بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Prevalence and Risk Factors of camel theileriosis in Northern State, Sudan

نسبة الإصابة وعوامل الخطر لمرض الثايليريا في الابل بالولاية الشمالية – السودان

A Thesis Submitted to the College of Graduate Studies in full fulfillment of the Requirements for the Degree of Master of Science in Preventive Medicine (M.P.V.M)

By

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2018

الآية

بسْمِ اللهِ الر َحْمنِ الر َحِيمِ

DEDICATION

To my mother

To my father

To my brother

To my sister's

To all my

Great family

Acknowledgements

Firstly, praise to Almighty Allah for giving me the strength and stamina to finish this work. With a great touch of pleasure and gratitude, I would like to express thanks to my

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Abstract

A cross sectional study was conducted for determination of camel theileriosis and investigation of associated potential risk factors in Northern State, Sudan. A total of 202 blood samples from camel were collected and examined using Direct smear and Geimsa stain method.

The result indicated that camel theileriosis infection was prevalent among camel at Northern State with an overall prevalence of 3%. The following risk factors showed association with camel theileriosis in the univariate analysis under significant level of P-value ≤ 0.25: sex (P-value= 0.219), age (P-value =0.236), body condition (P-value= 0.000), previous history of disease of the animals (p-value=0.000), present of ticks in the animals (p-value=0.000) .

Using multivariate analysis to determine possible significant association between theileriosis and potential risk factors, the result showed that there was significant association with the investigated risk factors.

In conclusion the disease should be tackled seriously and control programme can make and contribute towards prevention at Northern state.

المستخلص

أجريت دراسة مقطعية بالولاية الشمالية لتقدير معدل انتشار مرض الثايليريا في الجمال والتقصي حول عوامل الخطر المرتبطة به ، تم تشخيص المرض على عدد 202 عينة باستخدام المسحة الدموية مصبوغة بصبغة جيمسا .

أظهرت الدراسة ان نسبة انتشار مرض الثايليريا في الجمال في الولاية الشمالية يعادل 3% في التحليل بإستخدام اختبار الطفح لتحليل عوامل الخطر وجدت علاقة معنوية تحت قيم معنوية أقل من او يساوي 0.25 – بين حدوث المرض وكل من عوامل الخطر التالية : جنس الحيوان (القيمة المعنوية =0.219) ، عمر الحيوان (القيمة المعنوية = 0.236) ، البنية الجسمانية (الصحة البدنية) للحيوان (القيمة المعنوية = 0.000) اصابة الحيوان بالأمراض في السابق (القيمة المعنوية = 0.000) وجود ناقل المرض (القراد) علي جسم الحيوان (القيمة المعنوية = 0.000) .

باستخدام التحليل متعدد المتغيرات المتعدد لمعرفة درجة الارتباط بين مرض الثايليريا في الجمال وبين عوامل الخطر المحتملة اظهرت النتيجة ان هناك ارتباطا كبيرا مع عوامل الخطر التي اجريت في الدراسة .

في الختام يجب معالجة هذا المرض بصورة جادة ويمكن السيطرة عليه والوقاية منه والحد من انتشاره وذلك بتقليل العوامل التي تساعد على زيادة انتشاره في الولاية الشمالية .

Introduction

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

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

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

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

Theileria are tick-transmitted, obligate intracellular parasites that are important pathogens of livestock in the tropical and subtropical regions of the world. Like all intracellular organisms, host cell invasion is a critical aspect of Theileria biology and the various stages (Sporozoites and merozoites) in the mammalian host, (the zygote and kinete in the tick vector) are highly adapted to invade and survive in their specific host cells in both the vertebrate and tick host **(** Mckeever, 2002).

Theileria parasites enter the host during tick infestation as sporozoites, which rapidly invade mononuclear leukocytes , where, they mature into macroschizonts and induce proliferation of the host cell . Macroshizonts develop further into microschizonts and ultimately into merozoites, which are released from

the leukocyte (lymphocyte). The merozoites invade erythrocytes and develop into piroplasms **.**

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

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

Objectives

Therefore, the present study was conducted to:

- To detect the causative agent of theileria in camel – in Northern state – and use the correct method for prevention .
- To determine the prevalence of theileriosis in camel in Northern state .
- To use good prevention ,control method and the risk factor of the disease .

Chapter One

Literature Review

1.1.1 Classification of the causative agent

Genus *Theileria*, Family Theileriidae, Order Piroplasmida, Subclass Piroplasmia, Phylum Apicomplexa.

Theileriae are obligate intracellular protozoan parasites that infect both wild and domestic Bovidae throughout much of the world .

• Some species also infect small ruminants.

• They are transmitted by ixodid ticks, and have complex life cycles in both vertebrate and invertebrate hosts .

• There are six identified *Theileria* spp. that infect cattle; the two most pathogenic and economically important are *T. parva* and *T. annulata* .

• *T. parva* occurs in Eastern and Southern Africa and causes East Coast fever (ECF or Corridor disease) .

• *T. annulata* causes tropical theileriosis (TT), also known as Mediterranean theileriosis and occurs in North Africa, southern Europe and Asia .

• *T. lestoquardi* (*T. hirci*) is the only species of economic significance infecting small ruminants, and it occurs in the Mediterranean basin, North Africa and Asia .

