EPIDEMIOLOGY OF TROPICAL THEILERIOSIS IN NYALA DAIRY FARMS IN SOUTH DARFUR STATE, SUDAN

By

BOTHINA BAKOR MOHAMMED GAAFAR
(B. V. Sc. 1998, U. of Nyala)

SUPERVISOR
DR. SHAWGI MOHAMED HASSAN

DEPARTMENT OF PARASITOLOGY
FACULTY OF VETERINARY MEDICINE
UNIVERSITY OF KHARTOUM

ATHESIS SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF KHARTOUM IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTER OF VETERINARY SCIENCE (M. V. Sc)

AUGUST 2008
DEDICATION

To my great parents
brothers & sisters
with love and gratitude
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of contents</td>
<td>I</td>
</tr>
<tr>
<td>List of maps and plates</td>
<td>Vi</td>
</tr>
<tr>
<td>List of tables</td>
<td>Vi</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>X</td>
</tr>
<tr>
<td>Abstract</td>
<td>Xii</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td><strong>CHAPTER ONE: LITERATURE REVIEW</strong></td>
<td>3</td>
</tr>
<tr>
<td>1.1. Definition of tropical theileriosis</td>
<td>3</td>
</tr>
<tr>
<td>1.2. Aetiology</td>
<td>3</td>
</tr>
<tr>
<td>1.3. Origin</td>
<td>3</td>
</tr>
<tr>
<td>1.4. Historical background</td>
<td>4</td>
</tr>
<tr>
<td>1.5. Taxonomy</td>
<td>4</td>
</tr>
<tr>
<td>1.5.1. Family Theileriidae</td>
<td>5</td>
</tr>
<tr>
<td>1.5.2. Genus <em>Theileria</em></td>
<td>5</td>
</tr>
<tr>
<td>1.5.3. <em>Theileria</em> species of cattle</td>
<td>5</td>
</tr>
<tr>
<td>1.6. Biology of <em>Theileria</em> species</td>
<td>6</td>
</tr>
<tr>
<td>1.6.1. Life cycle of <em>Theileria</em> spp in the vertebrate hosts</td>
<td>6</td>
</tr>
<tr>
<td>1.6.2. Life cycle of <em>Theileria</em> spp. in the tick vector</td>
<td>7</td>
</tr>
<tr>
<td>1.7. Morphology</td>
<td>8</td>
</tr>
<tr>
<td>1.8. Transmission</td>
<td>9</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.8.1.</td>
<td>Vector of tropical theileriosis in the Sudan</td>
</tr>
<tr>
<td>1.9.</td>
<td>Geographic distribution of tropical theileriosis</td>
</tr>
<tr>
<td>1.9.1.</td>
<td>Distribution in the world</td>
</tr>
<tr>
<td>1.9.2.</td>
<td>Distribution in the Sudan</td>
</tr>
<tr>
<td>1.10.</td>
<td>Host susceptibility</td>
</tr>
<tr>
<td>1.11.</td>
<td>Incubation period</td>
</tr>
<tr>
<td>1.12.</td>
<td>Clinical signs</td>
</tr>
<tr>
<td>1.13.</td>
<td>Pathogenicity</td>
</tr>
<tr>
<td>1.14.</td>
<td>Pathological features</td>
</tr>
<tr>
<td>1.15.</td>
<td>Morbidity and mortality</td>
</tr>
<tr>
<td>1.16.</td>
<td>Immunology</td>
</tr>
<tr>
<td>1.17.</td>
<td>Diagnostic techniques</td>
</tr>
<tr>
<td>1.17.1.</td>
<td>Provisional diagnosis</td>
</tr>
<tr>
<td>1.17.2.</td>
<td>Laboratory diagnosis</td>
</tr>
<tr>
<td>1.17.2.1.</td>
<td>Microscopic examination</td>
</tr>
<tr>
<td>1.17.2.2.</td>
<td>Serological tests</td>
</tr>
<tr>
<td>1.17.2.2.1.</td>
<td>Indirect fluorescent antibody test</td>
</tr>
<tr>
<td>1.17.2.2.2.</td>
<td>The enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>1.17.2.2.3.</td>
<td>Other serological tests</td>
</tr>
<tr>
<td>1.17.2.3.</td>
<td>Molecular biology diagnosis</td>
</tr>
<tr>
<td>1.18.</td>
<td>Ticks of the Sudan and economically impact of ticks</td>
</tr>
<tr>
<td>1.18.1.</td>
<td>Economic importance of tropical theileriosis</td>
</tr>
<tr>
<td>1.19.</td>
<td>Treatment of tropical theileriosis</td>
</tr>
<tr>
<td>1.20.</td>
<td>Control measures of tropical theileriosis</td>
</tr>
<tr>
<td>1.20.1.</td>
<td>Management</td>
</tr>
<tr>
<td>1.20.2.</td>
<td>Tick control</td>
</tr>
<tr>
<td>1.20.3.</td>
<td>Immunization</td>
</tr>
<tr>
<td>1.20.3.1.</td>
<td>Infection and treatment</td>
</tr>
</tbody>
</table>
CHAPTER TWO: MATERIALS AND METHODS

2.1. The study area ...................................................... 31
2.2. Animal husbandry types ........................................ 33
2.3. Selection of cattle for sampling ................................. 34
2.4. Tick collection ..................................................... 34
2.5. Sampling of blood .................................................. 36
2.5.1. Sampling of whole blood ..................................... 36
2.5.2. Sampling for blood smears ................................. 36
2.5.3. Sampling for lymph nodes biopsy smears ............... 37
2.6. Haematological tests ............................................ 37
2.6.1. Determination of packed cell volume ...................... 37
2.6.2. Determination of haemoglobin concentration ........... 38
2.6.3. Red blood cells count ......................................... 38
2.6.4. White blood cells count ..................................... 39
2.6.5. The erythrocyte indices ...................................... 39
2.6.5.1. Mean corpuscular haemoglobin ......................... 39
2.6.5.2. Mean corpuscular volume ................................. 40
2.6.5.3. Mean corpuscular haemoglobin concentration ...... 40
2.7. Serum collection ................................................ 40
2.8. Indirect fluorescent antibody test ............................... 40
2.8.1. Phosphate buffered saline ................................ 41
2.8.2. Labelled conjugate ......................................... 41
2.8.3. Mountant ......................................................... 41
2.8.4. Control sera ................................................... 41
CHAPTER THREE:     RESULTS

3.1. Parasitoloical findings............................................. 45
3.2. Serological findings............................................... 45
3.3. Clinical features.................................................. 50
3.4. Prevalence of *Theileria* spp. among breeds of cattle..... 52
3.5. Prevalence of *Theileria* spp. among different age groups of cattle................................................ 52
3.6. Prevalence of *Theileria* spp. among different sex of cattle. ................................................................. 52
3.7. Prevalence of *Theileria* spp. according to systems management ............................................................... 56
3.8. Epidemiological indicators of tropical theileriosis....... 56
3.9. Haematogram indices of infected cattle....................... 59
3.9.1. Red blood cells.................................................. 59
3.9.2. Packed cells volume............................................. 59
3.9.3. Haemoglobin concentration................................... 59
3.9.4. White blood cells count....................................... 61
3.10. Erythrocyte indices............................................... 61
3.10.1. Mean corpuscular volume.................................... 61
3.10.2. Mean corpuscular haemoglobin................................. 61
3.10.3. Mean corpuscular haemoglobin concentration............. 61
3.11. Ticks infesting cattle............................................. 62
3.11.1. Boophilus decoloratus.......................................... 62
3.11.2. Boophilus annulatus............................................. 62
3.11.3. Hyalomma dromedarii........................................... 62
3.11.4. Hyalomma. impeltatum.......................................... 63
3.11.5. Hyalomma. marginatum rufipes.............................. 63
3.11.6. Hyalomma. truncatum.......................................... 63
3.11.7. Amblyomma variegatum ................................. 63

CHAPTER FOUR: DISCUSSION

5. CONCLUSIONS AND RECOMMENDATIONS...... 82
6. REFERENCES.......................................................... 83
7. ملخص الدراسة......................................................... 122
8. Appendix 1............................................................... 124
# LIST OF MAPS AND PLATES

<table>
<thead>
<tr>
<th>Map</th>
<th>Plate</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Sites from where samples were collected in Nyala town</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Thin blood smear showing erythrocytes containing <em>Theileria</em> spp. piroplasms from a calf suffering from theileriosis in Nyala</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Lymph node biopsy smear containing intracellular and extracellular <em>Theileria annulata</em> schizonts from a calf in Nyala</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>IFA detection of <em>Theileria annulata</em> antibodies using schizont antigens</td>
<td>49</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The meteorological data of Nyala area</td>
<td>32</td>
</tr>
<tr>
<td>2.</td>
<td>Prevalence rate of <em>Theileria</em> spp. piroplasms determined by blood smear and antibodies against <em>T. annulata</em> using IFA test among cattle in different localities in Nyala</td>
<td>46</td>
</tr>
<tr>
<td>4.</td>
<td>Prevalence of <em>Theileria</em> spp. piroplasms and <em>Theileria annulata</em> antibodies among local and cross breed cattle in Nyala farms using blood smears and indirect fluorescent antibody test from June 2006 to May 2007</td>
<td>53</td>
</tr>
<tr>
<td>5.</td>
<td>Incidence of <em>Theileria</em> spp. piroplasms and <em>Theileria annulata</em> antibodies using blood smears and indirect fluorescent antibody test, respectively among different age groups of cattle in Nyala during June 2006 to May 2007</td>
<td>54</td>
</tr>
<tr>
<td>6.</td>
<td>Incidence of <em>Theileria</em> spp. piroplasms and <em>Theileria annulata</em> antibodies using blood smears and indirect fluorescent antibody test, respectively among bulls and cows in Nyala during June 2006 to May 2007</td>
<td>55</td>
</tr>
</tbody>
</table>

57


58


60


64


67

6. Mean (± SE) monthly ticks infesting different age groups of cattle in Nyala during the period from June 2006 to May 2007.

69

7. Ticks collected in very low numbers from cattle in Nyala farms during the period from June 2006 to May 2007.

70
ACKNOWLEDGEMENTS

First of all, thanks and praise to Almighty Allah, The Compassionate, and The Most Merciful for giving me health and strength throughout the period of the study.

I would like to express my deepest gratitude and appreciation to my supervisor Dr. Shawgi Mohamed Hassan, Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum for his unfailing guidance, constructive criticism, continuous advice support, and encouragement during the study period. I am, also, greatly indebted to Dr. Khitma Hassan Elmalik, University of Khartoum, Prof. Musa Tibin Musa, Animal Resources Research Corporation, and Dr. Abbakar Adam Mohammed, University of Nyala, for their helpful guidance, advice valuable assistance and supported me.

I wish to express deep appreciation to the following for their considerable assistance: Dr. Mekki. M. Abdalla, Animal Resources Ministry, South Darfur State, Dr. Ali Siddig and Dr. Lymia Mubark El Hag, Department of Ticks and Tick-borne Diseases, CVRL and the entire staff members in the department with special thanks to Dr. Mohammed Tom, Mrs. Manal and Miss. Eiman. My special thanks are to Dr. Ahmed Hussein El Imam, University of El Imam El Mahdi and Dr. Mohamed Sayed Mohamed, University of Gezira.

My thanks are also extended to Dr. M. Bakri Abdulwhab, Dr. M. Adam Abdalla, and I would like express special thanks to Mr. Suliman Noja, and Mr. Adam Abdalla, Nyala Veterinary Research Laboratory for their unlimited help and assistance in collection of samples used in this study. My gratitude goes to my colleagues at Animal Resource Department, South Darfur State with special thanks to Dr. Nawal Abdel
Rahim, Dr. Wafa, M. Nasur. Dr. Awatif, M. Al sheikh. Dr. Kalthoum Yagoub.

Last but not least I extend my unlimited thanks to my father, mother, brothers and sisters for their encouragement, patience and moral support.
ABSTRACT

The current study of epidemiology of bovine tropical theileriosis has been conducted in dairy farms in and around Nyala town, South Darfur. Cattle under different husbandry systems, different age groups, different types and sex were randomly selected. Ticks infesting cattle, blood smears, biopsy smears and serum samples were collected on a monthly basis for one year from June 2006 to May 2007. Cattle were mainly indigenous and cross (Friesian X indigenous) while the husbandry systems practiced were semi-intensive and extensive systems. Four age groups of cattle were selected, viz: group one < one year old, group two one year to < two years old, group three two years to three years old and group four older than three years.

A total of 1200 blood smears and lymph node biopsy smears (780 samples from indigenous and 420 samples from Friesian cross bred cattle) were collected. The prevalence rate of *Theileria* spp. piroplasms was 43 (3.6%) in the Giemsa’s stained blood smears (BS). Using indirect fluorescent antibody (IFA) test, out of 100 serum samples, 31 samples (31%) were positive for *T. annulata* antibodies.

Prevalence rate of *Theileria* spp. piroplasms and *T. annulata* antibodies were highest in December followed by November, and the lowest prevalence rate was in August followed by April. Although the disease occurred throughout the year, but it was significantly higher in winter followed by summer and the lowest rate was in autumn. The prevalence of the piroplasms among cross bred cattle was higher than
indigenous cattle. However, the highest prevalence of antibodies against *T. annulata* was recorded among indigenous cattle.

The highest prevalence *Theileria* spp. piroplasms 9 (5.1%) was recorded among calves 1 < 2 years old, and lowest prevalence 3 (2.4%) was recorded among calves aged < 1 year old. The highest prevalence of *T. annulata* antibodies, 20 (4.1%), was recorded among old cattle (> 3 years old) and the lowest, 2 (1.8%) was recorded among young calves (< 1 year old). There was a slightly high significant difference (*P* < 0.05) between the prevalence rate of piroplasms and antibodies in bulls and cows.

Extensive management systems posed a greater risk than semi intensive systems and semi extensive systems.

There were four genera and thirteen species of ticks found feeding on cattle. These included *Amblyomma variegatum*, *A. lepidum*, *Boophilus annulatus*, *B. decoloratus*, *Hyalomma anatolicum anatolicum*, *H. anatolicum excavatum*, *H. dromedarii*, *H. impeltatum*, *H. impressum*, *H. marginatum rufipes*, *H. truncatum*, *Rhipicephalus evertsi evertsi*, and *R. sanguineus*. High abundance of *H. dromedarii*, *H. m. rufipes*, *H. truncatum*, *H. impeltatum* was found and the probable role of these tick species in transmission of theileriosis is discussed with emphasis on *H. dromedarii*.

It is strongly recommended not to introduce livestock from Central Sudan into South Darfur without applying strict tick control measures. At the same time, there must be planned programme of tick control in the farms where *H. a. anatolicum* was found for the purpose of eradicating this newly introduced tick species. Spread of this species to other farms, other towns and probably cross the international borders must be prevented.
INTRODUCTION

Tick–borne diseases (TBDs) constitute a major constraint of livestock production and have a considerable economic impact (Jongejan and Uilenberg, 2004). Tropical theileriosis is a parasitic disease caused by the haemo–protozoan *Theileria annulata* that is transmitted to cattle by ixodid ticks of the genus *Hyalomma* (Uilenberg, 1981). Two stages in the life cycle of the parasite are responsible for the pathogenesis of the disease. These are schizont in mononuclear cells of the reticuloendothelial system and the intraerythrocytic piroplasm. The disease occurs in a wide zone of Africa, Southern Europe and a large part of Asia (Neitz, 1957; Dolan, 1989). In the Sudan tick fauna comprises over 70 species prevalent in diverse ecological zones (Hassan, 2003). TBDs are widespread causing substantial economic losses and are a constant threat to the development of animal wealth (FAO, 1983). Tropical theileriosis is the most important TBDs in Northern Sudan. Although 14% of cattle in this region are infected with *Theileria annulata* but later serological tests using indirect fluorescent antibody assay (IFA) and ELISA suggest that the prevalence is much greater (over 30%) (Salih *et al*., 2007).

Livestock population constitutes some 40 million cattle, 50 million sheep, 42.5 million goats, 4 million camels and 0.5 million horses in the Sudan (Anon, 2005). South Darfur State in Western Sudan, one of the richest states concerning of animal population that is estimated to be 11 million consisting of 3.9 million cattle, 3.6 million sheep, 2.9 million goats, 53500 donkeys, 30700 horses and 8700 camels (Anon, 2004). Livestock farming is an important component of the agricultural sector in the Sudan for the provision of animal-based food products and as a source of income for resource-poor farmers. Key factors that hamper efforts to enhance production of indigenous cattle in Northern Sudan include
among others, poor indigenous animals with respect to nutritional status and lack of disease control measures. In particular, *T. annulata* infection has been shown as the major obstacle to cross breeding programmes. In Northern Sudan, the economic losses due to *T. annulata* infection were estimated at 30% of expected profitability (Salih, *et al.*, 2007). Moreover, in Khartoum State alone, Latif (1994) reported that 85% of farms investigated experienced clinical theileriosis and mortalities of 22% and 30%, respectively in young calves and heifers. He estimated the total losses to be between US$ 4 to 6 million annually. Later, Siddig *et al.* (2003) found the total losses due to outbreaks of tropical theileriosis in dairy farms in Khartoum State to be US$ 62,320,98. They found that costs of tick control and chemotherapy are the most expensive components.

