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Clinicopathological Studies on Camel Theileriosis

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ABSTRACT

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ABSTRACT

The present study was to investigate the incidence, hematological, biochemical and molecular changes associated with theileriosis in camels). A total of 243 camels, 3-5 years old from both sexes were investigated, clinical examination revealed there were no clinical signs but camels were either with or without tick`s infestation. The results revealed that 75 (30.86%) camels were infected with ***Theileria*** *species* in Geimsa stained blood smears. *Hyalomma dromedarii* was identified as the carrier tick of ***Theileria spp***. Multinucleated sporoblast and free sporozoit (the infective stage of **Theileria**) were observed in salivary gland smears from collecting ticks. Macrocytic hypochromic anemia was recorded in **Theileria** infected group. Leukocytosis associated with neutrophilia, eosinophilia and lymphopenia in **Theileria** infected group. Hypoproteinemia, hypoalbuminemia, significant increase in total globulins, α and γ globulins, with significant decrease in β globulins and A/G ratio were recorded in **Theileria** infected group. Hyperglycemia and marked increase in serum aspartate aminotransferase activity, creatinine and high density lipoprotein levels, whereas triglycerides and cholesterol concentration were decrease in **Theileria** infected group. Application of PCR assay was more sensitive and specific than conventional diagnostic techniques. In conclusion, high incidence of camel theileriosis was recorded in apparently healthy camels in Egypt. *Hyalomma dromedarii* were common ticks which transmitted **Theileria** infection to camels. Camels theileriosis caused by ***Theileria annulata*** greatly influenced the cellular and biochemical constituents.

CONTENTS

Page

1. INTRODUCTION..... **1**

2. REVIEW OF LITERATURE.....**5**

2.1. Incidence of camel theileriosis in the world.....**5**

2.2. The prevalence of camel theleriosis in Egypt.....**6**

2.3. Life cycle and transmission.....**9**

2.4. Clinical signs of theileriosois.....**10**

2.5. Parasitological examination of blood and lymph node smears.....**13**

2.6. Clinicopathological studies of theileriosis.....**16**

2.7. Polymerase chain reaction (PCR) for diagnosis of theileriosis.....**35**

3. MATERIAL AND METHODS.....**44**

3.1. Material **44**

3.1.1. Material used for blood, lymphnode, hemolymph, med gut and salivary gland smears.....**44**

3.1.2. Material used for clinicopathological examination **44**

3.1.3. Material for polymerase chain reaction.....**46**

3.2. Methods **51**

3.2.1. Examined animals.....**51**

3.2.2. Samping.....**51**

3.2.3. Parsitological examination.....**52**

3.2.4. Clinicopathological studies.....**55**

3.2.5. Method of polymerase chain reaction (PCR).....**62**

3.2.5. Statistical analysis.....**65**

4. RESULTS.....**66**

4.1. Clinical examination of the camels.....**66**

4.2. Parasitological examination.....**66**

4.3. Clinicopathological changes.....**73**

4.4. Diagnosis of blood parasites in camels using polymerase chain Reaction (PCR).....**93**

5. DISCUSSION.....**97**

6. SUMMARY AND CONCLUSION.....**.119**

7. REFERENCES.....**.123**

الملخص العربي

المستخلص

TABLE OF ABBREVIATIONS

ALP	**Alkaline phosphatase.**
ALT	**Alanine aminotransferase**
AST	**Aspartate aminotransferase**
B. ovis	***Babesia ovis***
Babesia spp	***Babesia species***
CBC	**Complete blood cell count**
DLC	**Differential leukocytic count**
EDTA	**Di-potassium salt of ethylene di-amine tetra-acetic acid**
GGT	**Gamma-glutamyl transferase**
HDL	**High density lipoprotein**
H.an.anatolicum	***Hyalomma anatolicum anatolicum.***
H.an.excavatum	***Hyalomma anatolicum excavatum***
H.impeltatum	***Hyalomma impeltatum***
H.marginatum	***Hyalomma marginatum***
H. rufipes	***Hyalomma rufipes***
H.schulzei	***Hyalomma schulzei***
Hb	**Hemoglobin**
MCH	**Mean corpuscular hemoglobin**
MCHC	**Mean corpuscular hemoglobin concentration**
MCV	**Mean corpucular volume**
MO	**Microscopic observation**
PBS	**Phosphate buffer saline**
PCR	**Polymerase chain reaction**
PCV	**Packed cell volume**
R. sanguines	***Rhipicephalus Sanguines***
RBCs	**Red blood corpuscles**
RFLP	**Restriction fragment length polymorphism**
RLB	**Reverse line blot**
SGOT	**Serum glutamic oxaloacetic transaminase**
SGPT	**Serum glutamic pyruvic transaminase**
T. annulata	***Theileria annulata***
T. hirci	***Theileria hirci***
T. lestoquard	***Theileria lestoquard***
T. buffeli	***Theileria buffeli***
T. ovis	***Theileria ovis***
T. parva	***Theileria parva***
T .sergonti	***Theileria sergonti***
T.evansi	***Theileria evansi***
TEC	**Total erythrocytic count**
T. ovis	***Theileria ovis***
T. spp	***Theileria species***
TLC	**Total leukocytic count**
WBCs	**White blood corpuscles**