• Most theileriae are confined to Asia or Africa corresponding to the geographical distribution of their vector ticks, except for the worldwide distribution of the apathogenic *T. buffeli.*

1.1.2 Life cycle and transmission

Both *T. parva and T. annulata* are spread by ticks .The most important vector for *T. parva* is *Rhipicephalus appendiculatus*. *R. zambeziensis* in southern Africa and *R. duttoni* in Angola can also spread East Coast fever. *T annulata* is transmitted by ticks of the genus *Hyalomma* .Ticks can remain infected on the pasture for up to 2 years depending on the climatic conditions. Disease is not maintained in the absence of these field vectors . *Theileria* sporozoites are transmitted to susceptible animals in the saliva of the feeding tick (OIE 2009.)

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

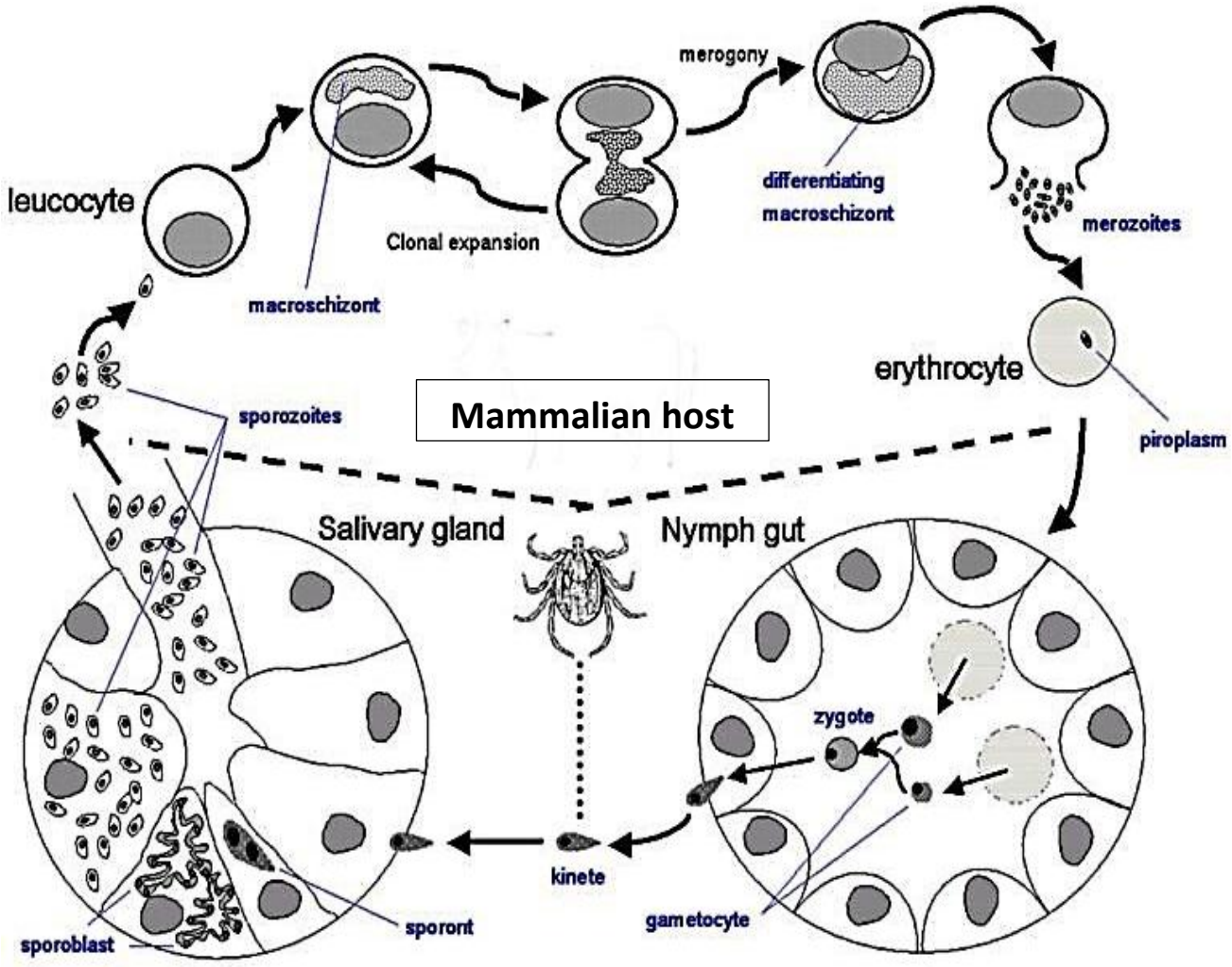


Figure (1): The life cycle of *Theileria*.

1.2 Diagnosis of theileriosis

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

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

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

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

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

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

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

Hamed *et al.* (2011) recorded the clinical signs on *Theileria* infected camels in Upper Egypt. Results revealed that 15 (6.75%) of a total 224 camels were harboring *Theileria* These 15 camels did not show any abnormal clinical signs except three cases that showed enlargement of superficial lymph nodes and fever.

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

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

• The first clinical sign of ECF is usually a swelling of the draining lymph node, usually the parotid, for the ear is the preferred feeding site of the vector; this is followed by a generalized lymphadenopathy in which superficial lymph nodes such as the parotid, prescapular, and prefemoral lymph nodes, can easily be seen and palpated, OIE (2009).

• Fever ensues and continues throughout the course of infection; this rise in temperature is rapid and may reach 106°F (42°C) , OIE (2009) .

• There is marked petechial and ecchymotic haemorrhage on most mucous membranes of the conjunctiva and the buccal cavity .

• Anorexia develops, and loss of condition follows .

• Other clinical signs may include lacrimation, corneal opacity, nasal discharge, terminal dyspnoea, and diarrhea .

• Before death the animal is usually recumbent, the temperature falls, and there is a severe dyspnoea due to pulmonary oedema that is frequently seen as a frothy nasal discharge , OIE (2009) .