**Objectives of the study:**

1. To determine prevalence of tropical theileriosis in dairy farms in and around Nyala.
2. To establish distribution and seasonality of tropical theileriosis in and around Nyala.
3. To study prevalence and seasonality of tick species infesting cattle in and around this town.
4. To identify the tick species incriminated in transmission of tropical theileriosis in Nyala.
CHAPTER ONE
LITERATURE REVIEW

1.1. Definition of tropical theileriosis

Tropical theileriosis is a severe often fatal protozoan disease of cattle Dschunkowsky and Luhs (1904 cited in Soulsby, 1982). It is an infectious, virulent, inoculable, non contagious disease of cattle (Robinson, 1982). Several different names have been used for this disease such as tropical piroplasmosis, Mediterranean Coast fever and Egyptian fever. However the most widely used name is tropical theileriosis (Gubbels, 2000).

1.2. Aetiology

*Theileria annulata* is the causative agent of tropical theileriosis (Soulsby, 1982; FAO, 1983; El Neima, 1983; OIE, 2004).

1.3. Origin

The origin of *T. annulata* is the Asiatic buffalo (Uilenberg, 1981). Though the parasite causes mild infection in buffalo, it is highly pathogenic to cattle (Losos, 1986). In Africa, the origin of *T. annulata* parasite was not established until late 1950s. However, Lewis (1943) recognized the importance of the African buffalo (*Syncerus caffer*) in the epidemiology of theileriosis in Africa. (Neitz, 1955; 1957) proved that the African buffalo acts as a reservoir host for *Theileria* spp. infection to domestic animals. Furthermore, Young *et al.* (1973; 1978) showed that African buffalo acts as an important source of bovine theileriosis in many areas. They found that the carrier state of buffalo theileriasis might extend up to two years in the absence of re-infection.
1.4. Historical background

Dschunkowsky and Luhs (1904 cited in Soulsby, 1982) identified *Theileria annulata* in Caucasia and named it *Piroplasma annulaum*. It was placed in the genus (*Theileria* by Wenyon, 1926, it has the synonyms of *T. dispar* by Sergent *et al.*, 1924 and *T. turkestanica* by Oboldoueff and Galouzo, 1928, cited in Wikle, 1999).

1.5. Taxonomy

The taxonomic status of the genus *Theileria* has been controversial for many years. This revolved particularly around the question of whether a sexual cycle occurs in the tick vector or not. Ultrastructural studies and description of sexual forms of *Theileria* and *Babesia* led to the following classification according to the revision of the Committee on Systematic and Evolution of the Society of Protozoologists (CSESP) (Levine *et al.*, 1980).

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Apicomplexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>Sporozoea</td>
</tr>
<tr>
<td>Subclass</td>
<td>Piroplasmia</td>
</tr>
<tr>
<td>Order</td>
<td>Piroplasmida</td>
</tr>
<tr>
<td>Family</td>
<td>Theileriidae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Theileria</em></td>
</tr>
</tbody>
</table>

This classification is essentially a modification of the classification of Honigberg *et al.* (1964). The main objective of another classification scheme proposed by Mehlhorn and Schein (1984) is to separate all parasites with blood invading forms (Sub order Haemosporida) from gut-inhabiting parasites (Order Eucoccidiidae), believed that a close relationship exists between *Theileria* and plasmodia.
1.5.1. Family Theileriidae

Members of this family are round, ovoid, rod-like or irregular forms found in lymphocytes and erythrocytes. They do not produce pigment. They are transmitted by ixodid ticks to cattle, sheep, goats and other ruminants (Soulsby, 1982). Three genera are recognized in the family Theileriidae, namely: *Theileria*, *Haematoxenus* and *Cytaxzoon* with *Theileria* spp. According to Levine (1973) but Barnett (1977) considered the genera to be separate.

1.5.2. Genus *Theileria*

*Theileria* was first identified by (Theiler 1904 and was created as new genus by Bettencourt *et al*, 1907 cited in Soulsby, 1982). The organism multiplies by schizogony in lymphocytes and finally invades erythrocytes. It is transmitted by the ticks of the family Ixodidae.

1.5.3. *Theileria* species of cattle

Differentiation between several species of the genus *Theileria* depends on the morphology of piroplasms and schizonts, geographic distribution, epidemiological, serological, and biological, infectivity characterization, host and vector specificity, drug sensitivity, biological and molecular biological differences (Levine, 1971; Uilenberg, 1981; Irvin, 1987; Norval *et al.*, 1992; Seifert, 1996). There are six identified *Theileria* spp. that infect cattle, the two most pathogenic and economically important of which are *T. parva* and *T. annulata*, *T. taurotragi* and *T. mutans*. The latter two species generally cause no disease or mild disease while *T. velifera* is non pathogenic (OIE, 2004). These parasites are mainly found in Africa and overlap in their distribution complicating the epidemiology of theileriosis in cattle. The parasite group referred to as *T. sergenti / T. buffeli / T. orientalis* complex
is now thought to consist of two species; *T. sergenti* and *T. buffeli / T. orientalis*. The latter may be referred to as *T. buffeli* (Fujisaki *et al*., 1994).

1.6. Biology of *Theileria* species

The life cycle of *Theileria* spp. involves two components, the vertebrate host and the vector tick. Mehlhorn and Schein (1984) have made a full description of the life cycle of *Theileria* spp.

1.6.1. Life cycle of *Theileria* spp. in the vertebrate host

*Theileria* spp. use, successively, WBCs and RBCs for completion of their life cycle in the mammalian hosts. The sporozoite stage of the parasite is transmitted in the saliva of infected ticks as they feed. Sporozoites invade lymphocytes (and also monocytes in the case of *T. annulata*) and, within a few days develop into schizonts. The injection of the parasite commences from day 4 to 5 of tick attachment on the host. It is considered to be a period for maturation of the parasites within the tick salivary glands (Walker, 1990). Within 10 - 30 minutes after injection sporozoites invade different leukocytes sub types depending on the *Theileria* species. *T. annulata* sporozoites preferentially invade major histocompatibility complex (MHC) class II (B) and not class T cells (Glass *et al*., 1989; Spooner *et al*., 1989). The sporozoite initially resides in a vacuole, but the membrane of the vacuole disappears in 24 hours post infection leaving the parasite free in the cytoplasm and associated with the host's spindle apparatus (Tilney and Tilney, 1996 cited in Gubbels, 2000). Inside the leukocytes, the parasite develops into macroschizont that induces transformation and proliferation of the host cell (William and Dobbelaelene, 1993). In the case of *T. annulata*, cell cycles of parasite and host cell are not synchronized leading to the formation of multinucleated
parasites, the microschizonts (Shiels et al., 1997), which are equally divided over the daughter cells upon host cell division (Irvin et al., 1982). The microschizonts differentiate into merozoites, which increase in number. The lymphocyte ruptures and releases merozoites. The merozoites become free and penetrate erythrocytes 8 - 10 days post infection with *T. annulata* (Mehlhorn and Schein, 1984). Inside the red blood cells, the parasite develops into the piroplasm stage, and that depending on the species appears as red, comma or round shaped organisms. After penetrating the erythrocyte, the organisms are, at first covered by the membrane of the erythrocyte, but after the decay of the host membrane, they emerge freely into the plasma. Some of the very small (0.5 - 1.0 µm) merozoites change into ovoid forms which are only forms able to develop within the gut of a feeding tick (Mehlhorn and Schein, 1984).

Although the lymphoproliferation may have pathogenic consequences on the infected animals, it is possible that the anaemia caused by the invasion, the immune reactions and destruction of the erythrocytes constitute the major factor that determines the clinical picture (Julla, 1994). Piroplasms replicate inside the red blood cells. This process is thought to occur at a low frequency in *T. annulata* as replicative stages consisting of four piroplasms, visible as a cross with the apical sides in the middle (Malteze cross) but they are rare (Conrad et al., 1985). The piroplasm stage is infective to ticks.

1.6.2. Life cycle of *Theileria* spp. in the tick vector

When a clean tick (larva or nymph) feeds on an infected vertebrate host, the piroplasms – infected erythrocytes are ingested in the tick gut. Sexual reproduction of the parasite begins with the release of piroplasms in the gut of the tick resulting in the fusion of macro- and microgametes
The fusion of the two gametes forms a zygote, which is the only diploid stage in the parasite life cycle (Gauer et al., 1995). Subsequently, the parasite invades the epithelial cell of the tick gut and undergoes differentiation into motile kinetes (Shein, 1975) which migrate through to the haemolymph till they reach and invade the tick salivary glands (Mehlhorn and Sehein, 1977). The sporogony stage begins when a kinete invades the salivary gland acini usually type III acinus (Fawcett et al., 1982; Binnington et al., 1983) and becomes rounded (sporont in shape). The sporont then differentiates into sporoblasts. When the tick moults into the next stage (nymph or adult) and starts feeding on a vertebrate host, the sporoblasts become mature and form sporozoites (the infective stage). The sporozoites are injected into the host through the saliva of the tick during the feeding process.

1.7. Morphology

The piroplasm forms in the red blood cells more commonly occur as round, oval, or ring shaped (0.5 – 1.5 µm) forms. Rod, comma (1.6 µm) shapes and anaplasma - like organisms may also be found, the latter measuring 0.5 µm. Macroschizonts and microschizonts are found in the lymphocytes of the spleen and lymph nodes, that are known as schizonts (Koch's blue bodies), being circular or irregularly shaped structures about 8 µm in diameter, but vary from 2 to 12 µm or more. Two forms of schizonts are recognized, those which contain large chromatin granules (0.4- 2 µm in diameter with a mean of 1.0 µm) that are referred to as macroschizonts and produce macromerozoites (2-2.5 µm diameter). The other forms contain smaller chromatin granules (0.3- 0.8 µm in diameter with mean of 0.5 µm) and are referred to as microschizonts that produce micromerozoites (Soulsby, 1982; Seifeat, 1996).
1.8. Transmission

*Theileria annulata* is transmitted trans-stadially by several tick species of the genus *Hyalomma* (Levine, 1973; Purnell, 1978; Soulsby 1982; Uilenberg, 1981; Walker *et al*., 2003). The important vectors of *T. annulata* in the field are *H. anatolicum anatolicum* and *H. detritum* (Morel, 1989; Flach *et al*., 1995). *H. dromedarii* has been confirmed to be the main vector of tropical theileriosis in Mauritania, where neither *H. a. anatolicum nor H. detritum* occurs (d’Oliveria *et al*., 1997). *H. marginatum* and *H. lusitanicum* may be the main vectors for tropical theileriosis in parts of Spain (Habela *et al*., 1997; Viseras *et al*., 1999). However, several tick species of the genus *Hyalomma* are capable of transmitting *T. annulata* experimentally. These are *H. excavatum* (Daubney and Said, 1951), *H. dromedarii* (Mazlum, 1969), *H. marginatum rufipes* (Jongejan *et al*., 1983), and *Hyalomma impeltatum* (Walker *et al*., 2003). However, since the immature stages of the two species feed on rodents, hares and birds they are not likely to be vectors of theileriosis in the field (Jongejan *et al*., 1983; El Neima *et al*., 1983). *H. truncatum* plays no role as a vector in nature (Neitz, 1957).

1.8.1. Vector of tropical theileriosis in the Sudan

According to FAO (1983), El Neima *et al*., (1983), *H. a. anatolicum* is an efficient tick vector of *T. annulata* in the Sudan. *H. a. anatolicum* is the most economically important ixodid tick in the Sudan. *T. annulata* occurs within the range distribution of the vector *H. a. anatolicum*, that it is dominant north of Sennar (Latitude 14° 35' N) (FAO, 1983). Over some years, changes have occurred in distribution pattern of *H. a. anatolicum* in the Sudan (Jongejan *et al*., 1987). It was noticed that *H. a. anatolicum* has apparently extended distribution southwards from latitude 14° N to 13° 31' N for the first time in the Sudan (Ahmed, 1999).
*dromedarii*, which probably transmits *T. annulata* (Walker *et al*., 2003) ranges extensively north, west, east and central parts of the Sudan (Hoogstraal, 1956).

1.9. **Geographic distribution of tropical theileriosis**

1.9.1. **Distribution in the world**

Tropical theileriosis is prevalent in the South – Eastern Europe Southern Europe (Portugal, Spain, Italy, Bulgaria, Greece, and Turkey) the Near and Middle East, India, China, Central Asia and former USSR (OIE, 2004). It is also distributed in a wide belt of tropical and subtropical zones, Northern Africa including Mauritania and surroundings of the Nile into the central Sudan and Middle East to South of the Caucasus splitting round the Himalaya into south branch (Pakistan and India) and a north branch (Afghanistan, Southern Russia, North- Eastern China) (Dolan, 1989; d’Oliverira, 1997; El Metenawy, 2000).

The distribution is determined by the presence of the tick vectors. Therefore, the incidence of the disease has a seasonal occurrence, which is modulated by the ecology of its vectors (Pipano and Hadani, 1974; Pipano, 1976). Disease outbreaks occur mainly during the wet season as the ticks require a humid environment for their development (Flach and Ouhelli, 1992; Bouattour *et al*., 1996).

The prevalence in cattle is about 18% in Turkey (Tait and Hall, 1990), 25% in Mauritania (Jacquiet *et al*., 1994), 95% in Tunisia (Darghouth *et al*., 1996), 80% in Spain (Martin- Sanchez *et al*., 1999) 95% in Morocco (Kachani *et al*., 1996) and 76.5% in Saudi Arabia (El Metenawy, 2000). Sporadic cases have been encountered in various geographic regions all year round (Pipano, 1989).

1.9.2. **Distribution in the Sudan**

In the Sudan, tropical theileriosis was reported for the first time in 1908 as piroplasmosis by Weynon (1908 cited in Osman, 1989). Later,
the presence of *T. annulata* was confirmed (Anon, 1914- 1939). Thereafter, it was reported from several districts of the country and since then assumed a considerable economic importance (Shommein and Obeid, 1973; Shommein, 1976b; Latif, 1994; Gamal and El Hussein, 2003). *T. annulata* and *T. mutans* infections in cattle were routinely diagnosed in Khartoum State (Anon, 1923- 1924; Osman, 1976; FAO, 1983; Mohamed et al., 1996).

The disease occurs across a wide zone of the Sudan that extends from Northern Sudan, particularly in Khartoum, Gezira, Kassala up to Sennar in the South (FAO, 1983). Serological detection of *T. annulata* antibodies was reported in various parts of the country, Kurdoфан Darfour, Gadarif (Kheir et al., 1994), and in River Nile State (El Hussein et al., 1991) but the disease occurs within the range of distribution of the vector *H. a. anatolicum* (FAO, 1983; El Neima, 1983).

1.10. Host susceptibility

Domestic cattle, zebu, the water buffalo (*Bubalus bubalis*), and yak are susceptible (Dhar et al., 1973; Robinson, 1982). Attempts to infect sheep and goats with *T. annulata* produced only macro and microschizonts in the lymphocytes and no piroplasms developed (FAO, 1983). The pure bred of Friesian cattle and cross-bred with zebu cattle are particularly susceptible with mortality rates of 40–60% (Brown, 1990) reaching up to 80% in some areas (Gill et al., 1977; Hashemi- Fesharki, 1988).

In the Sudan under normal conditions, indigenous zebu cattle (*Bos indicus*) are normally resistant to TBDs, but they may be severely affected or even die if stressed (Osman, 1976). This is usually due to early exposure of these animals to natural infection and that survivors may either become carriers or immune (Shommein and Hagir, 1988).
However, exotic cattle (*Bos taurus*) are more susceptible to *Theileria* spp. infection than the local zebu cattle (Barnett *et al*., 1960; Prasad *et al*., 1972; Gill *et al*., 1977; FAO, 1983; Shammein and Hagir, 1988).

### 1.11. Incubation period

The incubation period varies from 4 to 14 days after attachment of the infected ticks to the host (Boulter and Hall, 2000). The disease may last as little as three to four days in the acute form or may be prolonged for about 20 days (Soulsby, 1982).