List of Tables

No. of Table	Title	Page
Table (1)	List of primers used for PCR assay.	47
Table (2)	Incidence of ***Theileria species*** in Geimsa stained blood smears.	68
Table (3)	Erythrogram of camels in control and infected with ***Theileria species*** (Means ± SE).	74
Table (4)	Leukogram of camels in control and infected with ***Theileria species*** (means ± SE).	76
Table (5)	Protein profile of control and infected camels (mean ± SE).	79
Table (6)	Some serum enzymes and glucose of camels in control and infected with ***Theileria species*** (mean ± SE).	84
Table (7)	Serum lipids profile of camels in control and infected with ***Theileria species*** (means ± SE).	88
Table (8)	Serum urea and creatinine of camels in control and infected with ***Theileria species*** (means ± SE).	88
Table (9)	Serum calcium, inorganic phosphorus and iron of camels in control and infected with ***Theileria species*** (means ± SE).	89
Table (10)	Number and percentage of infected camels with **Theileria** using PCR.	94
Table (11)	Incidence of camel theileriosis by microscopic examination of blood smears and specific PCR assay. .	96

List of Figures

No. of Figures	Title	Page
Figure (1)	The life cycle of **Theileria**.	10
Figure (2)	Erythrogram in control and infected camels with ***Theileria species.***	75
Figure (3)	Leukogram in control and infected camels ***Theileria species.***	77
Figure (4)	Serum proteins levels (g/dl) and A/G ratio in control and infected camels with ***Theileria species***.	80
Figure (5)	Serum globulins levels (g/d) in control and infected camels with ***Theileria species***.	81
Figure (6)	Some serum enzyms in control and infected camels with ***Theileria species***.	85
Figure (7)	Levels of serum glucose in control and infected camels with ***Theileria species***.	86
Figure (8)	Serum triglycerides, cholesterol and high density lipoprotein levels in control and infected camels with ***Theileria species***.	90
Figure (9)	Serum urea and creatinine levels in control and infected camels with ***Theileria species***.	91
Figure (10)	Serum calcium, inorganic phosphorus and iron levels in control and infected camels with ***Theileria species***.	92

List of Photoes

No. of Photoes	Title	Page
Photo (1a)	Multinucleated sporoblast in Geimsa stained salivary gland smear from collected ticks (x1250).	69
Photo (1b)	Free sporozoite in Geimsa stained salivary gland smear from collected ticks (x 1250).	69
Photo (2)	Camel lymphocyte contains macroschizont in Geimsa stained med gut smears from collected ticks (x1250).	70
Photo (3)	***Theileria annulata*** (ring form piroplasm) inside camel erythrocyte in Geimsa stained blood smear (x 1250).	71
Photo (4)	***Theileria annulata*** piroplasm (bipolar shape) inside camel erythrocyte in Geimsa stained blood smear (x 1250).	71
Photo (5)	Two camel lymphocytes contain macroschizont in Geimsa stained blood smear (x1250).	72
Photo (6)	Camel lymphocyte contains microschizont (purple bluish fine bodies) in Geimsa stained blood smear (x1250).	72
Photo (7)	Representative electrophoretic patterns of serum proteins (Albumin, α-globulins, β-globulins and γ-globulins) in control and infected camels.	82
Photo (8)	The electrophoretic pattern of PCR amplicon on blood samples using general **Theileria** primer and ***Theileria annulata*** primer.	95

1. INTRODUCTION

Camels represented a great investment in agriculture and desert areas. It has been an important source of meat, milk, wool and transportation (**El-Fayoumy et al. 2005).**

The signs of illness in camel may be masked because of the normal variations in physiological functions but specific disease or parasite related symptoms are generally easy to recognize (**Higgins, 1986**).