• Mortality in fully susceptible cattle can be nearly 100% .

• The severity and time course of the disease depend on, among other

factors, the magnitude of the infected tick challenge (ECF is a dose dependent disease), and on the strain of parasites , OIE (2009) .

• Some stocks of parasites cause a chronic wasting disease .

• In recovered cattle, chronic disease problems can occur that result in stunted growth in calves and lack of productivity in adult cattle, however, this syndrome tends to be in the minority of recovered clinical cases , OIE (2009) .

• In a majority of cases, subclinical carriers can be recognized with apparently little or no effect on their productivity.

• A fatal condition called ‘turning sickness’ is associated with the blocking of brain capillaries by infected cells and results in neurological signs .

• Tropical theileriosis resembles East Coast fever, but jaundice and anaemia may also occur .

• In the acute disease of tropical theileriosis, death occurs 15–25 days after infection .

• Clinical signs might include pale mucous membranes (anaemia) or jaundice, as the piroplasms will precipitate destruction of red blood cells.

• During the stage when there is great production of macroschizonts within macrophages, there could be enlarged lymph nodes, and a generalised loss of condition and muscle wasting due to massive release of cytokines from infected cells , OIE (2009) .

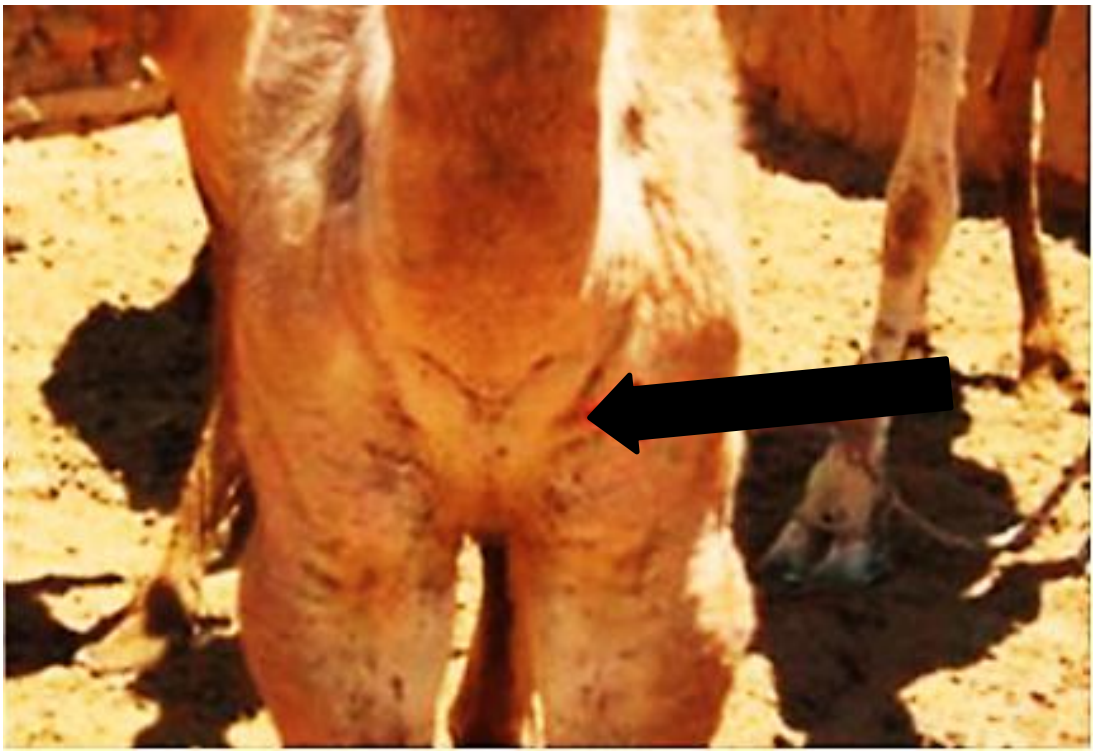


Figure (2): Bilateral enlargement of superficial cervical lymph nodes in a camel .

1.3.1 Parasitological examination of blood and lymphnode smears

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

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

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

1.3.2 Laboratory diagnosis

• In live animals, theileriosis is diagnosed by the identification of schizonts in thin smears from blood, lymph node .

• At necropsy, schizonts may be found in impression smears from most internal organs .

• Polymerase chain reaction (PCR) tests and DNA probes are sometimes used to detect and identify *Theileria* species .

• Antibodies to *T. parva* and *T. annulata* can be detected with an enzyme-linked immunosorbent assay (ELISA), but not commercially available anymore) or an IFA test .

• Serological tests may not be sensitive enough to detect all infected cattle, and cross–reactions can occur with other species of *Theileria* ,laboratory diagnosis according to that in OIE (2009).

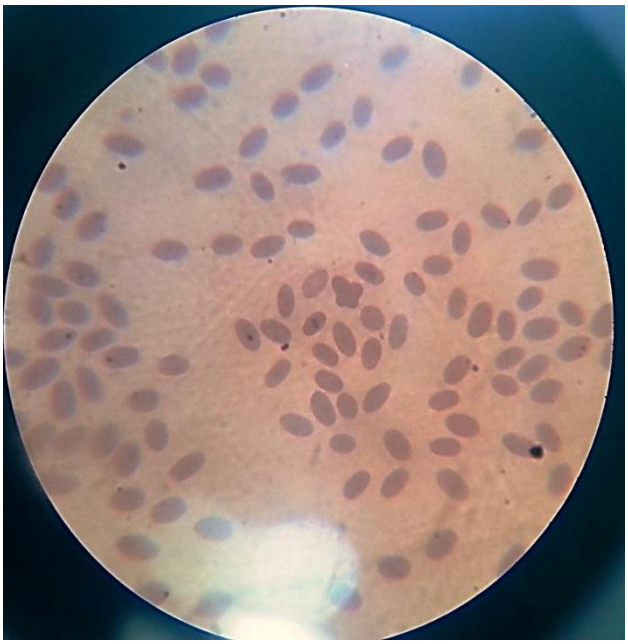


Figure (3) : Blood film from infested camels stained by Giemsa stain showing schizont of Theileria camelensis.