### 1.12. Clinical signs

The occurrence of the disease varies depending on the parasite strain, the host’s susceptibility and the quantity of sporozoites inoculated (Boulter and Hall, 2000). The severity of the disease is directly proportional to the initial inoculum of sporozoites injected (Cunningham, *et al*., 1974; Gill *et al*., 1977). The clinical signs include marked rise in body temperature, reaching 40 - 41.5°C followed by depression (Soulsby, 1982), enlargement of superficial lymph nodes namely, parotid prescapular and precrural (Robison, 1982), dyspnoea and frothy exudate from the nostrils (Boulter and Hall, 2000). Nasal and ocular discharges exophthalmia, lacrimation which may lead to corneal opacity or complete blindness and loss of appetite are often present (Norval *et al*., 1992) icteric and pale mucous membranes (Mira and Ralph, 1989). Anaemia jaundice and diarrhoea occur in the later stages of the disease. Yellow or Black coloured loose faeces and haemoglobinuria may be observed in some cases (Gill *et al*., 1977; Uilenberg, 1981; Hashemi – Fesharki, 1988; Sandhu *et al*., 1998). The animal becomes dull, recumbent and death may follow within two to three weeks of infection (Gill *et al*., 1977; Uilenberg, 1981). However, infection with *T. annulata* and other species
of bovine theileriosis could also result in rare syndromes which are characterized by signs of CNS involvement, development of skin lesions (Losses, 1986) or ocular lesions (Khalifa and Kadhim, 1967). Other symptoms, which accompany the disease, are inappetance ruminal atony, tachycardia and weakness (Norval et al., 1992).

1.13. Pathogenicity

The severity of infection depends upon virulence of the causative strain, the quantum of infection, the susceptibility status, age and health of the host (Gill et al., 1980; Irvin and Mawamchi, 1983; Preston et al., 1992).

The parasite replicates in both lymphocytes and erythrocytes causing severe lymphocytopenia, anaemia and jaundice. Norval et al. (1992) stated that *T. annulata* had a lymphoproliferative and a lymphodestructive phase as well as a haemolytic phase. The severity of the disease does not necessarily correspond with the extent of the parasitaemia. An animal may be seriously ill when less than 25% of the blood cells are infected with piroplasm stages, and less severe reactions may occur where 45% of the red blood cells are infected (Soulsby, 1982). For many years it was assumed that *T. annulata* resembled *T. parva* in causing a lymphoproliferative disease. However, *T. annulata*, unlike *T. parva*, infects and transforms myeloid cells, which then multiplies and metastasizes (Forsyth et al., 1999). It is not a lymphoproliferative disease resulting from the spread of multiplying schizont – infected T cells (Preston et al., 1999). The intraerythrocytic stages, as well as the schizonts, are pathogenic, and anaemia is a clinical consequence of infection (Trees, 1999). The cytokines and other factors like nitric oxide and matrix metalloproteinases, produced by the infected macrophages and
uninfected responding cells might contribute to pathogenesis (Preston et al., 1999).

1.14. Pathological features

The most prominent pathological features of tropical theileriosis are enlargement and swelling of the superficial and internal lymph glands and spleen, and lesions are generally in the form of ecchymotic and petechial haemorrhages of most of the internal organs, kidney infarcts, liver degeneration, lung oedema, serous and mucous membranes and ulcers on the abomasal mucosa which may extend to intestines (Irvin and Mwamochi, 1983). Heart infarcts in experimental infection are rarely seen in natural infection. Gum and dental erosions are occasionally seen in some cases. During the terminal stages there is pulmonary oedema, bilirubinurea, and bilirubinaemia (Yadav and Sharma, 1986; Mohammed et al., 1996; Boulter and Hall, 2000). The lung is emphysematous with frothy exudates on cut section with ulceration of the intestinal tracts (Salih et al., 2003).

1.15. Morbidity and mortality

Morbidity and mortality vary depending on the strain of parasite and the susceptibility of the host (OIE, 2004). Mortality varies considerably, being 10% in some areas and up to 90% in others (Soulsby, 1982).

1.16. Immunology

*Theileria annulata* are multistage parasites. All stages of theilerial parasites found in the mammalian host elicit humoral and cellular immune responses, which regulate host–parasite interaction and determine whether or not the host survives (Irvin, 1985). The infective
stage, sporozoite, when injected by ticks into host animal elicits a humoral immune response. On repeated exposure to ticks, these antibodies can reach a high titre to neutralize sporozoite infectivity. Sporozoites infect both B and T lymphocyte cells, multiply by schizogony and transform lymphocytes into lymphoblastoid cells. Macroschizonts synchronize their division with the host cell and thus remain protected from humoral response (Siddig, 1994). In general, cattle should be immunised in the first few months of life, and tick challenge under natural conditions reinforces the immunity (OIE, 2004). Some differences in antigenicity exist among strains, although there is an antigenic component common to all.

There is no cross immunity with other theilerial species, recovery from primary *T. annulata* infection results in the development of persistent carrier state (Ilhan *et al.*, 1998). Animals recovered from primary *T. annulata* infection are solidly immune against homologous and often heterologous challenge (Gill *et al.*, 1980). Indigenous cattle contract the disease early in life and they either die at calfhood or survive and became immune. This immunity is believed to be boosted annually by infected tick challenge (Osman, 1976; Shommein and Hagir, 1988). Treatment with immune serum or concentrated globulins did not protect recipient cattle against fatal East Coast fever (Muhammed *et al.*, 1974). However, serum from calves immune to *T. annulata* inhibited sporozoite – induced infection *in vitro* (Preston and Brown, 1985). Monoclonal antibodies (MABs) have been raised against the surface antigens of the sporozoite and many were found to neutralize sporozoite infectivity for normal peripheral blood leucocytes (PBL) *in vitro* (Williamson *et al.*, 1989). These MABs were used to identify the genes coding for the antigens recognized. The gene for the sporozoite surface antigen (SPAG-1) of *T. annulata* has been well characterized (Boulter *et al.*, 1994).
Serum from calves immune to *T. annulata* inhibited transformation of PBL into macroschizont infected lymphoblastoid cells (Perston and Brown, 1985). The effect of various recombinant cytokines on *in vitro* development of *T. annulata* and *T. parva* trophozoite – infected cells showed that tumour necrosis factor a (TNF- a), interferon y (IFN- y), interferon a (IFN- a), interleukin I (IL-I) interleukin 6(IL – 6) were inhibitory (Preston *et al.*, 1992). There is an increasing evidence that protective immunity against *T. annulata* depends upon the cooperation between innate (T cell independent) and adaptive/acquired (T cell depended) immune systems (Preston *et al.*, 1999). The innate immune responses include cytostatic macrophages and natural killer cells (NK). The cytostatic macrophages suppress the proliferation of schizont – infected cell (Preston and Brown, 1988) and produce tumour necrosis factor a (TNF- a) (Preston *et al.*, 1993) and nitric oxide (No) (Visser *et al.*, 1995). (NK) cells are now recognized as major effectors of innate resistance to protozoan parasites. The principal mechanism by which they control the growth of these pathogens is indirect, involving cytokine production rather than cytolytic activity (Scharton - Kersten and Sher, 1997). However, in *T. annulata* NK cells are believed to act by direct lysis of schizont – infected cells (Preston *et al.*, 1983). The adaptive immune responses include T cells cytotoxic for schizont – infected cells, antigen sensitized lymphocytes and CD4+ T cells (Preston *et al.*, 1999). The humoral immune response appears to be effective against the extracellular stages of *T. annulata* within the bovine host, the sporozoite the extracellular schizont and the merozoite (Innes, 1992).

Culture of a lymphoblastoid cell line infected with the macroschizont stage of *T. annulata* at 41°C included differentiation to the next stage, the merozoite and this development resulted in the loss of monoclonal antibody epitopes associated with the macroschizont stage
and the appearance of epitopes associated with the piroplasm (the intra-erythrocytic stages) (Glascodine et al., 1990).

1.17. Diagnostic techniques

Several conventional diagnostic procedures have been described to detect *Theileria* spp. (Irvin and Mwamachi, 1983; FAO, 1984; Young, 1987; Norval et al., 1992). Advanced laboratory techniques using molecular diagnostic tools have been developed (Stiller, 1990; Williamson et al., 1994; Gubbels et al., 1999).

1.17.1. Provisional diagnosis

This includes case history, clinical signs, postmortem findings and knowledge of disease and vector distribution (OIE, 2000). In the case of *T. annulata*, the disease is maintained exclusively in cattle and occurs within the range distribution of *H. a. anatolicum*. If the disease is diagnosed out of the vector area, some epidemiological factors should be considered such as recent movement of cattle from an enzootic area. Tentative diagnosis in enzootic areas depends on rise in body temperature, enlarged superficial lymph nodes and other clinical signs (FAO, 1983; El Neima, 1983). Differentiation between *T. annulata* and *T. parva* is not easy, and diagnosis is based on the evaluation of the enzootic parasitic condition in the area (Soulsby, 1982). In *T. parva*, the clinical syndrome and postmortem lesions are like *T. annulata*. However, *T. parva* occurs in areas where its vector *Rhipicephalus appendiculatus* is present. Usually *T. annulata* does not overlap with *T. parva* except may be in Southern Sudan a case that needs to be confirmed (Morzaria et al., 1981).

1.17.2. Laboratory diagnosis
Laboratory diagnosis of theileriosis is more accurate and confirmatory. *Theileria* spp. can be easily detected in mammalian hosts and the vector ticks (FAO, 1984).

1.17.2.1. Microscopic examination

Examination of stained tissue preparation to detect the parasite in mammalian host is by preparation of blood, lymph node biopsy and postmortem impression smears that are stained by Giemsa's stain (Norval *et al.*, 1992; Forsyth *et al.*, 1999). Microscopic examination shows *Theileria* spp. schizonts in white blood cells and piroplasms in erythrocytes. The morphological characteristics of the parasite may help to distinguish between the species. However, detection of piroplasms in blood films in the absence of clinical data and lymph node biopsy is of no value, as it may merely indicate that the animal is a carrier (Norval *et al.*, 1992). The schizont is a characteristic diagnostic feature of acute infections with *T. parva* and *T. annulata* in Giemsa's stained biopsy or tissue impression smears of lymph nodes, liver and spleen. The schizont is the pathogenic stage of *T. parva* and *T. annulata*. It initially causes a lymphoproliferative, and later a lymphodestructive disease. The piroplasms of *T. parva*, *T. annulata* and *T. mutans* are similar, but those of *T. annulata* and *T. mutans* are generally large and may be seen to divide. However, for practical purposes schizonts and piroplasms of different *Theileria* spp. are difficult to discriminate in Giemsa's stained smears. In *T. annulata* both the schizont and piroplasm stages may be pathogenic. Schizonts are scarce in the peripheral blood of acutely sick animals. The gross pathology caused by schizonts of *T. annulata* resembles that of *T. parva*, while anaemia and jaundice are features of the piroplasm pathology (OIE, 2004). The gross pathology caused by *T. annulata* is also of value in clinical diagnosis. Parasitized cells may be
found in impression smears from all tissues (Forsyth et al., 1999). Piroplasms of most of Theileria spp. may persist for months or years in recovered animals, and may be detected intermittently in subsequent examinations. However, negative results of microscopic examination of blood films do not exclude latent infection (OIE, 2004).

1.17.2.2. Serological tests

Antibody detection depends on antigen antibody reaction. Serum antibodies appear 2 to 4 weeks after infection, reach high titres during and shortly after infection subsides. They stabilize and decline to variable levels after 24 to 26 weeks. Antibodies against TBDs can be detected by different serological tests (Burridge and Kimber, 1973).

1.17.2.2.1. Indirect fluorescent antibody test

The indirect fluorescent antibody (IFA) test is the most widely used serum antibody assay for Theileria spp. (Burridge, 1971; Burridge and Kimber, 1972; Burridge et al., 1974; Goodeeris et al., 1982; OIE, 2004; 2005). The IFA test with either schizont antigen prepared from T. annulata infected lymphoblastoid tissue culture cell lines or piroplasm antigen obtained from highly parasitaemic animals has been used (Pipano and Cahana, 1969). Both schizont and piroplasm antigens may be prepared on slides or in suspension and preserved by freezing at less or equal to –20°C except in the case of the piroplasm suspension, which is stored at 4°C. Test sera are diluted with bovine lymphocyte lysate and incubated with the antigen in suspension, and anti-bovine immunoglobulin conjugate is then added. Using the test as described, the fluorescence is specific for the causative agent. IFA test is sensitive fairly specific, usually easy to perform, and useful for identifying herds that contain carriers of T. annulata, but is not always sufficiently sensitive to
detect all infected individuals. Both schizonts and merozoite IFA antigens have failed to detect antibody in some animals carrying patent infection with piroplasms (Darghouth et al., 1996). However, because of cross reactions between some *Theileria* spp. the test has a limitation for large scale surveys in areas where these species overlap (Burridge et al., 1974; Kiltz et al., 1986; OIE, 2004). IFA test has been widely applied in epidemiological studies in different countries of Africa including Sudan (FAO, 1983; Salih, 2003).

### 1.17.2.2.2. The enzyme linked immunosorbent assay

Serological tests based on the enzyme linked immunosorbent assays (ELISA) are being increasingl y used for detection of parasite-specific antibodies, antigens and immune complexes (Dolan, 1989; Tait and Hall, 1990). ELISAs for serodiagnosis have the advantage above IFA test in that they are less laborious, relatively easy to perform and a large number of animals can be tested in quite a short time, and hence are more useful in epidemiological studies than the IFA test. Both schizont and piroplasm lysates have been used as antigens (Manuja et al., 2000). ELISAs have been shown to detect antibodies for a longer period of time than the IFA (Kachani et al., 1992a), and have been successfully adopted for the detection of antibodies to *T. annulata* (Gray et al., 1980). These ELISAs provide higher sensitivity and specificity than the IFA tests (Morzaria et al., 1999; Musoke et al., 1994). ELISA based on schizont antigen functioned well in case of enrichment of the cellular fraction with the soluble fraction (Manuja et al., 2000). ELISA based on piroplasm lysates resulted in a sensitivity of 94% and a specificity of 100% (Kachani et al., 1992b; 1996). The field performance of this ELISA in Morocco was promising and the test was shown to be suitable to study *T. annulata* epidemiology (Flach et al., 1995). In spite of relatively good
performance of crude antigens, the disadvantages of this approach are the requirement of experimental animals for piroplasm antigen production and batch-to-batch variation, besides, the need to standardize the antigen derived from crude parasite material (Gubbels et al., 2000). These problems have been circumvented by the use of several recombinant parasite antigens in ELISA. Two ELISAs based on recombinant proteins have been developed. Firstly, the sporozoite antigen (SPAG-1) has been demonstrated to detect exposure to \textit{T. annulata}. However, sensitivity and specificity of this ELISA has not been evaluated (Boulter et al., 1998). Secondly, \textit{T. annulata} merozoite surface (Tams-1) rhobtry antigen, which has been tested as a candidate antigen for a diagnostic ELISA (Ilhan et al., 1998) Tams 1, the major merozoite/piroplasm surface antigen of \textit{T. annulata}, has the potential to be a component of a diagnostic ELISA and to be included in a recombinant subunit vaccine. However, the observation that this antigen displays diversity could constrain these applications (Katzer et al., 1998). Nevertheless, on indirect Tams 1 ELISA for diagnosis of \textit{T. annulata} infections in cattle have been introduced (Gubbels et al., 2000). Moreover, \textit{T. annulata} surface protein (TaSP) has been characterized and proposed for use in diagnostic tests and subunit vaccines (Schnittger et al., 2002b).

\textbf{1.17.2.3. Other serological tests}

Other serological tests which have been developed, but not widely used, are indirect haemagglutination. The complement fixation test and capillary agglutination test (Duffus and Wanger, 1974; Brown et al., 1990).

\textbf{1.17.2.3. Molecular biology diagnosis}
Development of molecular biology has made accurate tools available for detection of parasite molecules. These techniques are important in veterinary diagnostic parasitology. Since antibodies can remain circulating for sometime after the parasite has been cleared from the animals, serological assays do not always provide information about the actual presence of the parasite (Zarlenga and Higgins, 2000). The polymerase chain reaction (PCR) is the most commonly used tool. Target sequences are DNA and ribosomal RNA as well as fragments derived from random amplified polymorphic DNA (RAPD) (Comes et al., 1996). The first PCR application for *T. annulata* diagnosis in the bovine host was based on the *Tams*-1 gene (d'OliverIa et al., 1995). Sensitivity of 10^6% *T. annulata* parasitaemia was achieved by combining RCR with Southern blot hybridization enabling detection during the carrier state. Moreover, it was also possible to detect *T. annulata* in *Hyalomma* ticks using the same technique (d'Oliveria et al., 1997b).

In addition, two integrated approaches were developed to detect several *Theileria* or *Babesia* spp. in one assay (Figueroa et al., 1993; Allsopp et al., 1993). Using these approaches multiple species can be detected in one assay without performing independent PCR for each parasite (Gubbels et al., 1999). One such technique, namely reverse line blot hybridization, combines a genus specific PCR with hybridization to membrane bound type/species specific oligonucleotides for differential detection (Gubbels et al., 1999). A similar approach to detect and differentiate all known *Theileria* and *Babesia* spp. of importance in cattle in the subtropics on the basis of their differences in 18S subunit rRNA gene sequences has been developed (Gubbels et al., 1999).