Although wide range of parasitic infections and work related diseases are found in camels few deaths are attributed directly to parasites but undoubtedly are major cause of economic loss (**Chemuliti et al., 2003**).

Theileriosis is one of the most common tick-borne diseases, which have been studied and described in a wide range of ruminants such as cattle, sheep and goats, but in camels, only a few literatures were published. *Hyalomma species* especially, *Hyalomma dromedarii* and *Hyalomma annatolicum excavatum* are the common ticks infesting camels in Egypt **(Abd El-Baky, 2001).**

Theileria are tick-transmitted, obligate intracellular parasites that are important pathogens of livestock in the tropical and subtropical regions of the world. Like all intracellular organisms, host cell invasion is a critical aspect of

Theileria biology and the various stages (Sporozoites and merozoites) in the mammalian host, (the zygote and kinete in the tick vector) are highly adapted to invade and survive in their specific host cells in both the vertebrate and tick host **(Dobbelaere and Mckeever, 2002).**

Theileria parasites enter the host during tick infestation as sporozoites, which rapidly invade mononuclear leukocytes. Where, they mature into macroschizonts and induce proliferation of the host cell. Macroshizonts develop further into microschizonts and ultimately into merozoites, which are released from the leukocyte (lymphocyte). The merozoites invade erythrocytes and develop into piroplasms **(Radostitis et al., 2000)**

Diagnosis of theileriosis in acute cases is mainly based on clinical signs (fever, occular discharge, severe emaciation, diarrhea and enlargement of superficial lymph nodes) present on the infected animals and confirmation of the diagnosis depends on microscopic examination of Geimsa stained thin blood and lymph node smears **(Aktas et al. 2006)**. Serological tests such as the indirect fluorescent antibody technique (IFAT) and enzyme-linked immunosorbent assay (ELISA) were used to detect antibodies to schizont found in carrier status in the population, thereby the sensitivity and specificity of this IFAT in comparison with ELISA were 66.6% and 95.5% respectively **(Golchinfar *et al.*, 2003).** Polymerase chain reaction (PCR) assays are more

sensitive and specific than conventional diagnostic techniques. So, it has been the most preferred method for detection of tick-borne diseases (**Aktas et al., 2006) and Mahmmod et al., (2010).**

Some authors studied the prevalence of theileriosis in camels in the world as **Mishra et al. (1987)** in India, **El-Fayoumy et al. (2005)** in Libya and **Al-Saad et al. (2006)** in Iraq, the prevalence were 26.1%, 6.2% and 80% respectively. Other authors in Egypt as, **Abd El-Wahab (2009)** and **Hamed et al. (2011)** recorded that the prevalence was 44.23% and 6.75% respectively. While few authors as **El-Seragany et al. (1991), El-Fayoumy et al. (2005) and Al-Saad et al. (2006)** studied the associated hematological changes while **Al-Saad et al. (2006)** studied the serum biochemical changes. Also, the different parasitological aspect were studied by many authors as **El-kady (1998), El-Refaii et al. (1998), El-Kammah et al. (2001)** and **El-Fayoumy et al. (2005)** while **Abd El-Wahab (2009)** studied the molecular diagnostic tests.

Therefore, the present study was conducted to

1. Determine the incidence of theileriosis in camels from some localities in Egypt.
2. Investigate the effect of ***Theileria species*** naturally infected camels on hemogram, serum biochemical paramters.
3. Application of PCR for diagnosis of theileriosis in camels.

2. REVIEW OF LITERATURE

2.1. Incidence of camel theileriosis in the world

Boid et al. (1985) reported that a number of protozoan parasites, including **Theileria** have been reported to occur in camels throughout camel rearing areas of the world but their economic impact appears to be small.

Mishra et al. (1987) found ***Theileria dromedarri*** in (26.1%) of examined camels (114) and the parasitaemia was less than 1% at a breeding farm in Bikaner, India.