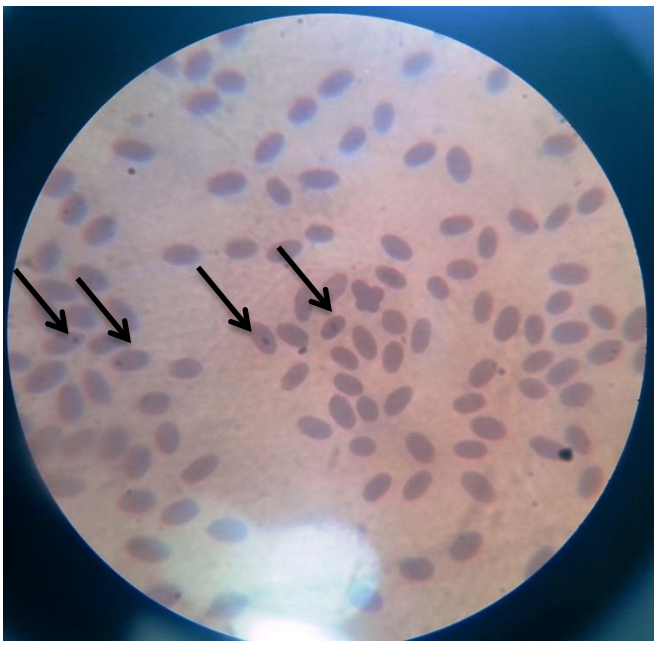
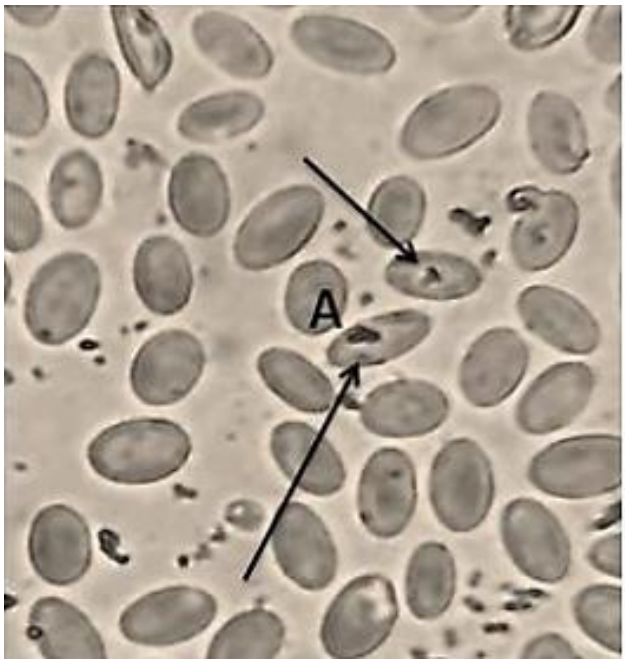


Figure (4) : arrows pointed to schizonts

*The study below reveal same results :

Twenty four from 241 examined camels were found infection with *Babesia* spp at infection rate of9.95%based on Giemsa stained blood smears ,more over the *Babesia* spp was identified as large pear shape and arranged in pairs with acute or wide angles near the margin of the infected RBCs figure (1). On the other hand the microscopic examination revealed that only 14 from 241 examined camels were found infected with *Theileria* spp at infection rate of 5.8%. *Theileria* spp were detected in RBCs with two forms include ;rod and comma shape as in figure (2). Hussein *et al* .(2015).



Figure(5) :Blood smear of camel infected with *Babesia spp (*A: large pear shape and arranged in pair inside RBCs)(Giemsa stain, 1000x)

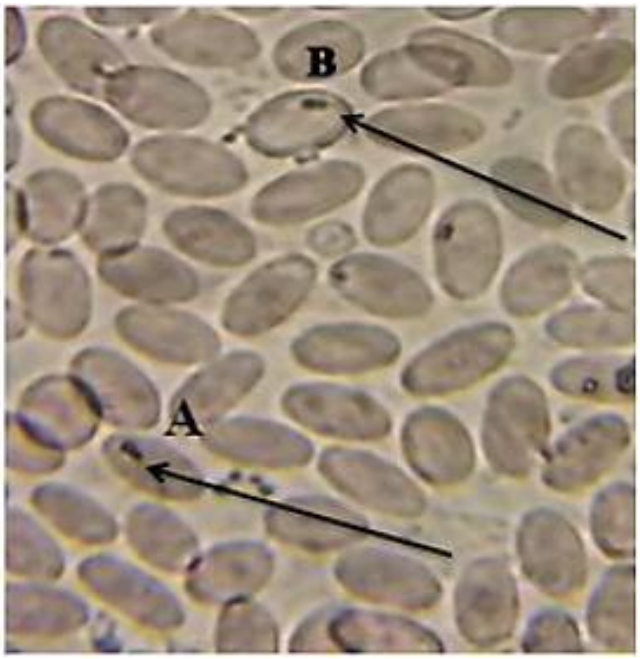


Figure (6): Blood smear of camels infected with *Theileria spp* (A: coma shape, B: rod shape inside RBCs) (Giemsa stain, 1000x)

A) Samples:

The schizont is the pathogenic stage of *T. parva* and *T. annulata.* It initially causes lymphoid proliferation, and later lymphoid destruction. Schizont-parasitised cells may be found in

• Blood or Buffy coat smears air-dried and fixed in methanol for demonstration of schizonts .