1.18. Ticks of the Sudan and economically impact of ticks
About 68 ixodid tick species under eleven genera infesting a variety of animal species have been recorded in the Sudan (Hoogstraal, 1956; Osman, 1978; Jonjegan et al., 1987). The distribution of certain species is restricted to specific ecological zone (Punyua et al., 1991). According to Hoogstraal (1956), Karrar et al. (1963), Osman et al. (1982) ticks infesting livestock in the Sudan are mainly *H. a. anatolicum*, *H. dromedarii*, *H. marginatum rufipes*, *H. impressum*, *H. impeltatum*, *H. truncatum*, *Rhipicephalus evertsi evertsi*, *R. sanguineus* group, *R. simus* group, *R. appendiculatus*, *Boophilus decoloratus*, *B. annulatus* *Amblyomma lepidum* and *A. variegatum*.

Ticks and TBDs are economically important parasites, with adverse effects on animal production. They cause irritation to animals, damage to udder and teats, cause damage to hide and skin and transmit bacterial, viral, rickettsial and protozoan diseases (FAO, 1983; Kettle, 1995). About 1600 million of livestock suffer from tick infestation throughout the world (Bram, 1975).

### 1.18.1. Economic importance of tropical theileriosis

The economic impacts of livestock diseases are calculated in form of animal loss, decline in productivity, retarded growth, failure of conception, abortions, sterility and disease control and management costs (Gamal and El Hussein, 2003). The disease results in heavy economic losses due to loss of weight, diminution of milk yield and growth retention (Niyongabo, 1987). The disease is highly fatal especially in high producing improved cattle (El Hussein et al., 1991).

In the Sudan, tropical theileriosis represents a major threat to livestock causing severe and often a lethal disease to foreign breeds and their crosses, while local breeds show considerable resistance (Shommein, 1976a; FAO, 1983). With the advent of large scale cross
breeding programmes for the promotion of milk production, the disease assumes serious dimensions. Over 80% mortality rate was observed among exotic cattle and their crosses in the Sudan FAO (1983). The losses due to theileriosis according to Latif (1994) are of direct losses which include mortality, costs of drug used for treatment, costs of control of ticks and losses due to morbidity where sick animals produce less milk, recovered animals fail to improve for 18 months, abortion and cows remain infertile for up to 12 months and 5 - 10% of the affected calves remain stunted. The indirect losses are that recovered animals become *Theileria* spp. carriers and act as sources of infection to other cattle and the disease acts as constraints to animal production and livestock development. He also reported the government losses which include loss of foreign currency for the importation of cattle, drugs and acaricides, money spent annually on research training, and importation of milk.

In the Sudan, no accurate economic evaluations have been made. However, economic losses due to tropical theileriosis have been estimated to be between US$ 4 and 6 million in Khartoum State (Latif, 1994).

**1.19. Treatment of tropical theileriosis**

A variety of drugs has been used with varying success against theileriosis. Long acting tetracyclines are effective only when they are concurrently administered with the infection (Brown *et al.*, 1977). Primaquine (Primaquine phosphate) is only effective against the piroplasm stage in the erythrocytes (Brown, 1990). Synthetic naphthoquinine from which parvaquone was derived (Clexon, Parvexon) are effective against the schizont stage (McHardy *et al.*, 1985). Parvaquone was followed by another naphthoquinine. Buparvaquone (Butalex), which is effective against both schizont and piroplasm stages
of *Theileria* spp. (McHardy *et al*., 1985) whereas Butalex proved most effective. Parvexon was unable to eliminate the parasite completely *in vitro* (Rintelen *et al*., 1990).

Although the efficacy of the drugs buparvaquone and parvaquone is well established, animals can still die from peracute tropical theileriosis when treatment is often too late, due to lack of prompt diagnosis (Hashemi–Fesharki, 1988; Dhar *et al*., 1988). In addition, for many developing countries the cost of chemotherapy, involving also the cost of diagnosis, is considered to be very high in relation to economics and systems of livestock rearing.

1.20. Control measures of tropical theileriosis

Several methodologies are currently available for the control of theileriosis (Young *et al*., 1988). Each of these methods has a role to play and effective control is most efficiently and economically achievable by utilizing these methods is a balanced and integrated way to provide an economically viable and sustainable system, robust to breakdowns of individual control methods (Irvin, 1986; Perry and Young, 1995). The philosophy suggested is that series of complementary control measures should be introduced which will allow cattle of high potential productivity to be kept successfully in areas endemic or epidemic for TBDs (Young *et al*., 1988).

1.20.1. Management

Management involves restriction of livestock movement and implementation of quarantine measures to keep the tick–free and disease susceptible cattle apart from the tick-infested and infected animals. This is also to ensure that ticks and the pathogens they transmit are not transported to ecologically suitable but currently uninfected areas (Norval
et al., 1992). The overall objective is to ensure that the target population is entirely free of the disease or there is endemic stability (Brown, 1990).

1.20.2. Tick control

Tick control is one of the most important factors influencing the epidemiology of bovine theileriosis. It has been achieved mainly by application of acaricides by dipping or spraying. Dipping is considered the most effective method for acaricide application (Norval, 1989). Earlier, acaricides such as arsenic and chlorinated hydrocarbons, known for high toxicity, have been replaced by organophosphates, amidines synthetic pyrethroids and ivermectins, (de Castro, 1997). Later, major advances have been made in the development of novel methods and strategies for the control of ticks. Slow–release rumen boluses, “pour–on” and “spot–on” formulations and acaricides- impregnated ear–tags, neck bands and tail bands have been used (Pegram et al., 1993; de Castro, 1997). A mechanical applicator has been developed and applied successfully in tick control on eland (Taurotragus oryx) and buffalo (Syncerus caffer) in Zimbabwe (Duncan and Monks, 1992). Despite these novel methods, disadvantages still exist including high costs in foreign exchange of acaricides, contamination of the environment of food with toxic residues and acaricide resistance of the ticks (Dolan, 1989; Frish, 1999). Intensive use of acaricides can also result in the breakdown of endemic stability and leave the cattle more susceptible to infection (Norval et al., 1992). This has encouraged the search for less expensive and environmentally more acceptable tick control alternatives.

The use of natural enemies such as predators, parasitoids and pathogens has been examined (Mwangi et al., 1991a; 1991b; Hassan et al., 1991; Kocan et al., 1998; Samish, 2000; Samish and Alekseev, 2001). The use of tick repellents on livestock is limited. However, several
studies have indicated the potential benefits of using tick–repellent grasses and plants such as *Melinis minutefora*, *Gynandropsis gynandra*, *Stylosanthes* species and *Cassia absus* (Kaaya, 1992; Malonza et al., 1992; Mwangi et al., 1995; Kaaya, 2000). Application of tick attractants such as pheromones, in combination with acaricides has also been considered (Norval et al., 1991; 1996). Pasture spelling and withdrawal of domestic hosts are used to control tick species (Kaaya, 1992).

Tick control strategies vary according to the breed, type of cattle and the management system. The three main options are i) intensive dipping or spray for purebred *Bos taurus* dairy cattle, ii) strategic tick control for crossbred cattle, and iii) minimum or threshold tick control under conditions of enzootic stability (Pegram et al., 1993). Chemical acaricides tick control has been widely applied to prevent tropical theileriosis and other TBDs (Jongejan and Uilenberg, 1994).

Most chemical acaricides are toxic, leave residues in meat and milk and cause environmental pollution (Drummond, 1976). The extensive use of chemical acaricides leads to the development of resistance (Wharton, 1976). Moreover, they are costly and constitute a major constraint on the development of livestock industry, particularly in many African countries (Young et al., 1988).

### 1.20.3. Immunization

The observation that animals recovered from infection with *T. annulata* are solidly immune to subsequent challenge, at least with the homologous stock, has encouraged research on vaccine development (Burridge et al., 1972). The most important and effective control method against *T. annulata* is the use of attenuated cell line vaccine (Brown, 1983).

#### 1.20.3.1. Infection and treatment
Immunization against tropical theileriosis by the infection-treatment method has been successfully used but with limited field application (Pipano, 1981). In this method, infections were induced by inoculation of ground-up tick supernate. Effective treatment must be applied early in the course of the disease (Dolan et al., 1984; Chema et al., 1986). Such prompt action will require a good diagnostic ability and high standards of management by the farmer as well as proximity to effective veterinary services. Antitherilerial drugs should be available on a long term basis and the treatment should be affordable. The treated animals show mild reactions and develop a solid immunity to homologous challenge (Gill et al., 1978).

1.20.3.2. Cell culture schizont vaccine

The successful *in vitro* cultivation of *T. annulata* macroschizonts by Tsur (1945) was an achievement that served as the basis for developing a schizont vaccine against *T. annulata*. Successful cell culture vaccines have been developed in many countries e.g. Iran (Hashemi-Fesharki, 1988), Israel (Pipano, 1989), Russia (Stepanova and Zablotski, 1989), China (Wenshum and Hong, 1994), Morocco (Ouhelli et al., 1997), Turkey (Sayin et al., 1997) and Spain (Viseras et al., 1998). Attenuation of schizonts is achieved by prolonged *in vitro* passaging of schizonts infected cultures (Pipano, 1989). A culture is defined attenuated when, upon inoculation in susceptible cattle, neither schizonts nor piroplasms are detected as well as no development of clinical signs. The duration of immunity varies from six months (Ilhan et al., 1998) to 3 - 5 years (Pipano, 1989). However, application of a tissue culture vaccine has a number of drawbacks. It is not clear whether a carrier state is produced or whether the parasite is cleaned (Ilhan et al., 1998). Also testing the long culturing period needed to produce a vaccine and requirement of a
cold chain for delivery of the vaccine are limitations that make the vaccine very expensive. Furthermore, although cattle are protected by the attenuated cell line vaccine, it does not prevent the erythrocytic stage to develop after animals are challenged (Pipano, 1981). In addition, it may also be possible to transfer other pathogens by the use of such live vaccines. Many of these drawbacks could be overcome by vaccination with defined, recombinant antigens that would provide better stability and quality control (Trees, 1999).

1.20.3.3. Recombinant vaccines

To circumvent the drawbacks of the tissue culture vaccine subunit–vaccines composed of several recombinant antigens have been developed (Boulter and Hall, 2000). Most likely, one antigen is not capable of inducing 100% protection. Therefore, several antigens of different life cycle stages should be included to cover as much as possible each developmental stage of the parasite. The first component of such a vaccine is the sporozoite derived SPAG-1 antigen, which blocks sporozoite penetration of bovine peripheral blood mononuclear cell *in vitro* (Boulter *et al*., 1994). Experiments with a second vaccine component were conducted with two allelic forms of the merozoite/piroplasm *Tams* 1 antigen. *Tams* 1-1 and *Tams* 1-2 were delivered in lSCOMs, as naked DNA plasmids encoding *Tams* 1-1 and *Tams* 1-2 or were expressed in recombinant *Salmonella typhimurium* aroA vaccine strains (d'Oliveira *et al*., 1996; 1997a). Challenge infection of blood containing 30% parasitaemia resulted in mild reaction in the lSCOMs group and moderate reaction in the DNA – vaccinated animals.

As the schizont vaccine confers the most efficient protection inclusion of schizont derived antigens is a prerequisite for a functional sub-unit vaccine. Trials for schizont derived *T. annulata* antigens have
not yet been, but one candidate has been identified, sporozoite and macro schizont antigen (Spm2), which is expressed in all bovine stages (Knight et al., 1998). Later, Tasp has been identified as another candidate. This gene is present as a single copy within the parasite genome and transcribed in the sporozoite and schizont stages, codes for a protein of about 315 amino acids and has a predicted molecular weight of 36 kDa (Schnittger et al., 2002a).

CHAPTER TWO
MATERIALS AND METHODS

2.1. The study area
The present study was carried out in and around Nyala town, capital of South Darfur State which is located in the south west of the Sudan. The state covers 139800 km² between the latitude 8° 30’ N to 13° 30’ N longitude 23° 15’ E to 28° 53’ E, and altitude 655 m above sea level (Map 1). The climate of South Darfur State is of Savanna type with low rainfall (300 - 800 mm) in the northern parts to the clay high rainfall (rainfall 400 - 1300 mm) woodland in the southern parts where the lowland is covered with broad leaves wooded savanna trees and grass. There are three distinct seasons in the State with summer season starting in March and ends in May. Early rainfall starts in May but the regular is from June to October. Winter covers the period from November to February.

Meteorological data obtained from Nyala Airport Station (Table 1) shows that the monthly mean minimum and mean maximum ambient temperatures in 2006 and 2007 were 13.9°C (December 2006), 13.5°C (January 2007) and 38.3°C (May 2006), 39.5°C (April 2007) respectively. The monthly mean minimum relative humidity (10%) was recorded in April 2006 while the mean maximum monthly relative humidity (84%) was in August 2006. The highest total rainfall (140.7 mm) was recorded in August 2006.

A dense vegetation cover in the state includes different species of grasses such as Cynodon dactylon, Dactyloctenium aegyptium, Sorghum sudanensis, and Ipomoea cardiosepal. Other Acacia spp. include Ficus spp., Tamarindus indica, Cordia abssinica, Khaya senegalensis, Ziziphus Table (1). The meteorological data of Nyala area during the study in the period 2006-2007.

<table>
<thead>
<tr>
<th>Year</th>
<th>Months</th>
<th>Temperature (°C)</th>
<th>Relative humidity (%)</th>
<th>Total rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>January</td>
<td>33.3</td>
<td>18.7</td>
<td>21</td>
</tr>
<tr>
<td>2006</td>
<td>February</td>
<td>35.3</td>
<td>19.8</td>
<td>15</td>
</tr>
</tbody>
</table>
spp., *Balanites aegyptiaca*, *Borassus aethiopum*, *Piliostigma reticulatum*...etc (Musa et al., 1996). Such grasses and trees modulate the environmental conditions by their effects on microclimate (temperature and humidity). Cattle, camels, sheep and goats from near by localities and even from Chad spend the dry season in these areas to make use of the grasses, crop residues, fodder trees surface water and shade. All these circumstances are conducive for ticks to reproduce and survive long and transmit diseases (Musa et al., 1996).
2.2. Animal husbandry types

Three types of animal husbandry and breeding systems are practiced for livestock production around Nyala. These include semi-intensive, semi-extensive and extensive systems of husbandry. The semi-intensive system started recently in the state, mainly in Nyala town and in other few main towns. Cattle types in these farms of *Bos taurus* (Friesian) crossed with *Bos indicus* (Kenana and Butana) that had been introduced from central Sudan. Calves and bulls are always maintained in the farm while adult cattle graze nearly provided with concentrates. These farms are mainly located in south eastern parts of Nyala (Museah area) and a few of them are in the different sites around Nyala (e.g. Domaya, Al geer Al masanea). Nine farms were selected in this system.

The other system of husbandry are managed under the semi-extensive system where animals are kept in the back yards, inside or close to the homesteads, where cattle graze on natural pasture early in the morning, but are returned to the farm at the end of the morning, while the calves tethered in the homesteads. Sixteen farms were selected in this study. But in the extensive traditional system animals graze on natural pasture early in the morning, but are returned the evening. In this systems the local types (zebu cattle) dominated by Baggara type (a few of Kenana and Butana types). Most of these farms are located in southern parts of Nyala town (Um dam area) Eastern parts (Algabal area) and South eastern of Nyala (Belial area).

2.3. Selection of cattle for sampling

Random sampling was adopted for this study. Four age groups of cattle were selected. These were group one that was less than one year old (123 calves), group two of one year to less than two years old (178 calves), group three of two years to three years old (360 heifers) and
group four (539 adult) which were older than three years. A total of 1200 cattle in the four groups (780 cattle of locals breed and 420 cattle of local crosses with Friesian) were sampled from each farm for blood smears lymph node biopsies, and serum samples. Sampling was carried out at monthly intervals for a period of 12 months from June 2006 to May 2007. Cattle were sampled in seven sites of Nyala. These were Museah (289 cattle), Al gabal (142 cattle), Domaya (95 cattle) Belial (165 cattle) Al geer (75 cattle), Um dam (352 cattle) and Al masanea (82 cattle). These farms were selected from semi-intensive type of husbandry (9 farms), semi-extensive system (16 farms) and extensive system (10 farms).

2.4. Tick collection

Whole body tick collection was carried out from 1200 cattle. All visible ticks were collected after each animal was cast and restrained. The collected ticks were preserved in 70% ethyl alcohol. On each farm the cattle population was divided in three age groups of cattle were selected. These were group one that was less than two years old, group two two to three years old and group three more than three years old. Cattle type husbandary type, locality, farm and date of collection were recorded for each sample. Ticks were identified under a dissecting microscope.
Map (1) Nyala town indicating sites (1 – 7) from where samples were collected (modified by researcher from webe site of HIC)
according to Hoogstraal (1956), Walker et al. (2001), Walker et al. (2003) methods. The identified adult ticks of each animal were recorded according to sex and species.