Johannes (1996) reported that ***Theileria camelensis*** is a non-pathogenic ***Theileria species*** found in erythrocytes of camels in Egypt and Eritrea. The vector is *Hyalomma dromedarii* (*H. dromedarii*).

Wernery and Kaaden (2002) reported that ***T. camelensis*** and ***T. dromedarri*** are the forms of ***T. species*** in Turkmenistan, Egypt and Somalia. They also mentioned that although ticks are often on camels in large numbers, very few reports have been published concerning tick-borne pathogens in camels. These few case reports are not considered reliable as they usually fail to give adequate taxonomic description.

Karimi et al. (2004) examined blood smears from 350 native camels (*Camelus dromedaries*) and impression smears from the pre-scapular lymph node from 150 camels slaughtered at Yazd slaughter house in Iran**.** Both smears stained with Giemsa stain and examined for ***T. species*** infection. The study concluded that no infection with **Theileria** was observated.

Al-Saad et al. (2006) performed a study on a total of 35 male and female camels, 4-12 years old in Iraq. Twenty eight (28) camels were naturally infected with ***T. camelensis***, while the 7 were clinically normal.

El-Maghrabi (2007) found 14 (6.2%) of a total 225 camels from Tripoli abattoirs in Libya were infected with ***T. species***. No other blood parasites were detected.

Jean Larson (2010) mentioned that there are several species of ticks are dangerous arthropod pests of camels as they can transmit a number of protozoan diseases such as ***T. species*** in the dromedary camels living in Africa and Asia.

2.2. The prevalence of camel theleriosis in Egypt

El-Saragany *et al.* (1991) recorded that **Theileria** infection was (12.1%) in lymph nodes, erythrocytes, and lymphocytes of camels.

Nassar (1992) found that 30% out of total 200 camels examined were infected with ***T. species***.

El-kady (1998) recorded that *H. dromedarri, H. impeltatum* and *H. anatolicum excavatum* were most common tick species in a survay of ticks infesting camels which was carried out in seven localities of Sinai, El-Arish, Beer El Abd, Nakhel, Ain Mousa, Sant Catherine, Wadi Hadra and Dahab. ***T. spp.*** were recorded in both tick guts and hemolymph in all species of ticks all over the studied areas.

El-Refaii et al. (1998) performed a study on lymph nodes, livers and lungs of 74 slaughtered camels in El-Bassatin abattoir for screening **Theileria** infection by parasitological and electeron microscopy examinations. Eighteen blood films from the same cases were taken as well as, female ticks were collected from the investigated camels.Forty six camels out of 74 (62.1%) were infected with **Theileria**. Likewise, microscopical examination of blood films revealed oval and ring forms of theileria in the erythrocytes of 6 camels (33.3%).

El-Kammah et al. (2001) collected 19 species and subspecies of ticks from different localities in five governorates Giza, Sharkia, Ismailia, El Behira and Sinai. *H. species* (98.6%) were found on camels. Examination of camels infected with ticks showed ***T. annulata*** (rod and ovoid).

Mazyad and Khalaf (2002) recorded ***Theileria ovis*** in 24 (12.6%) camels from total 190 camels in Al-Arish and El-Hasanah, North Sinai government.

Mahran (2004) performed a survey on 450 camels from Shalatin City Red Sea Governorate, Egypt, to detect the incidence of blood parasite in camels. He found that 6.2% were infected with ***T. camelensis***.

El-Fayoumy et al. (2005) studied a total of 125 camels belonging to two districts (Sidibarani and Mersa Matrouh). The camels were examined for ***T. camelensis***, fifty six camels out of 125 examined (44.8%) were naturally infected with ***T. camelensis***. They found that 68 (54.4%) camels out of 125 were infected with *H.species* (*H. dromedarii* and *H. anatolicum excavatum)*.

Taher (2005) performed a study on 174 camels slaughtered at different slaughter houses in Assiut Governorate, during the period from February 2003 to January 2004. The prevalence of ***T. camelensis*** was 8.62%.

Abd El-Wahab (2009) performed a study on a total one hundred and four (104) camels, forty six camels (44.23%) were naturally infected with ***T. annulata*** by microscopic examination of Geimsa stained blood smears.

Hamed et al. (2011) performed a study on a total of 224 camels infested with *H. dromedarii* ticks to investigate the presence of ***T. camelensis*** infection in Upper Egypt. Results revealed that 15 (6.75%) of 224 camels were harboring ***T. camelensis*** by microscopic examination of Geimsa stained blood smears.