• Lymph node for demonstration of schizonts .

• Impression smears from lung, spleen, kidney and lymph node, air-dried and fixed in methanol, for demonstration of schizonts .

• Lung, kidney, brain, liver, spleen, and lymph nodes for histopathology: demonstration of schizonts and infiltrations of immature lymphocytes .
• A nervous syndrome called ‘turning sickness’ is sometimes observed and intravascular and extra vascular aggregations of schizont-infected lymphocytes are observed, causing thrombosis and ischaemic necrosis throughout the brain .
• Serum for antibody detection ,OIE (2011) .

B) Procedures

Identification of the agent

• The presence of multinucleate intracytoplasmic and free schizonts, in lymph node biopsy smears, is a characteristic diagnostic feature of acute infections with *T. parva* and *T. annulata* .
• The demonstration of schizont-infected cells in Giemsa-stained blood smears, lymph node impression smears, or histological sections, is diagnostic of ECF .
• Small piroplasms in erythrocytes are suggestive of ECF, but diagnosis must be confirmed by the detection of schizonts .
• Schizonts can be detected in sections but are best seen in smears of lymph node biopsies .

As there is considerable similarity between schizonts of other theileria parasites (*T. mutans*, *T. velifera*, *T. taurotragi* and *T. buffeli*), which may co-infect an animal, it is important to differentiate the infecting species; this can be done by using serological and DNA-based assays .

• Piroplasms of most species of *Theileria* 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 .

• Relapse parasitaemia can be induced with some *Theileria* species by splenectomy .

• Piroplasms are also seen in prepared smears at post-mortem, but the parasites appear shrunken and their cytoplasm is barely visible .

• A range of probes is available to detect all the *Theileria* species that are known to infect cattle and are based on 18S ribosomal RNA gene sequences .

• A number of PCR methods (targeting sequences TpR, p104, p67, PIM) can be used to detect *T. parva* and *T. annulata* .

Serological tests

• The most widely used diagnostic test for *Theileria* species is the IFA test; both schizont and piroplasm antigens may be used the IFA test is sensitive, fairly specific, and usually easy to perform because of the problems of cross-reactivity among some *Theileria* species, the test has limitations for large-scale surveys in areas where species distribution overlaps the IFA test for *T. parva*, does not distinguish among the different immunogenic stocks .

• The new indirect ELISAs for *T. parva*, and *T. mutans*, based on recombinant parasite-specific antigens, have demonstrated higher sensitivity and specificity and have largely replaced the IFA tests

previously used in Africa .

• Serological tests based on the ELISAs are being used increasingly for the detection of parasitespecific antibodies .

• ELISAs have been successfully adapted for the detection of antibodies to *T. annulata,* and have been shown to detect antibodies for a longer period of time than the IFA. Indirect ELISAs for *T. parva* and *T. mutans* have been extensively evaluated in the laboratory and the field, and are now being used in large parts of Africa .

These tests provide higher (over 95%) sensitivity and specificity than IFA tests but are not available commercially .

laboratory diagnostic methodologies, OIE (2011).

1.4 Prevention and control

1.4.1 Sanitary prophylaxis

• Bovine theileriosis is generally controlled by the use of acaricides to kill ticks, but this method is not sustainable .

• Acaricides are expensive, they cause environmental damage, and over time ticks develop resistance to them requiring newer acaricides to be developed .

• More sustainable and reliable methods for the control of theileriosis that deploy a combination of strategic tick control and vaccination are desirable, however, these are yet to be successfully applied on a large scale in endemic areas .

• Sanitation and disinfection measures are not generally effective in preventing transmission of theileriosis .

1.4.2 Medical prophylaxis

• Chemotherapeutic agents such as buparvaquone are available to treat *T. parva* and *T. annulata* infections .

• Treatments with these agents do not completely eradicate theilerial infections and lead to the development of carrier states in their hosts

• Recovery from one strain of *T. annulata* confers cross-protection against most other strains .

• Complete cross-protection does not occur with *T. parva* .

1.4.3 Vaccination

I) Inactivated vaccines

• None are available

II) Live attenuated vaccines

• Reliable vaccines of known efficacy have been developed for *T. parva* and *T. annulata* .

• For *T. annulata*, the vaccine is prepared from schizont-infected cell lines that have been isolated from cattle and attenuated during in-vitro culture .

• The vaccine must remain frozen until shortly before administration .
• Vaccination against *T. parva* is based on a method of infection and treatment in which cattle are given a subcutaneous dose of tick derived sporozoites and a simultaneous treatment with a longacting tetracycline formulation .This treatment results in a mild or inapparent East Coast fever reaction followed by recovery . Recovered animals demonstrate a robust immunity to homologous challenge, which usually lasts for the lifetime of an animal .
• Immunisation of animals with a stock(s) engendering a broad-spectrum immunity is desirable to cover a range of immunological *T. parva* strains that exist in the field.
• Immunised animals usually become carriers of the immunising parasite stock(s) .
• Consideration should be given to the risk of introducing new isolates into an area where they may then become established through a carrier state .

III) Recombinant vaccines

• Experimental subunit vaccines are being developed for ECF, and . ideally will contain antigens from both sporozoite (as the p67 protein) and schizont stages. An improved p67 vaccin has been tested in the field and might be available soon , OIE (2011).