2.5. Sampling of blood

2.5.1. Sampling of whole blood

Whole blood for haematological studies was collected using disposable syringes with needles from the jugular vein into clean dry test tubes containing Ethylene Diamine Tetra Acetic acid (EDTA). The skin at the punctured area was cleaned with a piece of cotton soaked in 70% ethanol. Another volume of whole blood (10 ml each) for collection of serum was collected in dry test tubes that do not contain EDTA.

2.5.2. Sampling for blood smears

A drop of blood (1200 blood samples) was taken on a clean grease free microscope glass slide, spread by the edge of another slide at an acute angle, air dried and fixed by absolute methanol for 2–3 minutes. The slide was labeled in pencil indicating animal number, farm and date of collection. The fixed smears were stained by 10% Giemsa's stains (one ml of stock Giemsa's stain was diluted in 9 ml of distilled water or phosphate buffered saline - PBS) for 30 minutes. Excess stain was removed by PBS or distilled water. The slides were air dried and examined under a compound microscope, using oil immersion objective × 100. Each slide was examined covering about 50 microscope fields for detection of *Theileria* spp piroplasms.
2.5.3. Sampling for lymph node biopsy smears

Lymph node biopsy smears were prepared by aspiration of 73 head of cattle with enlarged superficial lymph nodes (prescapular lymph nodes). The enlarged lymph nodes were immobilized between two fingers and punctured using G 18 needles. The aspirated samples were put on a glass slide, spread by the edge of the same needle, air dried, fixed in absolute methanol for 2-3 minutes and stained by 10% Giemsa’s stain. They were examined under a compound microscope, using oil immersion objective × 100 for detection of schizonts.

2.6. Haematological tests

All the haematological parameters from the 22 positive samples for tachizont *Theileria annulata* were schizonts performed as described by Schalm (1965).

2.6.1 Determination of packed cell volume

The packed cell volume (PCV) of erythrocytes was determined by the microhaematocrit method using microhaematocrit centrifuge. Capillary tubes (75 mm × 1.0 mm) were filled by capillarity action from whole blood sample with EDTA up to ¾ the length of the tubes. The outside of each tube was cleaned with absorbent gauze and one end was sealed off with special clay (cristaseal). The filled tubes were centrifuged at 1200 rpm for 5 minutes. The PCV was measured as percentage of whole blood using the microhaematocrit reader (Howksley and Sens Itd England).
2.6.2. Determination of haemoglobin concentration

Haemoglobin (Hb) concentration was measured by the cyanome-haemoglobin by Drabkin solution (0.2 gm potassium cyanide, 0.2 gm potassium ferric cyanide and 1.0 gm sodium bicarbonate per one litre of distilled water). A volume of 0.02 µl of EDTA treated whole blood avoiding vigorous agitation and after wiping the tip of the pipette was added to 4 ml of Drabkin solution (at dilution of 1/200), mixed well and allowed to stand for 10 minutes to ensure the completion of the reaction. The test solution was compared with the standard and reagent blank at 450 nm in haemoglobin–metre (CIBA Corning, 950 Hb metre, England). It was read in a colorimeter with a tube of Drabkins solution as a blank and the standard solution of cyanmethaemolobin. The Hb concentration was calculated by the use of the standard Hb solution and results were expressed in g/dl.

2.6.3. Red blood cells count

An erythrocyte pipette was filled with EDTA – treated whole blood to the point of 0.5 and then to 101 with Hymen’s solution (0.5 gm) mercuric chloride (0.5 gm sodium sulphate and 1.0 gm sodium chloride dissolved in 200 ml distilled water) and mixed well by inversion. The first drop was discarded, then an improved Neubauer haematocytometer (Hawskey and Sons Ltd. England) was filled and red blood cells (RBCs) were counted as described by Baker and Silverton (1980) using the following formula (expressed in million/ microlitre).

Total RBCs count = number of cells counted × 5 × 10 × 200.

Where:

5 is the correction factor as only 0.2 square mm area was counted.
10 is the correction factor as the depth of the dilution is 0.1 mm.
200 is the correction factor as the blood dilution is 1/200.

2.6.4. White blood cells count

The leukocyte pipette was filled with EDTA- treated whole blood to the point of 0.5 and then to 11 with Turk's solution (1% glacial acetic acid, tinged with gentian violet) mixed and the first drop was discarded. The Neubauer was filled and the white blood cells (WBCs) were counted as described by Baker and Silverton (1980) using the following formula (expressed in thousands / microlitre).

Total leukocytes count = number of cells counted \( \times 10 \times 20 \times 4 \).

Where:

10 is the correction factor as the fluid in each primary square is 1/10 cm\(^3\).
20 is the diluting factor as the blood dilution is 1/20.
4 is the correction as four primary squares were counted.

2.6.5. The erythrocyte indices

2.6.5.1. Mean corpuscular haemoglobin

Mean corpuscular haemoglobin (MCH) was calculated from the Hb and RBCs as follows:

\[
MCH \text{ (Pg)} = \frac{\text{Hb (g/dl)}}{\text{RBCs (10}^6/\mu\text{l})} \times 100
\]
2.6.5.2. Mean corpuscular volume

Mean corpuscular volume (MCV), was calculated from the PCV and RBCs count as follows:

$$MCV (fl) = \frac{PCV \times 10^6}{RBCs (10^6/\mu l)} \times 100$$

2.6.5.3. Mean corpuscular haemoglobin concentration

The mean corpuscular haemoglobin concentration (MCHC) was calculated from the average concentration of haemoglobin in a given volume of packed cell volume as follows:

$$MCHC (g/dl) = \frac{Hb (g/dl)}{PCV\%} \times 100$$

2.7. Serum collection

Whole blood for serum was withdrawn from the jugular vein. Plain glass vacutainers free from anticoagulant, with a tube holder were used. The vacutainer tubes were labelled indicating locality, date and animal number prior to arranging on a rack. The blood was allowed to clot for more than three hours at room temperature away from direct sunlight. The tubes were, then, kept overnight in a refrigerator at +4°C. Serum was separated after centrifugation at 1500 rpm for 10 minutes. Each serum sample was collected using a different sterile Pasteur pipette for each sample and transferred into eppendorf tubes. These were labelled indicating locality, farm, date and animal number then stored at –20°C unit used.

2.8. Indirect fluorescent antibody test

The indirect fluorescent antibody (IFA) test was performed in this survey. This test is the most widely serological test used for T. annulata
antibodies detection (OIE, 2005). The IFA test protocol was described by Burridge et al. (1974), FAO (1984) and Darghouth et al. (1996).

2.8.1. Phosphate buffered saline

One tablet of phosphate buffered saline (PBS) Dulbecco A (Oxoid, England) pH 7.2 was dissolved in 1000 ml distilled deionised water and autoclaved at 15 lbs/sq in pressure for 15 minutes.

2.8.2. Labelled conjugate

Labelled conjugate is available commercially as lyophilised powder usually rabbit anti- bovine IgG (Nordic or Miles) stored at (+4ºC). To make up and reconstitute in 2 ml sterile distilled water it was mixed well, centrifuged to precipitate insoluble lumps divided in 50 µl aliquots in 0.5 ml micro-capped centrifuge tubes, and stored at –20ºC in the dark bottles. When needed, these aliquots were thawed and diluted to the required strength (usually 1/80 by adding 3.95 ml PBS).

2.8.3. Mountant

A volume of 50 mM Tris buffer pH 9.2 was made up 50 ml and the pH was adjusted with NHCl (121 mg /l = 6.05 mg in 50 ml). A volume of 10 ml was mixed with 20 ml glycerol to make a 66% solution of glycerol in Tris buffer and stored at 4ºC.

2.8.4. Control sera

The positive control serum (C+) for *T. annulata* was kindly provided by Dr. Hashemi-Fesharki, Head Department of Protozology and Protozoa vaccine, Razzi Institute, Iran. The serum collected prior to the infection with *T. annulata* was used as a negative control (C-).

2.8.5. Preparation of antigen
The antigen slides were prepared from *T. annulata* Soba strain infected cell lines isolated from a pure Friesian 4 month old calf.

### 2.8.5.1. Culture techniques

According to the method described by Brown (1980), when the parasiteaemia in the Friesian calf exceeded 10%, 10 ml of the blood from jugular vein and biopsy tissue materials were aseptically aspirated from the prescapular lymph node. The cell suspension was centrifuged at 400 g for 5 minutes. The supernatant was discarded and the cell pellets were resuspended in 20 ml of growth media containing Glasgow MEM that contains fungizone 5 μg/ml, penicillin 200 IU/ml, streptomycin 100 μg/ml lactalbumin hydrolysate 5%, yeast extract 1%, Heps buffer 15 mM sodium bicarbonate 7.5% and calf serum 20%. The cell suspension was dispensed into two sterile 25 cm³ tissue culture flasks (Falcon®) prior to incubating at 37°C.

The culture was daily examined under an inverted microscope. Subculture was attempted when signs of transformation occurred, as evidenced by presence of pleomorphic refractile mononuclear cells singly or in clumps both in suspension and/or attached to the inner surface of the tissue culture flask as monolayer.

### 2.8.5.2. Schizont antigen preparation

The content of two schizont culture bottles (approximately 150 ml) of cell suspension was centrifuged at 150 g for 10 minutes. The pellet was resuspended in 10 ml PBS and centrifuged again at 150 g for 10 minutes. Washing was carried out three times before the cells were resuspended in a small volume of PBS containing 1% bovine serum albumin (Fraction-v (Sigma®, USA).

The antigen suspension was applied onto the PTFE-coated multi-spots slides (Highved Biological (PTY) LTI), Lyndhurst, RSA). This was done
by pipetting 200 µl of the schizont suspension into a well and immediately aspirating the excess and transferring into the next well using a single channel micropipette (BDSL), adjusted to 200 µl. The slides were allowed to air dry for one hour, fixed in cold (+4°C) acetone (Analar) for 10 minutes and again allowed to air dry. The slides were individually wrapped in tissue paper and packed in aluminum foil with 4 slides in each packet. The slide packets were labelled indicating date of preparation, type of antigen and number of the slides and stored at –20°C until used.

2.8.6. Indirect fluorescent antibody test procedure

Antigen slides were removed from –20°C and allowed to thaw at 4°C for 30 minutes, then placed at room temperature for another period of 30 minutes. The thawed sera (test and control sera) were diluted (1/80) in PBS by adding 5 µl serum into the corresponding tubes (1 ml Polypropethere, Micronic®), each contained 395µl PBS by using automatic pipette with a different tip for each serum sample. Antigen slides once thawed and dried were placed in humidity chamber (Petri dishes on a moist filter paper). A volume of 10 µl of the sample, using an automatic pipette, was put in the designated wells on each antigen spot the pipette tip was changed before going on to the next sample. The samples stayed within the spot and were not allowed to run into each other. A PBS control was included on at last antigen slide, and the slides were left undisturbed in a plastic box with moist filter paper and incubated at 37°C for 30 minutes to allow antigen/antibody reaction take place. Then the slides were thoroughly washed three times and rinsed by dipping into PBS in a staining jar. Each wash was for 10 minutes to remove excess sera, while the buffer being renewed after the first 15 minutes, drained and the slides were dried on the rack. Meanwhile, FITC conjugate was
thawed at room temperature and diluted in PBS. This was achieved by dilution to 1/80 (5 µl conjugate in 395 µl PBS) according to the manufacturer recommendations. Evans Blue (50 µl) at a concentration of 0.1% was added to the conjugate as a counter stain in order to improve definition of specific fluorescence. A volume of 10 µl of the diluted conjugate was applied to each test well of the slides and they were incubated at 37°C for 30 minutes in the dark, washed with PBS as described above and air dried. Examination of the slides was carried out in a dark room using Olympus Vanox incident-light excitation fluorescent microscope (Japan). The slides were examined under ×40 immersion objective to detected microscopic fluorescent as positive indications.

2.9. Statistical analysis:

Statistical analysis was performed using SAS package. Ticks collected were subjected to appropriate general liner model (GLM). The SAS was used to perform analysis of variance (ANOVA) and mean separations were performed using Ryan-Einot-Gebriel-Welsch multiple range test (REGWQ) according to Day and Quinn (1989). Tick counts were transformed to $\sqrt[\log_{10}(X+1)+0.5}$ scale before being subjected to (ANOVA) according to Hassan et al. (1992). Correlation analysis was carried out the relate tick abundance to meteorological data.

CHAPTER THREE
RESULTS
3.1. Parasitological findings

A total of 1200 blood smears were collected from dairy cattle during the survey period from June 2006 to May 2007. The prevalence rate of piroplasms in Nyala dairy farms are shown in (Table 2). Out of 1200, 43 (3.6%) *Theileria* spp piroplasm forms were detected in the blood smears (BS) (Plate 1), and 22 macroschizonts form were detected in the biopsy smears (Plate 2). *Theileria* spp were generally low in all the localities ranging from 0/82 (0%) to 18/352 (5.1%) in Al masamea and Um dam respectively.

The highest prevalence rate of *Theileria* spp. piroplasms was in December 7/98 (7.1%), followed by November 6/100 (6%), June 6/103 (5.8%), and July 5/105 (4.7%). The lowest prevalence rate was in August 1/111 (0.1%), followed by April 1/99 (1.0%) and February 1/95 (1.1%) (Table 3).

Since it is not possible to identify *Theileria* spp. using piroplasm form, (FAO, 1983) it was not established whether these piroplasm forms were of *T. annulata*, *T. mutans* or *T. velifera*.

3.2. Serological findings

Indirect fluorescent antibody (IFA) test was performed using *T. annulata* schizont antigens for detection of antibodies against *T. annulata* (Plate 3). Out of 100 serum samples, 31 (31%) were found positive for *T. annulata* antibodies (Table 2). Prevalence of *T. annulata* antibodies was unexpectedly high in all the localities ranging from 1/7 (14.3%) to 5/11 (45.5%) in Al masamea and Algabal, respectively.

Table (2) Prevalence rate of *Theileria* spp. piroplasms determined by blood smears (BS) and antibody against *T. annulata* using indirect fluorescent antibodies (IFA) test among cattle in different localities in Nyala during June 2006 to May 2007.
<table>
<thead>
<tr>
<th>Locality</th>
<th>BS</th>
<th></th>
<th>IFA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animals examined</td>
<td>Positive <em>Theileria</em> spp. piroplasms (%)</td>
<td>Animals examined</td>
<td>Positive <em>T. annulata</em> antibodies (%)</td>
</tr>
<tr>
<td>Museah</td>
<td>289</td>
<td>8(2.8)</td>
<td>24</td>
<td>4(16.7)</td>
</tr>
<tr>
<td>Algabai</td>
<td>142</td>
<td>7(4.9)</td>
<td>11</td>
<td>5(45.5)</td>
</tr>
<tr>
<td>Domaya</td>
<td>95</td>
<td>2(2.1)</td>
<td>8</td>
<td>2(25)</td>
</tr>
<tr>
<td>Belial</td>
<td>165</td>
<td>6(3.6)</td>
<td>14</td>
<td>5(35.7)</td>
</tr>
<tr>
<td>Al geer</td>
<td>75</td>
<td>2(2.7)</td>
<td>6</td>
<td>2(33.3)</td>
</tr>
<tr>
<td>Um dam</td>
<td>352</td>
<td>18(5.1)</td>
<td>30</td>
<td>12(40)</td>
</tr>
<tr>
<td>Al masanea</td>
<td>82</td>
<td>(0)</td>
<td>7</td>
<td>1(14.3)</td>
</tr>
<tr>
<td>Total</td>
<td>1200</td>
<td>43(3.6)</td>
<td>100</td>
<td>31(31)</td>
</tr>
</tbody>
</table>
Plate (1) A thin blood smear showing erythrocytes containing *Theileria* spp. piroplasms from a calf suffering from thileriosis in Nyala.
Plate (2) Lymph node biopsy smear containing (a) intracellular and (b) extra-cellular schizonts of *Theileria annulata* from a calf in Nyala
Plate (3) Detection of *Theileria annulata* antibodies using schizont antigens in IFA test. Arrows indicate positive reactions.
Using IFA test, the highest monthly prevalence rate of *T. annulata* antibodies was revealed in December 6 (75.0%), November 4 (50.0%), June 4 (50.0%), and July 3 (37.5%). The lowest prevalence rate was in August 1 (11.1%) followed by April 1 (11.1%), and February 1 (12.5%) (Table 3).

Blood smears examination was less sensitive in detection of *Theileria* spp. compared to IFA test which detect *T. annulata* antibodies. However, it was noticed that in localities where detection of piroplasms was high the antibodies detection was also high e.g. Al gabal and whenever the piroplasms were low antibodies were also low e.g. Al masamea.