2.3. Life cycle and transmission

Dobbelaere and Mckeerver (2002): mentioned that the cycle of ***Theileria*** in the mammalian hosts begins when sporozoites are inoculated by a tick as feeds. The sporozoites enter lymphoid cells (leukocytes) and develop in to a multinucleate schizont, and at the same time induce host cell transformation and proliferation. A proportion of schizonts eventually differentiate into merozoites and these invade erythrocytes. Infected erythrocytes are ingested by a tick and in the lumen of the tick gut gametogenesis and fertilization occurs. The resulting zygote invade the gut epitheilial cells where it remains during the tick molt cycle and develops into a single motile kinete. The motile kinete egresses the gut cell and subsequently invades the salivary glands where another round of a sexual multiplication. Sporogny occurs, producing many thousands of sporozoites. These are injected into a mammalian host when the tick feeds.

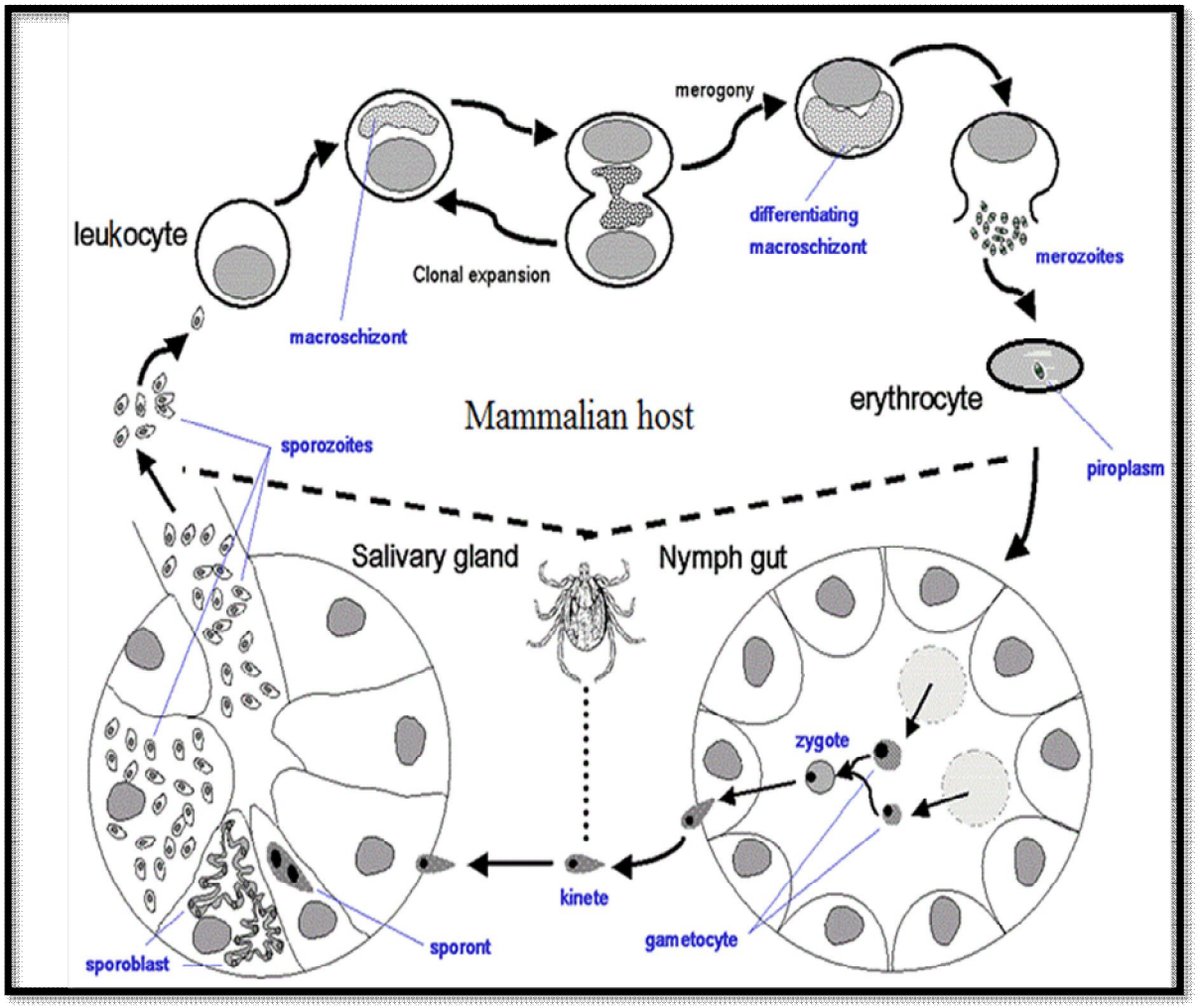


Figure (1): The life cycle of ***theileria***.