1.5 PUBLIC HEALTH

There is no evidence that *T. parva* or *T. annulata* are hazards to humans.

1.6 Incidence of camel theileriosis in the world

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

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

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

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

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

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

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

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

1.7 The prevalence of camel theleriosis in Egypt

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

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

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

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

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

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

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

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

anatolicum excavatum).

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

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

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

Chapter Two

Materials and Methods

2.1 Study area

The study was carried out in Northern State, Sudan , which is located about 310 Km north of Khartoum . 20◦32 E longitudes and 16◦22 N latitude. The temperature ranges from 5◦C in at winter and 49◦C at summer and the humidity less than 20% . the mean annual rainfall of the state is about 250mm/year .

2.2 Study design and sampling

A cross-sectional study design was conducted in Northern State for determination of theileriosis in export camels. Information regarding age, sex, origin of the animals was recorded during collection of samples . The ages of the animals were determined based on owners' information. Furthermore, previous history of treatment with any theilerial drugs was also recorded. Sampling technique was applied to select animals at the study area.

2.3 Sample size determination

Sample size was calculated according to the formula given

$$N = \frac{4 \times P \times Q}{L2}$$

N= sample size P=expected prevalence L=desired absolute precision

Q=(1-P) (Martin *et al* .1987)

* After calculation the result was 101head of camels and it multiplied by two to be 2 X 101 = **202** head of camels – Just to strengthening the result of the study .

2.4 Sample collection and transportation

Blood sampling were done after proper restraining of the animal according to Urquhart *et al.* (1996). Before blood collection, the area of puncture was cleaned, hair removed and disinfected with 70% alcohol. Thin smears was prepared by applying the slide with blood on to a clean slide at an angle of 45° and then gently moving forward. The slide was air dried and fixed for 2 minutes in methyl alcohol (absolute methanol). Soon after the slides were fixed and air dried it was entered into slide box and transported to Veterinary Parasitology Laboratory for examination of the parasites.

2.5 Laboratory investigation procedures

Giemsa staining procedures and microscopic examination of slides was conducted according to OIE .(2009) .The slides was immersed in Giemsa stain (1:10 solution) in staining rack for 30 minutes. Then the slides was washed with distilled water to remove excess stain and made air dry. The stained blood smears was examined under oil immersion lens of microscope (100X) for appreciation and identification of different *Theileria spp* according to their morphological characteristics.

2.6 Examination of camels

Camels were clinically inspected for the presence of ticks according to Köhler-Rollefson *et al* . (2001) and the infested cases were subjected to detailed clinical examination and samples collection. Abnormal clinical findings were recorded. Blood samples were collected from 202 camels into clean and dry sterile tubes containing Ethylene Diamine Tetra-acetic Acid (EDTA) as an anticoagulant. These samples were used for preparation of blood films. Thin blood films from each camel were prepared, fixed by methanol stained with Giemsa stain (1:10),by tap water and upright air dried and then examined microscopically Burgdorfer,(1970) **.**The erythrocytic form of *Theileria* were rod, rounded and ring shaped.

2.7 Data analysis

The data collected and was analyzed by the statistical software called SPSS for Windows (Stata Corp. College Station, USA). The prevalence was calculated by dividing the number of camel found to be positive for *Theileria* by the total number of camel examined for *Theileia* spp. The association of risk factors like age, sex, and history of treatment with positivity for theileiosis was assessed using Chi-square test.

Chapter Three

Results

Thin blood smears and Geimsa stain method were used in 202 blood of camels for examination of theileriosis. Six animals were found positive (3%) and 197 animals were negative (97%) to camel theileriosis (Table: 1). Therefore the overall prevalence of camel theileriosis in Northern state was 3%.

Table 1: The Prevalence of camel theileriosis in 202 camels in Northern State-Sudan.

	Frequency	Percent
Positive	**6**	**3.0**
Negative	**196**	**97.0**
Total	**202**	**100.0**

3.1 Sex of animal

Total number of female examined was 11 animals. Among these, 1 animal were found infected. The rate of infection was 16.7%. Total number of males examined was 191 . Among these, 5 animals were found infected. The rate of infection was 83.3% (Table 2) and (Table 2, 3).

The Chi-square test, showed that there was significant association between theileriosis and sex of animals (p-value =0.219) (Table 4).

3.2 Age of animal

The number of less than or equal 5 year of age was 87 animals. Among these, 4 animals were found infected. The rate of infection was 4.6 %. While the number of

animals more than 5 years was 115 animals. Among these, 2 animals were found infected. The rate of infection was 1.7% (Table 2,3).

In the Chi-square test, the result showed that there was significant association between theileriosis and age of animals (p-value =0.236) (Table 4).

3.3 Body condition of animal

The body condition of animals and the presence of the theileriosis had been investigated. 151 animals were found in good condition. Among these no animals were found infected and the rate of infection was 0.0 %. Fifteen animals in poor condition among these 3 animals were found infected and the rate of infection was 20.0%, while 36 animals in moderate condition and among these 3 animals was found infected and the rate of infection was 8.3% (Table 2,3). The Chi square test showed that there was highly significant association between theileriosis infection and body condition (p-value=0.00) (Table 4).

3.4 Previous history of disease of animal

About 12 animals were found had previous history of disease among these 5 animals were found infected. The rate of infection was 41.7%.and 190 animals were found without previous history of disease among these 1 animal was found infected and the rate of infection was 0.5% .The Chi square test showed that there was highly significant association between theileriosis infection and previous history of disease (p-value=0.00) (Table 4).