Apparently, tropical theileriosis occurs throughout the year. The prevalence rate varied between different seasons during the study period.

### 3.3. Clinical features

The clinical signs encountered from naturally infected dairy cattle included elevated body temperature (39–42°C) and enlargement of superficial lymph nodes. Other symptoms observed were pale congested to icteric mucous membranes, lacrimation, nasal discharge difficulty in breathing, loss of appetite were often present. The animals become dull and weak.
Table (3) Monthly prevalence of *Theileria* spp. piroplasms as determined by blood smears (BS) and *T. annulata* antibodies using indirect fluorescent antibody (IFA) test among cattle in Nyala during June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Month</th>
<th>Animals examined</th>
<th>Positive <em>Theileria</em> spp. piroplasms(%)</th>
<th>Animals examined</th>
<th>Positive <em>T. annulata</em> antibodies(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2006</td>
<td>103</td>
<td>6(5.8)</td>
<td>8</td>
<td>4(50.0)</td>
</tr>
<tr>
<td>July</td>
<td>105</td>
<td>5(4.7)</td>
<td>8</td>
<td>3(37.5)</td>
</tr>
<tr>
<td>August</td>
<td>111</td>
<td>1(0.9)</td>
<td>9</td>
<td>1(11.1)</td>
</tr>
<tr>
<td>September</td>
<td>100</td>
<td>2(2)</td>
<td>8</td>
<td>1(12.5)</td>
</tr>
<tr>
<td>October</td>
<td>84</td>
<td>3(3.6)</td>
<td>8</td>
<td>3(37.5)</td>
</tr>
<tr>
<td>November</td>
<td>100</td>
<td>6(6)</td>
<td>8</td>
<td>4(50.0)</td>
</tr>
<tr>
<td>December 2006</td>
<td>98</td>
<td>7(7.1)</td>
<td>8</td>
<td>6(75.0)</td>
</tr>
<tr>
<td>January 2007</td>
<td>107</td>
<td>3(2.8)</td>
<td>9</td>
<td>2(22.2)</td>
</tr>
<tr>
<td>February</td>
<td>95</td>
<td>1(1.1)</td>
<td>8</td>
<td>1(12.5)</td>
</tr>
<tr>
<td>March</td>
<td>104</td>
<td>4(3.8)</td>
<td>8</td>
<td>3(37.5)</td>
</tr>
<tr>
<td>April</td>
<td>99</td>
<td>1(1)</td>
<td>9</td>
<td>1(11.1)</td>
</tr>
<tr>
<td>May 2007</td>
<td>94</td>
<td>4(4.3)</td>
<td>9</td>
<td>2(22.2)</td>
</tr>
<tr>
<td>Total</td>
<td>1200</td>
<td>43(3.6)</td>
<td>100</td>
<td>31(31.0)</td>
</tr>
</tbody>
</table>
3.4. Prevalence of *Theileria* spp. among breeds of cattle

*Theileria* spp. were prevalent among different breeds of cattle (Tables 4). Using (BS) technique, the prevalence of the piroplasm in cross bred cattle was higher 22/420 (5.2%) than in local 21/780 (2.7%). However, using IFA the highest prevalence of antibodies against *T. annulata* was in local breeds compared with the cross breed. Those were 22/52 (40.7%) and 9/48 (19.6%) for local and cross bred cattle respectively.

3.5. Prevalence of *Theileria* spp. among different age groups of cattle

Within any herd of cattle examined for *Theileria* spp. using BS, the highest prevalence was 9/178 (5.1%) recorded among calves (1 to 2 years old), and the lowest prevalence was 3/123 (2.4%) recorded among calves (< 1 year old) (Table 5). Using IFA test the highest prevalence of antibodies against *T. annulata* 20/49 (4.1%) was recorded in older groups of cattle (> 3 years old) and the lowest prevalence 2/11 (1.8%) was recorded among calves (< 1 year old).

3.6. Prevalence of *Theileria* spp. among different sex of cattle

Slightly high significant difference was observed between the prevalence rate of disease in females in comparison with males by using two diagnostic techniques (BS and IFA) (Table 6). The prevalence values were 7/230 (3.4%), and 36/970 (3.7%) in bulls and cows, respectively using BS, and 5/21 (2.4%), and 26/79 (3.3%), respectively by using IFA test.
Table (4) Prevalence of *Theileria* spp. piroplasms and *T. annulata* antibodies among local and cross bred cattle in Nyala farms using blood smear (BS) and indirect fluorescent antibody (IFA) test from June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Breed of cattle</th>
<th>BS</th>
<th>IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animals examined</td>
<td>Positive <em>Theileria</em> spp. Piroplasms (%)</td>
</tr>
<tr>
<td>Zebu</td>
<td>780</td>
<td>21 (2.7)</td>
</tr>
<tr>
<td>Zebu × Frisian</td>
<td>420</td>
<td>22 (5.2)</td>
</tr>
<tr>
<td>Total</td>
<td>1200</td>
<td>43 (3.6)</td>
</tr>
</tbody>
</table>
Table (5) Incidence of *Theileria* spp. piroplasms and *T. annulata* antibodies using blood smears (BS) and indirect fluorescent antibody (IFA) test, respectively among different age groups of cattle in Nyala during June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Age group</th>
<th>BS Number examined</th>
<th>Positive piroplasms (%)</th>
<th>IFA Number examined</th>
<th>Positive <em>T. annulata</em> antibodies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 year</td>
<td>123</td>
<td>4(3.2)</td>
<td>11</td>
<td>2(1.8)</td>
</tr>
<tr>
<td>1 &lt; 2 years</td>
<td>178</td>
<td>9(5.1)</td>
<td>22</td>
<td>5(2.3)</td>
</tr>
<tr>
<td>2 to 3 years</td>
<td>360</td>
<td>13(3.6)</td>
<td>18</td>
<td>4(2.2)</td>
</tr>
<tr>
<td>&gt; 3 years</td>
<td>539</td>
<td>17(3.2)</td>
<td>49</td>
<td>20(40.8)</td>
</tr>
</tbody>
</table>
Table (6) Incidence of *Theileria* spp. piroplasms and *T. annulata* antibodies using blood smears (BS) and indirect fluorescent antibody (IFA) test, respectively among bulls and cows in Nyala during June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Diagnostic technique</th>
<th>Bulls</th>
<th>Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number examined</td>
<td>Positive (%)</td>
</tr>
<tr>
<td>Theileria <em>spp. piroplasms using BS</em></td>
<td>230</td>
<td>7 (3.0)</td>
</tr>
<tr>
<td><em>T. annulata</em> antibodies using IFA</td>
<td>21</td>
<td>5 (2.4)</td>
</tr>
</tbody>
</table>
3.7. Prevalence of theileriosis according to systems of management

Concerning the systems of management practiced in Nyala dairy farms (Table 7) semi–extensive and extensive management systems had higher prevalence than intensive or semi intensive systems. There was significant difference in the prevalence rate between different management systems, using BS and IFA test.

3.8. Epidemiological indicators of tropical theileriosis

Three endemic situations were identified based on incidence of theileriosis, abundance of ticks namely: enzootic stability, low endemic instability and high endemic instability. The enzootic stability was recorded in indigenous breed cattle under semi-extensive and extensive management system, while endemic instability was mainly observed among cross bred under semi - intensive system. The exposure of calves to infection was shown to be significantly higher than in older cattle in enzootic stability, while in endemic instability clinical cases of tropical theileriosis were observed in different age categories. Density of tick population, in endemically stable situations high tick infestation rates were manifested on cattle, while in endemically unstable situations lower infestation rates were recorded (Table 8).
Table (7) Prevalence of *Theileria* spp. piroplasms and *T. annulata* antibodies using blood smears (BS) and indirect fluorescent antibody (IFA) test, respectively in Nyala among different husbandry systems of cattle during June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Type of system</th>
<th>BS</th>
<th></th>
<th></th>
<th>IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number examined</td>
<td>Number positive (%)</td>
<td>Number examined</td>
<td>Number positive (%)</td>
</tr>
<tr>
<td>Semi-intensive</td>
<td>260</td>
<td>6 (1.9)</td>
<td>14</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>Extensive</td>
<td>760</td>
<td>15 (1.97)</td>
<td>39</td>
<td>10 (2.6)</td>
</tr>
<tr>
<td>Semi-intensive</td>
<td>528</td>
<td>22 (4.2)</td>
<td>47</td>
<td>18 (3.8)</td>
</tr>
<tr>
<td>Total</td>
<td>1200</td>
<td>43 (3.6)</td>
<td>100</td>
<td>31</td>
</tr>
</tbody>
</table>
Table (8) Epidemiology indicators of *Theileria* spp. piroplasms and *Theileria annulata* antibodies in farms in Nyala from June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Type of farm</th>
<th><em>Theileria</em> spp. piroplasms (%)</th>
<th><em>T. annulata</em> antibodies (%)</th>
<th>Total prevalence (%)</th>
<th>Mean tick abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzootic stable situation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Umdam, Algabal Museah, Belial)</td>
<td>39/948 (4.1)</td>
<td>26/79 (32.9)</td>
<td>65/1027 (6.4)</td>
<td>2.64±0.17</td>
</tr>
<tr>
<td>Medium stable situation</td>
<td>4/170 (2.4)</td>
<td>4/14 (28.6)</td>
<td>8/184 (4.3)</td>
<td>2.0±0.10</td>
</tr>
<tr>
<td>High instable situation</td>
<td>0/75</td>
<td>1/7 (14.3)</td>
<td>1/82 (1.2)</td>
<td>1.54±0.09</td>
</tr>
<tr>
<td>(Al masanea)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.9. Haematogram indices of infected cattle

Mean values of haematogram indices of naturally infected dairy cattle with *T. annulata* are shown below. These cattle (15 adults and 7 calves) exhibited tropical theileriosis clinical signs and schizonts were detected in lymph node biopsy smears.

3.9.1. Red blood cells count

The mean values of the red blood cells (RBCs) of the infected adult cattle were 4.6 million RBCs/µl. Those ones of the infected calves were 4.4 million cells/µl. The total mean RBCs of the infected animals was 4.5 \(10^6/\mu l\) (Table 9). That is less than normal mean values RBCs (7.2-11.6 \(10^6/\mu l\)) (Hematological Values, International Units).

3.9.2. Packed cell volume

The mean values of the packed cell volume (PCV) of the infected adult cattle were 17% while the PCV mean values of the calves were 15.8%, whereas the total mean values of the infected cattle were 16.4% (Table 9), while the normal PCV mean values of cattle are 28.07%.

3.9.3. Haemoglobin concentration

The mean values of haemoglobin of the infected adult cattle were 6.0 gram/deciliter while the mean values of the infected calves were 5.6 g/dl, and the mean total values of infected cattle were 5.8 g/dl (Table 9) while the haemoglobin mean value of normal cases are 9.7 - 13.7 g/dl.
Table (9) Range and mean values of haematological indices of naturally infected cattle with *Theileria annulata* in Nyala from June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Animals examined</th>
<th>Haematogram indices</th>
<th>Erythrocytes indices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RBCs ((10^6/µl))</td>
<td>PCV (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rang (mean)</td>
<td>Rang (mean)</td>
</tr>
<tr>
<td>Adult cattle</td>
<td>15</td>
<td>4.5-4.8 (4.6)</td>
<td>16-21 (17)</td>
</tr>
<tr>
<td>Calves</td>
<td>7</td>
<td>3.5-4.6 (4.4)</td>
<td>12-17 (15.5)</td>
</tr>
<tr>
<td>Mean of all animals</td>
<td></td>
<td>4.5</td>
<td>16.4</td>
</tr>
</tbody>
</table>
3.9.4. White blood cells count

The mean values of the white blood cells (WBCs) count of the infected adult cattle were 3,300 thousand cells/µl. Those ones of the calves were 2,800 $10^3$ cells/µl. The overall mean values of infected cattle were 2900 cells/µl (Table 9), while the normal mean values are 5.24 – 9.84 $10^3$ cells/µl.

3.10. Erythrocyte indices

3.10.1. Mean corpuscular volume

The mean value of the mean corpuscular volume (MCV) (femtoliter = 9-10 microliter) of the infected adults was 35.9 fl. The infected calves showed value of 33.8 fl MCV. The mean values of the infected adult cattle were 36.96 fl (Table 9), while the mean values of normal cattle are 41 - 61 fl.

3.10.2. Mean corpuscular haemoglobin

The infected adult cattle showed mean value of 13.47 Pg mean corpuscular haemoglobin (MCH) while that of the infected calves was 12.72 Pg. The infected animals revealed mean values of 12.88 Pg MCH mean value (Table 9), while the mean values of normal cattle are 13.9 – 21.9 Pg.

3.10.3. Mean corpuscular haemoglobin concentration

The Mean corpuscular haemoglobin concentration (MCHC) value of the infected adult cattle was 35.29 g/dl. The infected calves showed 35.44 g/dl MCHC. In general mean value of infected cattle was 35.36 g/dl (Table 9), but mean values of normal cattle are 30.0 – 36.0 g/dl.
3.11. Ticks infesting cattle

All dairy cattle examined were found to be infested with four tick genera and thirteen (13) different species of ticks included *Amblyomma lepidum*, *A. variegatum*, *Boophilus annulatus*, *B. decoloratus*, *Hyalomma anatolicum anatolicum*, *H. a. excavatum* *H. dromedarii*, *H. marginatum rufipes*, *H. truncatum*, *H. impeltatum*, *H. impressum*, *Rhipicephalus evertsi evertsi*, *R. s. sanguineus* (Table 10). The highest and lowest mean monthly abundance throughout the year in the present survey is given as follows:

3.11.1. *Boophilus decoloratus*

The highest means totals body collection of this blue tick were recorded in August (0.40 ±0.06), July (0.39±0.07), followed by January (0.23±0.05), while the lowest means were recorded in February (0.03±0.02), May (0.05±0.03) and followed by November (0.08±0.03) (Table 10).

3.11.2. *Boophilus annulatus*

The highest means totals were in November (0.78±0.13) and December (0.51±0.11), followed by July (0.28±0.06), and March (0.33±0.08), while the lowest means were recorded in (0.04±0.05) and (0.08±0.03), were recorded in January, and, respectively (Table 10).

3.11.3. *Hyalomma dromedarii*

This camel tick was highly abundant in the traditional farms, which was mainly composed of local types. The highest means were recorded in March (0.71±0.09) and June (0.46±0.06), followed by July (0.25±0.05) while the lowest mean was recorded in December (0.06±0.03) and
January (0.12±0.04) followed by February (0.17±0.05). This species was not found in October (Table 10).

3.11.4. *Hyalomma impeltatum*

This tick species was numerous throughout the year in all farms. The highest means were recorded in May (1.18±0.08), April (0.89±0.08) November (0.89±0.11), and June (0.86±0.07), while the lowest means were recorded in October (0.21±0.05), September (0.23±0.05) and December (0.35±0.06) (Table 10).

3.11.5. *Hyalomma marginatum rufipes*

This tick species was present in high to moderate abundance in all months except in March. The highest means were in December (1.59±0.09) and November (1.23±0.011) followed by January (0.77±0.09) and October (0.63±0.07) and the lowest means were in July (0.05±0.03), February (0.12±0.09), and September (0.29±0.06) (Table 10).

3.11.6. *Hyalomma truncatum*

The highest means of this tick species were recorded in October (0.79±0.07), July (0.52±0.08), and December (0.24±0.06), while the lowest means were recorded in August (0.01±0.01), March (0.02±0.02) and April (0.03±0.02) (Table 10).

