine anaplasmosis.

In the current study, a total number of 110 dairy cattle from different zones of Qena governorate, Egypt were clinically and laboratory investigated for diagnosis of theileriosis, babesiosis and anaplasmosis using two methods: direct microscopy (stained blood smears) and polymerase chain reaction (PCR) during the period from January 2019 to December 2019.

PCR technique is the most sensitive and specific test used for diagnosis of the disease in either acute or chronic cases and also in carrier animals of theileriosis, babesiosis and anaplasmosis.

It is not accurate enough to determine the blood parasitic organisms by Giemsa staining as blood parasitic structures recognized in erythrocytes are often difficult to be differentiated from Heinz bodies, Howell-Jolly bodies or staining artifacts.

Hemato-biochemical alternations in theileriosis, babesiosis and anaplasmosis infected cattle were also detected in this study and can be considered one of the most important procedure that may confirm the infection.

In bovine piroplasmosis and anaplasmosis, there were changes in leucogram which might be attributed to persistent harmful effects of toxic metabolites of blood parasites on the haemopiotic organs especially bone marrow and their interference with the process of leucogenesis.

There were changes in hemogram could be due to destructive parasite's influence on RBCs, or because of lytic effect of intra erythrocyte parasite, immunemediated mechanism such as erythrophagocytosis might be responsible for RBCs count reduction eventually. Anemia proposed to be resulted from removal of piroplasm from infected erythrocytes by macrophages.

In bovine piroplasmosis and anaplasmosis, there were changes in liver enzymes activities which explained as a result of the harmful effect of toxic metabolites of blood parasites on liver cells.

There were also changes in the protein picture in infected animals which could be due to decrease protein production as a result of harmful effect of toxic metabolites of blood parasites on liver cells, deprivation of diet protein resulting from anorexia and fever accompanied infection also, disturbed hepatic functions and destructed RBCs and its excretion in urine can play a role. Also immune response against blood parasites may have a role in these changes. Due to the economic losses of livestock sector caused by hemoparasitic diseases, high costs of treatment and the high prevalence of carrier state infections, prevention is the best mean to control the infections; it consists of two types of actions which are control of the vector ticks and vaccination.