3.5 presence of ticks in the animals

One hundred and six animals were found infested by ticks among these 6 animals were infected .The rate of infection was 5.7% and 96 animals were found free of ticks and among these there is no animals were found infected with theileriosis and the rate of infection was 0.0% .The Chi square test showed that there was highly significant association between theileriosis infection and present of ticks in animals (p-value=0.018) (Table 4)

Table 2 : Summary frequency for the distribution of 202 camel examined for theileriosis in Northern State – Sudan.

Risk factor	Frequency	Relative frequency (%)	Cumulative frequency (%)
Sex of animal :			
Female	11	5.4	5.4
Male	191	94.6	100
Age of animal :			
≤year	87	43.1	43.1
>year	115	56.9	100
Body condition of animal:			
Good	151	74.8	74.8
Pour	15	7.4	82.2
Moderate	36	17.8	100
previous history of disease of animal:			
	12	5.9	5.9
Present	190	94.1	100
Not present			
present of ticks in animal :			
Yes	106	52.5	52.5
No	96	47.5	100

Table 3 : Summary cross-tabulation of theileriosis in 202 camel examined in Northern State – Sudan

Risk factor	Animals tested	Animals affected	Affected %
Sex of animal :			
Female	11	1	9.1
Male	191	5	2.6
Age of animal :			
≤year	87	4	4.6
>year	115	2	1.7
Body condition of animal:			
Good	151	0	0.0
Pour	15	3	20
Moderate	36	3	8.3
previous history of disease of animal:			
Present	12	5	41.7
Not present	190	1	0.5
present of ticks in animal :			
Yes	106	6	5.7
No	96	0	0.0

Table 4 : Summary of univariate analysis for risk factors associated with camel(N=202) theileriosis in Northern State, Sudan

Risk factor	No. inspected	No. affected (%)	Df	p- value
Sex of animal :				
Female	11	1 (9.1)	1	0.219
Male	191	5 (2.6)		
Age of animal :				
≤year	87	4 (4.6)	1	
>year	115	2 (1.7)		0.236
Body condition of animal:				
Good	151	0 (0.0)	2	0.000
Pour	15	3 (20)		
Moderate	36	3 (8.3)		
previous history of disease of animal:				
Present	12	5 (41.7)	1	0.000
Not present	190	1 (0.5)		
present of ticks in animal :				
Yes	106	6 (5.7)	1	0.000
No	96	0 (0.0)		

Chapter Four

4.1 Discussion

Pathogenic protozoa belonging to the order Piroplasmida include *Babesia* species and *Theileria* species are common pathogens transmitted by ticks and are of significant importance in many domestic animals, including camels Wernery and Kaaden,(1995).Theileriosis is considered to be the second most important hemoprotozoal disease following trypanosomosis affecting dromedary camels in tropical and subtropical countries (Gatt-Rutter, 1967, Mishra *et al.*, 1987, Nassar, 1992, El-Refaii *et al*.,1998, Mazyad and Khalaf, 2002) and there are different types of *Theileria spp* implicated as etiologic agents of the disease. However, *Theileria camelensis* appears to be the principal cause of camel theileriosis particularly in Egypt (Gatt-Rutter, 1967, Barnett, 1977, Boid *et al*., 1985, Mishra *et al*., 1987, El-Fayoumy *et al*., 2005). The current work indicated that 3 % (6 of 202) of the examined camels were harboring the erythrocytic forms of *Theileria spp* and most of the positive cases had no apparent characteristic clinical signs. This may be attributed to the chronic nature of *Theileria* infection and/or to the investigated *Theileria camelensis* was probably apathogenic Boid *et al.*, (1985)**.** Nassar (1992) examined 200 apparently healthy camels under Egyptian field conditions and found that 30% of them were infected with *Theileria camelensis*. This may indicate that theileriosis in camels is symptomless infection. However, there were three (1out of 6 animals , 16%) camels in the present study with characteristic enlargement of the lymph nodes, in particular, the superficial cervical nodes, in association with systemic reaction in the form of fever, polypnea and tachycardia. These infected cases were young and might be under stresses because they were debilitated. Similar clinical signs of theilerial infection in one-humped camels were previously reported by El-Fayoumy *et al*. (2005). The prevalence rate

of *Theileria* infection in one-humped camel reported by Nassar (1992), El- Refaii *et al*. (1998), El-Fayoumy *et al*. (2005) were 30 % (60 of 200), 62.1 % (46 of 74) and 44.8 % (56 of 125) respectively, which are higher than that reported by the present study. Such variations may ascribe to several reasons, including different localities, population density of camels, environment and hygienic measures. Furthermore, the sharply pendulous changes in the desert environment have a strong effect on the prevalence of various hemoprotozoal infections of small ruminants and camels Bahy *etal*., (2008). *Hyalomma dromedarii* is the principal vector in transmission of *Theileria camelensis* in vertebrates Hoogstraal, (1956). *Theileria* has various developmental stages of different shapes and forms inside the vector (ticks). These forms were observed in the haemolymph and gut smears as ring form, slender spine-like form, an elongated structure, round form, and enclosing centrally located nucleus surrounded by a cloud-like dispersed cytoplasm, which was reported by El-Refaii *et al*. (1998). From this results the disease should be controlled.