3.11.7. *Amblyomma variegatum*

The highest means were recorded in August (0.65±0.08), September (0.59±0.08) and July (0.52±0.08) while the lowest means (0.02±0.02) were found in February, March, and May, followed by June (0.05±0.03) and April (0.04±0.02) (Table 10).
Table (10) Mean (+ SE) monthly total tick species infesting cattle in Nyala during June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Months</th>
<th>B. decoloratus</th>
<th>B. annulatus</th>
<th>H. dromedarii</th>
<th>H. impeltatum</th>
<th>H. m. rufipes</th>
<th>H. truncatum</th>
<th>A. variegatum</th>
<th>Mean total</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2006</td>
<td>0.16±0.04bc</td>
<td>0.13±0.04cd</td>
<td>0.46±0.06a</td>
<td>0.86±0.07bc</td>
<td>0.50±0.06cd</td>
<td>0.08±0.03e</td>
<td>0.04±0.02c</td>
<td>2.23±0.32</td>
</tr>
<tr>
<td>July</td>
<td>0.39±0.07a</td>
<td>0.28±0.06c</td>
<td>0.25±0.05bc</td>
<td>0.60±0.08c</td>
<td>0.05±0.03e</td>
<td>0.52±0.08ab</td>
<td>0.52±0.08a</td>
<td>2.61±0.45</td>
</tr>
<tr>
<td>August</td>
<td>0.40±0.06a</td>
<td>0.15±0.04cd</td>
<td>0.11±0.04c</td>
<td>0.68±0.07bc</td>
<td>0.33±0.06de</td>
<td>0.01±0.01e</td>
<td>0.65±0.08a</td>
<td>2.33±0.36</td>
</tr>
<tr>
<td>September</td>
<td>0.21±0.06bc</td>
<td>0.13±0.04cd</td>
<td>0.18±0.05bc</td>
<td>0.23±0.05c</td>
<td>0.29±0.06de</td>
<td>0.13±0.04cd</td>
<td>0.59±0.08a</td>
<td>1.76±0.38</td>
</tr>
<tr>
<td>October</td>
<td>0.11±0.04bc</td>
<td>0.11±0.04d</td>
<td>0.21±0.05cd</td>
<td>0.63±0.07c</td>
<td>0.79±0.07a</td>
<td>0.21±0.05b</td>
<td>2.01±0.32</td>
<td></td>
</tr>
<tr>
<td>November</td>
<td>0.08±0.03bc</td>
<td>0.78±0.13a</td>
<td>0.06±0.03c</td>
<td>0.86±0.11bc</td>
<td>1.23±0.11b</td>
<td>0.14±0.04d</td>
<td>0.06±0.03c</td>
<td>3.21±0.48</td>
</tr>
<tr>
<td>December 2006</td>
<td>0.15±0.04bc</td>
<td>0.51±0.11b</td>
<td>0.11±0.04c</td>
<td>0.35±0.06cd</td>
<td>1.59±0.09a</td>
<td>0.24±0.06b</td>
<td>0.06±0.03c</td>
<td>3.01±0.43</td>
</tr>
<tr>
<td>January 2007</td>
<td>0.23±0.05b</td>
<td>0.04±0.05cd</td>
<td>0.12±0.04c</td>
<td>0.60±0.07bc</td>
<td>0.77±0.09c</td>
<td>0.19±0.05c</td>
<td>0.06±0.03c</td>
<td>2.01±0.38</td>
</tr>
<tr>
<td>February</td>
<td>0.03±0.02c</td>
<td>0.08±0.03cd</td>
<td>0.17±0.05bc</td>
<td>0.55±0.09cd</td>
<td>0.12±0.09e</td>
<td>0.12±0.04cd</td>
<td>0.02±0.02c</td>
<td>1.09±0.34</td>
</tr>
<tr>
<td>March</td>
<td>0.08±0.04bc</td>
<td>0.33±0.08c</td>
<td>0.71±0.09a</td>
<td>0.68±0.09cd</td>
<td>0.02±0.02e</td>
<td>0.02±0.02c</td>
<td>1.84±0.34</td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>0.08±0.04bc</td>
<td>0.08±0.03cd</td>
<td>0.12±0.04c</td>
<td>0.89±0.08b</td>
<td>0.55±0.08cd</td>
<td>0.03±0.02e</td>
<td>0.05±0.03c</td>
<td>1.8±0.32</td>
</tr>
<tr>
<td>May 2007</td>
<td>0.05±0.03bc</td>
<td>0.08±0.03cd</td>
<td>0.15±0.05bc</td>
<td>1.18±0.08a</td>
<td>0.42±0.08d</td>
<td>0.18±0.05c</td>
<td>0.02±0.02c</td>
<td>2±0.31</td>
</tr>
</tbody>
</table>

Means (+ SE) followed by the same letter in each column are not significantly different at 5% level based on Ryan’s Q test.
Correlation analysis between abundance of ticks and climatic values revealed that there was a significant negative correlation with minimum temperature \( (r = -0.105 \; P \leq 0.001) \), maximum temperature \( (r = 0.129 \; P \leq 0.001) \), rainfall \( (r = -0.006 \; P \leq 0.001) \) and positive significant relative humidity \( (r = 0.075 \; P \leq 0.001) \) (Table 11). There was a negative non significant correlation between \( B. \) *decoloratus* and rainfall, maximum and minimum temperature, while positively correlated with relative humidity \( (r = 0.192 \; P \leq 0.001) \). \( B. \) *annulatus* was negatively correlated with rainfall and maximum temperature, while negatively significantly correlated with rainfall \( (r = -0.021 \; P \leq 0.01) \) and highly significant correlated with minimum temperature \( (r = -0.145 \; P \leq 0.001) \). There was also a significant correlation between \( H. \) *dromedarii* and relative humidity \( (r = -0.164 \; P \leq 0.05) \), while significant positive correlation with rainfall, minimum and maximum temperature. \( H. \) *impeltatum* was significantly correlated with maximum temperature \( (r = 0.155 \; P \leq 0.05) \) with a non significant correlation with relative humidity, rainfall and minimum temperature. \( H. \) *m. rufipes* was highly significantly correlated with relative humidity \( (r = -0.126 \; P \leq 0.001) \) and minimum temperature \( (r = -0.369 \; P \leq 0.001) \), while negatively correlated with rainfall and maximum temperature. \( H. \) *truncatum* was highly significantly correlated with rainfall \( (r = -0.115 \; P \leq 0.001) \) and negatively correlated with relative humidity, maximum and minimum temperature. There was a highly significant correlation between \( A. \) *variegatum* and relative humidity \( (r = 0.348 \; P \leq 0.001) \), and was middle significant correlation with minimum temperature, but negatively correlated with rainfall and maximum temperature (Table 11).
Table (11) Correlation analysis between tick species in Nyala with climatic factors during the period from June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Tick species</th>
<th>R H</th>
<th>RAIN</th>
<th>MAX</th>
<th>MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. decoloratus</td>
<td>0.192***</td>
<td>0.248</td>
<td>-0.106</td>
<td>0.049</td>
</tr>
<tr>
<td>B. annulatus</td>
<td>0.062</td>
<td>-0.021**</td>
<td>0.163</td>
<td>-0.145***</td>
</tr>
<tr>
<td>H. dromedarii</td>
<td>-0.164*</td>
<td>0.071</td>
<td>0.136</td>
<td>0.154</td>
</tr>
<tr>
<td>H. impeltatum</td>
<td>0.099</td>
<td>-0.022</td>
<td>0.155*</td>
<td>0.116</td>
</tr>
<tr>
<td>H. m. rufipes</td>
<td>-0.126***</td>
<td>-0.072</td>
<td>-0.204</td>
<td>-0.369***</td>
</tr>
<tr>
<td>H. truncatum</td>
<td>0.112</td>
<td>-0.115***</td>
<td>-0.136***</td>
<td>-0.152</td>
</tr>
<tr>
<td>A. variegatum</td>
<td>0.348***</td>
<td>0.390</td>
<td>-0.067</td>
<td>0.171**</td>
</tr>
<tr>
<td>Total</td>
<td>0.075***</td>
<td>-0.006***</td>
<td>-0.129***</td>
<td>-0.105***</td>
</tr>
</tbody>
</table>

*P ≤ 0.05  **P ≤ 0.01  ***P ≤ 0.001.

Rain = Rainfall, RH = Relative humidity, Min and Max = mean minimum and maximum temperatures.
Analysis between monthly tick load infesting cattle and age of cattle revealed a significant difference among different groups (Table 12). The highest mean ticks (4.95± 0.2) in November and the lowest mean ticks (2.65± 0.3) in September infested cattle aged more than four years followed by heifers that were infested by the highest mean (3.12± 0.2) in November and the lowest mean (1.52 ± 0.2) in September. The lowest mean infestation (0.94± 0.1) in November was recorded among calves of less than two years old with the highest mean (1.161± 0) in November.

Ticks recorded in very low numbers in the present survey included *Amblyomma lepidum, Hyalomma anatolicum anatolicum, H. anatolicum excavatum, H. impressum, Rhipicephalus evertsi evertsi, R. s. sanguineus* (Table 13).
Table (12) Mean (±SE) monthly ticks infesting different age groups of cattle in Nyala during June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Month</th>
<th>Age groups of cattle</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 2 years</td>
<td>2 to 3 years</td>
<td>&gt; 3 years</td>
<td></td>
</tr>
<tr>
<td>June 2006</td>
<td>1.08± 0.1c</td>
<td>2.53± 0.2b</td>
<td>2.92± 0.2a</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>0.91± 0.2c</td>
<td>2.16± 0.3b</td>
<td>4.07±0.2a</td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>0.81 ±0.1c</td>
<td>2.31±0.3b</td>
<td>3.50± 0.4a</td>
<td></td>
</tr>
<tr>
<td>September</td>
<td>0.94 ± 0.1</td>
<td>1.52±0.2b</td>
<td>2.65± 0.3a</td>
<td></td>
</tr>
<tr>
<td>October</td>
<td>1.24± 0.1c</td>
<td>1.96± 0.2b</td>
<td>3.29± 0.2a</td>
<td></td>
</tr>
<tr>
<td>November</td>
<td>1.61± 0.2c</td>
<td>3.12± 0.2b</td>
<td>4.95± 0.2a</td>
<td></td>
</tr>
<tr>
<td>December 2006</td>
<td>1.23± 0.2c</td>
<td>2.96± 0.3b</td>
<td>4.77± 0.4a</td>
<td></td>
</tr>
<tr>
<td>January 2007</td>
<td>1.10±0.1c</td>
<td>1.78± 0.2b</td>
<td>3.12± 0.3a</td>
<td></td>
</tr>
<tr>
<td>February</td>
<td>1.21± 0.1c</td>
<td>1.71± 0.1b</td>
<td>3.00± 0.2a</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>0.89± 0.1c</td>
<td>1.79±0.2b</td>
<td>2.65± 0.2a</td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>0.94± 0.1c</td>
<td>1.59±0.1b</td>
<td>2.68± 0.1a</td>
<td></td>
</tr>
<tr>
<td>May 2007</td>
<td>1.17± 0.1c</td>
<td>1.92± 0.1b</td>
<td>2.69± 0.2a</td>
<td></td>
</tr>
</tbody>
</table>

Means (±SE) followed by the same letter in each row are not significantly different at 5% level based on Ryan’s Q test.
Table (13) Ticks collected in very low numbers from cattle in Nyala farms during June 2006 to May 2007.

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. lepidum</td>
<td>4</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>H. a. anatolicum</td>
<td>5</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>H. a. excavatum</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>H. impressum</td>
<td>12</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>R. e. evertsi</td>
<td>22</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>R. sanguineus</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>47</td>
<td>39</td>
<td>86</td>
</tr>
</tbody>
</table>
CHAPTER FOUR
DISCUSSION

Ticks-borne diseases of cattle remain an important impediment to livestock development in the Sudan (Gamal and El Hussein, 2003). The impact of ticks and tick-borne diseases (T&TBDs) in South Darfur State is tremendous due to high abundance of ticks throughout different climatic zones of the region (Abdallah, 2007). Recently, due to progressive development of South Darfur State and rapid growing of human population in Nyala town, there is increasing demand of milk and milk products. Many farmers introduced Friesian cattle and their crosses with indigenous breeds (Friesian × Kenana or Butana) from Central Sudan. However, T&TBDs are the main threat to these cattle in most localities within the State. Tropical theileriosis in particular is considered as a major obstacle to the development of dairy industry in Northern Sudan (Hassan and Osman, 1984; El Hussein et al., 1991; Mohammed 1992; El Hussein et al., 2002; Salih, 2003). The present study provides an epidemiological data regarding tropical theileriosis in and around Nyala town. The study, also, provides prevalence of the tick species and determination of the possible vectors of the disease in this area.

The study revealed that out of 1200 blood smears, 43(3.6%) were positive for *Theileria* spp. piroplasms Salih (2003) reported 8 (3.9%) and Abdallah (2005) reported 2.8% in the same region. The piroplasms detected in the blood smears were not necessarily indication of infection with *T. annulata* but could also be *T. mutans* or *T. velifera* piroplasms which are non pathogenic to cattle. These two parasites are transmitted by *Amblyomma lepidum* and *A. variegatum* which are prevalent in the region according to the current study. Species differentiation using blood smears is not possible (FAO, 1983).
The seropositivity for *T. annulata* antibodies was 31(31%) out of 100 serum samples. It is slightly higher than that recorded by Salih (2003) who found 13(6.3%) positive *T. annulata* antibodies using ELISA. The increase in the incidence may be due to the fact that Salih (2003) made a cross-sectional survey but the current study was a whole year round sampling. It could also be attributed to the fact that introduction of cattle with foreign blood into the region has intensified breaking the enzootic stability established. It is also possible that due to the introduction of the vector *H. a. anatolicum* with the exotic breeds of cattle that the disease has become widespread.

Blood smears (BS) examination revealed lower sensitivity in detection of *T. annulata* compared to serological tests. Thus, piroplasms were detected in 3.6%, while 31% *T. annulata* antibodies were positive by IFA. This finding is similar to previous studies by Salih (2003), Salih *et al.* (2005) working on *T. annulata* using BS, IFA and ELISA to determine piroplasms and sero-prevalence of *T. annulata*. The latter found that BS revealed 13.8% piroplasms and sero-positivity of 33.3% and 35.7% by ELISA and IFA test, respectively. They performed indirect ELISA using the recombinant *T. annulata* surface protein (*Ta*SP), while IFA test was performed using schizont antigen. Salih *et al.* (2007) also reported that the test the agreement between BS, a surface protein-detecting *Ta*SP ELISA and IFA for detection of *T. annulata* revealed that BS test was inferior to the serological tests. Ali *et al.* (2006) reported that BS revealed the lowest sensitivity in detection of carrier cattle to *T. annulata* compared to polymerase chain reaction (PCR) and reverse line blot (RLB). They concluded that the available serological tests, IFA or ELISA are helpful for detection of antibodies against *T. annulata* in cattle under field conditions in the Sudan. Nevertheless, molecular techniques are more accurate for detection of *T. annulata* particularly in carrier
cattle. Molecular techniques are recommended to be used all over South Darfur region to conduct an accurate mapping of the spread of the disease and evaluate the magnitude of problem of tropical theileriosis.

The status of the sick animals infected with theileriosis was defined as clinical cases in two groups; one group included those animals found sick with symptoms of tropical theileriosis during sampling and then confirmed by high parastaemia in BS and those animals not showing symptoms during sampling but were positive by blood smears or serologically. The clinical signs encountered were pyrexia, enlargement of superficial lymph nodes, icterus, pale mucous membrane, diarrhoea, laboured respiration, dehydration, pneumonia, and jaundice. This is similar to the observations by FAO (1983) which also reported hyperthermia for several days, dyspnoea and lacrimation, diarrhoea and occasionally corneal opacity. Similar findings were reported by Mira and Ralph (1989), Robison (1982) and Boulter and Hall (2000).

This study evaluated changes in haematological pictures in naturally infected with theileriosis. Low levels of PCV and Hb concentration were found compared with the normal rates of healthy cattle indicating that the disease caused anaemia. The anaemia with marked decrease in PCV, Hb, RBC counts, WBC counts was similar to the findings of El Haj (2000) who worked on *T. annulata* infected young calves in Khartoum State. The findings also agree with those of Sharma and Gautam (1971), Gautam *et al.* (1970), El Hussein *et al.* (1998). Despite the fact that anaemia is one of the main symptoms of tropical theileriosis, the precise mechanism is still unknown. It has been claimed to be as a result of removal of erythrocytes by phagocytosis rather than parasite-induced lysis (Boulter and Hall, 2000). Losos (1986) pointed out that *T. annulata* piroplasms have been found to cause RBCs destruction. Maxi *et al.* (1982) showed that theilerial infection was
associated with normocytic normochromic non-responsive anemia. The decrease of the RBCs, PCV, and Hb is the results of the destruction of the myeloid tissue by *T. annulata* infected lymphoblastoid cell toxins (Wilde, 1963; Laiblin, 1978).