2.4. Clinical signs of theileriosois

Nassar (1992) examined 200 apparently healthy camels and found 30% infected with ***Theileria species***. Ten ml of bovine blood containing high number of ***T. annulata*** parasites were injected intravenously into five (2 years-old) healthy dromedaries. The camels did not show any clinical signs and ***T. annulata*** was not observed in blood samples taken over a period of one month.

Darweach (1993) detected that ***Theileria*** infection cause fever, enlargement of lymph nodes, emaciation, high drop of milk yield and death among cattle.

Mansuer (1996) recorded that clinical symptoms of theileriosis in cattle were enlargement of superficial lymph node, inappetence and intermittent fever.

Sandhu et al., (1998) recorded that the clinical signs of ***T. annulata*** in experimentally infected crossbred calves were enlargement of the pre-scapular lymph nodes, lacrimation, anemia, icterus, and protrusion of eyeballs. Diarrhea was only observed in three animals. The experimental infected calves maintained their appetite until a day or two before death, when they became recumbent.

Radostitis et al. (2000) recorded that the most marked clinical signs of theileriosis in cattle were enlargement of the lymph nodes in the area draining the site of tick attachment followed by fever, depression, anorexia and drop in milk production. In later stages, there may be nasal and ocular discharge, dypsnea and generalized lymph node enlargement. Severe cases may be associated with diarrhea and dysentery.

Singh et al. (2001) recorded that ***T. annulata*** experimentally infected crossbred calves showed a rise in body temperature and enlargement of the prescapular lymph nodes from day 8 post infection. The lymph nodes were enlarged two or three times in size by day 10. Bilateral lacrimation, nasal discharge and palor of the conjuctiva and oral mucous membranes were noticed from day 12 onwards.

El-Fayoumy et al. (2005) recorded that the main clinical findings in camels infected with ***T. camelensis*** were fever, ocular discharge, severe emactiation, diarrhea, and enlargement of superficial lymph nodes.

Al-Saad et al. (2006) recorded that the infected camels with ***T. camelensis*** exhibited enlargement of superficial prescapular lymph nodes, emaciation, hind limb weakness, diarrhea, pale mucous membranes, watery eyes (lacrimation), inappetence, rough hair coat, high body temperature, tick (*H. anatolicum*) infestation on the different parts of the body, increase in body temperature, respiratory and heart rates, and slow ruminal contractions.

Hamed et al. (2011) recorded the clinical signs on ***T. camelensis*** infected camels in Upper Egypt. Results revealed that 15 (6.75%) of a total 224 camels were harboring ***T. camelensis***. These 15 camels did not show any

abnormal clinical signs except three cases that showed enlargement of superficial lymph nodes and fever.

Issi et al. (2011) detected anorexia, cough, growth in superficial lymph nodes and petechial blood blisters in conjunctivas and clearly anemic or slightly icteric mucosa in cattle with theileriosis.

Khan et al. (2011) detected high rise in body temperature, general debility, enlarged prescapular lymph nodes, mucosal hemorrhages, conjunctivitis, in cross bred cattle infected with bovine theileriosis in semi arid zone of Pakistan.

2.5.Parasitological examination of blood and lymphnode smears

Mishra et al. (1987) found ***T. dromedarri*** in 26.1% Giemsa stained blood smears from 114 camels at a breeding farm in Bikaner, India. The parasitaemia was less than 1%.

El-Sergany et al. (1991) recoded ***Theileria*** infection in (12.1%) lymph nodes of camels in both erythrocytes and lymphocytes.

Metwally (1992) mentioned that lymph nodes smears taken from enlarged lymph node of ***T. annulata*** infected bovine (fixed and stained like blood film) revealed macroschizonts stage inside lymphoblast (koch`s blue bodies) in Egypt.