4.2 Conclusion and Recommendation

Conclusion

The prevalence of infection of the camels with theileriosis in Northern state was (3%) . while the total animal tested was (202 head of camel) . From total animals tested (202) only 6 animals were found infected (+ve) and 196 animals were found non infected (-ve) .Total number of female examined was 11 animals. Among these, 1 animal were found infected. The rate of infection was 16.7%. Total number of males examined was 191 . Among these, 5 animals were found infected. The rate of infection was 83.3% .The number of less than or equal 5 year of age was 87 animals. Among these, 4 animals were found infected. The rate of infection was 4.6 %. While the number of animals more than 5 years was 115 animals. Among these, 2 animals were found infected. The rate of infection was 1.7% .The body condition of animals and the presence of the theileriosis had been investigated. 151 animals were found in good condition. Among these no animals were found infected and the rate of infection was 0.0 %. Fifteen animals in poor condition among these 3 animals were found infected and the rate of infection was 20.0%, while 36 animals in moderate condition and among these 3 animals was found infected and the rate of infection was 8.3% . About 12 animals were found had previous history of disease among these 5 animals were found infected. The rate of infection was 41.7%.and 190 animals were found without previous history of disease among these 1 animal was found infected .and the rate of infection was 0.5% .One hundred and six animals were found infested by ticks among these 6 animals were infected .The rate of infection was 5.7% and 96 animals were found free of ticks and among these there is no animals were found infected with theileriosis and the rate of infection was 0.0% .

Recommendation

- Using of cypermethrine for spraying camels especially transport camels .
- Awareness of breeder and helping them to fight all external and internal parasite cooperatingly .
- This examination can be fixed by using the PCR test.
- And also classifying of ticks can be do to demonstrate the real vector of the causative agent (*Theileria*) .
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4.4 Appendix

Frequency Table

result of diseases

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	+ve	6	3.0	3.0	3.0
	-ve	196	97.0	97.0	100.0
	Total	202	100.0	100.0	

age of animal

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	less than or equal 5years	87	43.1	43.1	43.1
	more than 5 years	115	56.9	56.9	100.0
	Total	202	100.0	100.0	

sex of the animal

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	female	11	5.4	5.4	5.4
	male	191	94.6	94.6	100.0
	Total	202	100.0	100.0	

Body condition

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	good	151	74.8	74.8	74.8
	pour	15	7.4	7.4	82.2
	modrate	36	17.8	17.8	100.0
	Total	202	100.0	100.0	

previous history of disease

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	present	12	5.9	5.9	5.9
	not present	190	94.1	94.1	100.0
	Total	202	100.0	100.0	

present of the ticks

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	106	52.5	52.5	52.5
	No	96	47.5	47.5	100.0
	Total	202	100.0	100.0	

Data analysis

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
result of diseases * age of animal	202	100.0%	0	0.0%	202	100.0%
result of diseases * sex of the animal	202	100.0%	0	0.0%	202	100.0%
result of diseases * body condition	202	100.0%	0	0.0%	202	100.0%
result of diseases * previous history of disease	202	100.0%	0	0.0%	202	100.0%
result of diseases * present of the ticks	202	100.0%	0	0.0%	202	100.0%

result of diseases * age of animal

Crosstab

			age of animal		Total
			less than or equal 5years	more than 5 years	
result of diseases	+ve	Count	4	2	6
		% within result of diseases	66.7%	33.3%	100.0%
		% within age of animal	4.6%	1.7%	3.0%
	-ve	Count	83	113	196
		% within result of diseases	42.3%	57.7%	100.0%
		% within age of animal	95.4%	98.3%	97.0%
Total		Count	87	115	202
		% within result of diseases	43.1%	56.9%	100.0%
		% within age of animal	100.0%	100.0%	100.0%

result of diseases * sex of the animal

Crosstab

			sex of the animal		Total
			female	male	
result of diseases	+ve	Count	1	5	6
		% within result of diseases	16.7%	83.3%	100.0%
		% within sex of the animal	9.1%	2.6%	3.0%
	-ve	Count	10	186	196
		% within result of diseases	5.1%	94.9%	100.0%
		% within sex of the animal	90.9%	97.4%	97.0%
Total		Count	11	191	202
		% within result of diseases	5.4%	94.6%	100.0%
		% within sex of the animal	100.0%	100.0%	100.0%

result of diseases * body condition

Crosstab

			body condition			Total
			good	pour	modrate	
result of diseases	+ve	Count	0	3	3	6
		% within result of diseases	0.0%	50.0%	50.0%	100.0%
		% within body condition	0.0%	20.0%	8.3%	3.0%
	-ve	Count	151	12	33	196
		% within result of diseases	77.0%	6.1%	16.8%	100.0%
		% within body condition	100.0%	80.0%	91.7%	97.0%
Total		Count	151	15	36	202
		% within result of diseases	74.8%	7.4%	17.8%	100.0%
		% within body condition	100.0%	100.0%	100.0%	100.0%

result of diseases * previous history of disease

Crosstab

			previous history of disease		Total
			present	not present	
result of diseases	+ve	Count	5	1	6
		% within result of diseases	83.3%	16.7%	100.0%
		% within previous history of disease	41.7%	0.5%	3.0%
	-ve	Count	7	189	196
		% within result of diseases	3.6%	96.4%	100.0%
		% within previous history of disease	58.3%	99.5%	97.0%
Total		Count	12	190	202
		% within result of diseases	5.9%	94.1%	100.0%
		% within previous history of disease	100.0%	100.0%	100.0%

result of diseases * present of the ticks

Crosstab

			present of the ticks		Total
			yes	no	
result of diseases	+ve	Count	6	0	6
		% within result of diseases	100.0%	0.0%	100.0%
		% within present of the ticks	5.7%	0.0%	3.0%
	-ve	Count	100	96	196
		% within result of diseases	51.0%	49.0%	100.0%
		% within present of the ticks	94.3%	100.0%	97.0%
Total		Count	106	96	202
		% within result of diseases	52.5%	47.5%	100.0%
		% within present of the ticks	100.0%	100.0%	100.0%