In the current study, tropical theileriosis occurred throughout the year with a minor seasonal variation. The highest prevalence was in December, followed by November, June, and July. The lowest prevalence was in August followed by April, and February (Table 3). This finding indicates that there are other risk factors that might affect incidence of the disease such as prevalence of vector of tropical theileriosis and host susceptibility. No seasonal variation of ticks was also reported by FAO (1983) in Central Sudan. This could be due to the fact that *H. a. anatolicum* is more adapted to their microclimates within the farms. In this region, the ambient temperatures are not too cold to force the vector to go into diapause clear seasonal variations are evident for *H. detritum* and for incidence of tropical theileriosis in North Africa (Danghouth et al., 1996)

Stress as well as introduction of highly susceptible hosts can greatly alter an animal status and render the animal susceptible to the disease breaking the enzootic stable situation. Gamal and El Hussein (2003) reported that high ambient temperatures in the summer stress of pregnancy and reduced appetite in post-calving stage, may lead to breakdown of carrier animals immunity Similarly, El Hussein et al. (1991) referred the high incidence of theileriosis during summer to stress of heat and lack of adequate feed supply. Ahmed (1999) concluded that the stress by sudden change in the macroclimate from autumn to winter besides the increased tick activity during this season cause a stress to carrier animals and emergence of outbreak of tropical theileriosis.
Zebu cattle had lower prevalence of *Theileria* spp. piroplasms in their blood smears but high antibodies against *T. annulata*. Ali *et al.* (2006) detected a low level of piroplasms but high numbers of *T. annulata* parasites using PCR and RLB indicating that most of the animals were carriers of *T. annulata*. This could hold true in this survey since prevalence of *Theileria* spp. piroplasms were found higher in zebu cattle. These carrier animals could be means of spread of the diseases in a wider geographic area in South Darfur if control measures are not strictly imposed. There was an increased prevalence of *T. annulata* antibodies in local breed in comparison with cross breed despite the fact that exotic cattle (*Bos taurus*) are more susceptible to *Theileria* spp. infection than zebu cattle (Osman, 1976; Shommein and Hagir, 1988). This is probably due to the application of acaricides to control ticks on exotic breeds besides the fact that exotic breeds are reared under good management systems. This result is substantiated by the finding that infestation of ticks were low on exotic cattle compared with zebu despite the fact that the former are more susceptible to tick infestation. Nevertheless, these acaricides did not save these animals from attracting the disease probably because these chemicals are indiscriminately applied. If not used in a regular and scientific manner, ticks develop resistance against these chemicals (Regassa and de Castro, 1993).

In the Sudan, indigenous cattle are normally resistant to tick–borne diseases, but they may be severely affected or even die if stressed (Osman, 1976). Bakheit and Latif (2002) demonstrated significant differences in disease resistance of the indigenous breed, Kenana compared to Friesian cattle. Salih *et al.* (2007) compared zebu and cross-bred (*Zebu × Friesian*) and detected a difference in a relative risk. In the current study, although 40.7% of zebu cattle (Table 4) had antibodies
against *T. annulata*, but, showed no signs of the disease and only 2.7% of them showed *Theileria* spp. piroplasms.

The occurrence of the theileriosis among cattle was dependant on husbandry systems. The highest prevalence of the disease was observed in semi extensive system in comparison with farms under semi–intensive systems. That is probably due to poor management in the traditional farms where cattle are mainly of local type (Baggara, Kenana and Butana). They are usually kept in the back yards or housed under badly managed and bad hygiene conditions. This situation of hygiene provides favourable conditions for survival of ticks throughout the year. Similar results were observed by Salih *et al.* (2007) who recorded semi–extensive management systems posed a greater risk than either intensive or extensive systems. On the other hand, low incidence of theileriosis among cattle reared under semi – intensive husbandry may be related to good management with application of acaricides, high level of nutrition maintenance of good hygienic conditions and fairy proper veterinary supervision. This observation coincided with Mohammed (1997) who reported that bad hygiene and poor management are important predisposing factors for theileriosis. Mohammed *et al.* (2001) pointed out that break in the tick control regime in such farms leads to fatal cases of the disease.

The results indicated higher susceptibility of older groups of cattle to theileriosis than young (Table 5). This finding is in agreement with that of Flach *et al.* (1995) and Darghouth *et al.* (1996) who conducted epidemiological investigations on tropical theileriosis in Morocco and Tunisia, respectively. They commented that calves are less heavily infested because of a lower attachment rate or higher detachment by grooming and adults become more tolerant as a result of repeated infestation. It is also possible that in the current study, since calves are
tethered or housed away from dams calves/ticks contact is less and hence become less infected with ticks. Punyu and Hassan (1992) found that cattle tethered up to midday had less tick infestation than another group allowed to graze for whole day. In addition, Salih *et al* (2007) found that the antibodies profile was increased significantly with the advance in age, and thus the age of the animal age could a risk factor for acquisition of the infection. However, it should be mentioned that this finding may indicate a cumulative risk factor rather than actual risk because as the animal lives longer, more antibodies could be serologically detected.

Four genera and thirteen species of ticks were found infesting cattle in Nyala. These were *Amblyomma variegatum*, *A. lepidum*, *Boophilus annulatus*, *B. decoloratus*, *Hyalomma anatolicum anatolicum*, *H. dromedarii*, *H. impeltatum*, *H. impressum*, *H. marginatum rufipes*, *H. truncatum*, *Rhipicephalus evertsi evertsi*, *R. s. sanguineus*. This result is similar more or less to previously reported in Darfur. Osman (1978) had reported twenty tick species in the same region on domestic animals under nomadic systems. Recently, Abdallah (2007) recorded 15 tick species from sedentary and semi-sedentary cattle in South Darfur State. However, there was a difference in tick abundance according to different animal husbandry type. The current study revealed lower tick load on cattle reared under semi-intensive than extensive and semi-extensive systems. This may be attributed to the occasional control of ticks by chemical acaricides in the former system.

The most predominant genus encountered in this survey was the genus *Hyalomma*. Among these *Hyalomma* spp., *H. a. anatolicum* found in several farms is a further confirmation that this tick species as the most efficient vector of tropical theileriosis has established in Nyala and probably in other towns of the Darfur. The first reports of this ticks species in Darfur were by Abdallah (2005) and Abdallah (2007). In
earlier investigations, Hoogstraal (1956) and Osman (1978) had not reported this tick species in Darfur. It is suspected that introduction of Friesian breeds of cattle from Central Sudan where H. a. anatolicum is highly abundant is the source of this tick species in the region. This is substantiated by the fact that these cattle are introduced indiscriminately in Darfur without regulations imposed to clean the introduced cattle from ticks. Outbreaks of tropical theileriosis are currently not uncommon in Nyala evidenced by the 22 cases of theileriosis in the current study where macroschizonts were detected in farms where H. a. anatolicum was identified. To make the situation even worse, cattle for the search of water and pasture cross the international borders to the neighbouring countries and, thus, it would not be surprising to find that this tick species has reached and established in the far west African countries.

However, detection of T. annulata antibodies in farms where H. a. anatolicum was not found was difficult to explain. Abdallah (2007) also reported incidence of the disease in some localities South Darfur in the absence of H. a. anatolicum. Nevertheless, since the disease has already been introduced, it is possible that other tick species such as H. dromedarii may be playing a role in transmission of the disease. Jacquiet et al. (1994) concluded that H. dromedarii appears to be the natural vector of tropical theileriosis over the whole Mauritanian territory. Similarly, Walker et al. (2003) reported that in Mauritania T. annulata infection of cattle is widespread because of the feeding of nymphs and adults of H. dromedarii on cattle where they mix with camels. In the current study, the most prevalent tick species encountered was H. dromedarii which was collected from cattle infected with theileriosis. This high abundance might be due to the close contact of cattle with camels and sheep. Osman (1978) collected H. dromedarii in this region more abundantly on camels and few collections from sheep. Abdallah
(2007) found cattle infested with *H. dromedarii* in large numbers in South Darfur State. There are no hitherto planned studies on the role of *H. dromedarii* in transmission of tropical theileriosis under field conditions. El Ghali (2005) found that this tick species behaves exclusively as a 2-host tick on camels. How it behaves when fed on cattle under field conditions and the degree of efficiency in transmission of this disease remains to be investigated.

Under laboratory conditions, El Neima (1983) found that *H. m. rufipes*, *H. truncatum*, and *H. dromedarii* could efficiently transmit *T. annulata* and concluded that these ticks may play a role in transmission of the disease in the field. Another tick species that could have been suspected in transmission of this disease is the northern African tick species *H. detritum*. Although this species had been earlier reported from this region by Hoogstraal (1956), but in later collections by Osman (1978), Abdallah (2005), Abdallah (2007) and the current survey, this tick species was not found. Probably it has disappeared from the area due to climatic changes. Jongejan et al. (1987) and Salih et al. (2004) pointed out that over the past years major changes in tick distribution have occurred in the Sudan.

In this study, *H. impeltatum* was found feeding on theileriosis infected cattle. Under laboratory conditions, FAO (1983) found that *H. impeltatum* larvae fed on a *T. annulata* infected calf and the subsequent nymphs transmitted fatal theileriosis. El Neima et al. (1983) reported that *H. impeltatum* nymphs were able to transmit *T. annulata*. Therefore, this species may be playing a role in the epidemiology of tropical theileriosis as a vector in South Darfur. Probably, this tick species behaves as 3-host tick species fed on cattle under field conditions (Hoogstraal, 1956). Under laboratory conditions, Osman (1979) found that this tick species behaved as 2-host tick when fed on rabbits. Further planned studies are required to
elucidate the role of this tick species in transmission of *T. annulata* under fielded conditions.

*Amblyomma* spp. were highly abundant during the rainy seasons with peak numbers in August. This observation is similar with that of El Imam (2003) who reported that *A. lepidum* was mostly affected by rainfall and relative humidity, and Osman *et al* (1982) who recorded that *A. lepidum* reached peak activity between August and December in the rainy season in Kordofan. *A. lepidum* is the main vector of heartwater (*Ehrlichia ruminantium*) (Karrar, 1968; Jongejan *et al*., 1984). Osman and Hassan (2003) reported that *A. lepidum* was found abundant in eastern parts of the Sudan from Torit and Kaepoetea in the south as far Kassala State in the north. In the current study, *A. lepidum* was found infesting in high abundance the local bred cattle in traditional farms. Abdallah (2007) found *A. lepidum* with higher prevalence in dry and wet sesons in all localities in South Darfur State. However, in an earlier study in Darfur, Osman (1978) in his intensive survey found only male of this tick species. This indicates that the limit distribution of this tick species has apparently extended from eastern regions to far western parts of the country. *A. variegatum* has been found highly abundance in the wet season, there was a clear pattern of seasonality for *A. lepidum*. *A. variegatum* was identified in this survey as similar to reported by Tatchell (1986), Salih *et al* (2004) and Abdallah (2007).

In the present study population density of *Boophilus annulatus* and *B. decoloratus* identified were numerous. This could be attributed to the environmental condition that is conducive to establishment of this species. Similar by Osman (1978) found *B. decoloratus* and he recorded *B. annulatus* for the first time in Darfur. The high abundance of *B. annulatus* in the current study similar to the finding of Abdallah (2007) is are evidence that this tick species was moved from southern parts of the
Sudan to as north as Nyala. This tick species is restricted to the southern parts of the central and southern Sudan (FAO, 1983; Salih et al., 2004). The highest tick count of this species was in wet season. The effect of rainfall and relative humidity was more pronounced with *A. lepidum*, *B. annulatus* and *B. decoloratus* (El Imam, 2003).
CONCLUSIONS AND RECOMMENDATIONS

The study revealed that tropical theileriosis has spread among dairy cattle in Nyala. *H. a. anatolicum* found in several farms is a confirmation that this tick species as the most efficient vector of tropical theileriosis has established in Nyala and probably in other towns of the Darfur. However, detection of *T. annulata* antibodies in farms where *H. a. anatolicum* was not found indicates that there is a possibility of *H. dromedarii* and *H. impeltatum* are playing a role in transmission of the disease. Further planned studies are required to elucidate the role of this tick species in transmission of *T. annulata* under field conditions. Molecular techniques are recommended to be used all over South Darfur region to conduct an accurate mapping of the spread of the disease and evaluate the magnitude of problem of tropical theileriosis.

It is strongly recommended not to introduce livestock from Central Sudan into South Darfur without applying strict tick control measures an animals brought to Nyala. At the same time, there must be planned programme of tick control in the farms where *H. a. anatolicum* was found for the purpose of eradicating this newly introduced tick species. Spread of this species to other farms, other towns and probably cross the international borders must be prevented.
REFERENCES


**Figueroa, J. V.; Chieves, L. P.; Johnson, G. S. and Buening, G. M. (1993).** Multiplex polymerase chain reaction based assay for detection of *Babesia bigemina*, *B. bovis* and *Anaplasma*


Kaaya, G. P. (2000). The potential for anti-tick plants as components of


**OIE. (2004).** The office international des epizootics: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Part 2 Section 2.3. Chapter 2.3.11.


development of animal resources in the Sudan. pp 163-167.


Organization for Agriculture.


Wenshum, C. and Hong, Y. (1994). Bovine and Ovine theileriosis in


contains neutralizing determinants in the C terminus. Parasite Immunology, 16: 97-104.


ملخص الأطروحة

نفذ هذا المسمح الخاص بدراسة وقائية معدل الحد المدارية في أبيض مزارع الألبان بمدينة نيالا بولاية جنوب دارفور في الفترة بهدف توفير معلومات عن وقائية اكتشاف المرض في مزارع الألبان تحت الأنظمة المختلفة المتغيرة للأبقار مع الأذن في اعتبار نوع سلالة الأبقار والجنس والعمر بالإضافة إلى دراسة وتصنيف أنواع القراد التي تنتشر بهذه المزارع وتحديد نوع القراد الناقل للمرض بكل المنطقة.

تم اختيار المزارع التي يمارس بها النظام تربية شبه المكثف وشبه الواسع والتي تتضمن بشكل رئيسي الأبقار ذات السلالات الهجين (القريزيان × السلالات المحلية) ودراسة وقائية للمرض في السلالات المختلفة، وتم تقسيم كل قطيع مستهدف إلى أربعة مجموعات عمودية هي: 

أظهرت نتائج الفحص المجهري للمسحات الدموية انتشار ملفالثاثا في عدد 43 عينة بنسبة 3.6% كما تم تحديد الثالثا أنيلاتا في 22 حالة مرضية بفحص المسحات الخزانية للليمفاوية. كما أظهرت نتائج انتشار الجسم المضادة المشع غير المباشر 31 صحة موجبة للأجسام المضادة للثالثا أنيلاتا بنسبة 31%. أظهرت الدراسة أن نظام الرياح والبيئة المدارية في المزارع بالإضافة إلى كثافة القراد في المنطقة من أكثر العوامل المساعدة في التأثير على الاصابة وانتشار المرض. إلا أن جنس الحيوان والموسم السنوي لهما تأثيراً طفيفاً على إصابة الحيوان بهذا المرض.

دلت الدراسة على نقص في كمية الهيموفيلوزي، عدد كريات الدم الحمراء وكتدس كريات الدم الحمراء في كل من الحالات التي أظهرت نتائج أسبابية للمرض بواسطة الفحص المجهري للمسحات الخزانية المفاهيمية.

أظهرت الدراسة وجود ثلاثة عشر نوع من القراد بالمنطقة، والأنواع التي سجلة بكثافة أكبر هو Boophilus annulatus، B. decoloratus، Hyalomma dromedarii، H. impeltatum، H.m. rufipes، H. truncatum، Amblyomma variegatum، Amblyoma lepidum، Hyalomma impressum، أما الأنواع التي سجلت بكثافة أقل هي H. a. anatolicum، H. a. excavatum، Rhipicephalus evertsi evertsi، R. sanguineus.

تمت مناقشة إحتمال وجود نواقل أخرى للمرض من جنس القراد هايلوما غير النوع هايلوما H. impeltatum في H. a. anatolicum، H. a. excavatum، R. sanguineus.
توصي الدراسة بعدم إدخال الأبقار ذات الدم الأجنبي من وسط السودان إلا بعد التأكد من خلوها من القراد وتشجيع التوسع والقضاء عليه في القراد الموبوءة بقراد الهالولوما أنثوي. ووضع برنامج علمي دقيق لمكافحة القراد دقيق، وانتزاع أنثوي القارع لحد من إنتشار هذا النوع من القراد إلى المدن الأخرى في الولاية.

Appendix (1) ANOVA mean squares (MS), R. squares (RS), Coeffecient varience (CV) and F values (F). for tick species infesting dairy cattle in Nyala during June 2006 to July 2007.
<table>
<thead>
<tr>
<th>Tick species</th>
<th>MS</th>
<th>RS</th>
<th>C.V</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. decolori</em></td>
<td>1.62</td>
<td>0.17</td>
<td>0.35</td>
<td>12.9***</td>
</tr>
<tr>
<td>B. annulatus</td>
<td>2.84</td>
<td>0.18</td>
<td>0.45</td>
<td>14.2***</td>
</tr>
<tr>
<td><em>H. dromedarii</em></td>
<td>1.81</td>
<td>0.19</td>
<td>0.37</td>
<td>13.2***</td>
</tr>
<tr>
<td><em>H. impeltatum</em></td>
<td>3.65</td>
<td>0.68</td>
<td>0.63</td>
<td>09.3***</td>
</tr>
<tr>
<td><em>H. m. rufipes</em></td>
<td>7.54</td>
<td>0.71</td>
<td>0.63</td>
<td>18.9***</td>
</tr>
<tr>
<td><em>H. truncatum</em></td>
<td>0.44</td>
<td>0.12</td>
<td>0.31</td>
<td>04.5***</td>
</tr>
<tr>
<td><em>A. variegatum</em></td>
<td>3.09</td>
<td>0.21</td>
<td>0.39</td>
<td>20.5***</td>
</tr>
</tbody>
</table>

*** $P \leq 0.001$