Sudan University of Science and Technology

College of Graduate Studies

Prevalence and Risk Factors Associated with Bovine Theileriosis and Ticks Infestation in Northern State-Sudan

نسبة الإصابة وربطها بعوامل الخطر لمرض الثايليريا في الأبقار وانتشار القراد بالولاية الشمالية ـ السودان

A thesis Submitted to the College of Graduate Studies in the Fulfillment of Requirements for Master Degree in Preventive Medicine (M.P.V.M)

By

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Dedication

To my Mother

To my Father

To My Wife

To My Brother

To my Sister

To my Sons

To All My

Great Family

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Firstly thanks Allah for giving me the strength to finish this work, then i would like to express many thanks to Professor, Mohammed Abd Elsalam Abdella and professor Siham Elias Suleiman For Advice, encourage and continues support during practical and theses .I would like thank to general director of animal resources, fisheries northern state , central veterinary research laboratory staff, and Dongula research laboratory, for facilities.

My heart fell thanks to Professor Ahmed Elghali Elmakhtar, who facilitated all difficulties during laboratory effort.

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Abstract

This study was conducted for determination of bovine theileriosis and investigation of associated potential Risk Factors and cattle tick infestation in Northern State, Sudan. A total of 329 blood samples were collected randomly from cattle from January 2018 to January 2019 and examined for blood parasite using direct smear Geimsa stain method. Questionnaire was included; locality, breed, sex, age and season. Also 1252 tick samples were collected from 313 cattle in 70% ethanol for identification. Blood smears were reveled 11.6% prevalence rate of the disease. The following risk factors showed association with cattle theileriosis in the univariate analysis under significant level of P-value $\leq$ 0.25: sex (P-value= 0.432), age (P-value =0.001), breed (P-value= 0.000) , previous history of disease of the animals (p-value=0.005), present of ticks in animals (p-value=0.000) , there was two genera of ticks were identified which were *Hyaloma* and *Rhibocephalus*. Six species of these two genera were *H.rufipus*, *H.trancatum* *H.dromadarii*, *H.impltatum, H.anatolicum* and *R.evansi*. In conclusion, the disease is prevalent in Northern state.

المستخلص

أجريت هذه الدراسة لتحديد انتشار داء الثيلريا البقري والتحقيق في عوامل الخطر المحتملة المصاحبة وانتشار قراد الماشية في الولاية الشمالية بالسودان. تم جمع 329 عينة دم من الماشية بشكل عشوائي من يناير 2018 إلى يناير 2019 وتم فحصها بحثًا عن الطفيليات باستخدام طريقة المسحة المباشرة لصبغة Geimsa. تم تضمين الاستبيان المكان, النسل، النوع ، الجنس ، العمر والموسم. كما تم جمع 1252 عينة قراد من 313 ماشية في 70٪ الإيثانول لمعرفتها. تم الكشف بواسطة مسحة الدم عن معدل انتشار المرض 11.6 ٪. وأظهرت عوامل الخطر التالية الارتباط مع داء الثيلريا الماشية في التحليل وحيد المتغير تحت مستوى كبير من قيمة $P \leq 0.25$: الجنس (قيمة P =0.432) ، والعمر (قيمة P = 0.001) ، والسلالة(قيمة P = 0.000) ،وإصابة الحيوان بالأمراض في السابق (قيمة 0.005) ، وجود ناقل المرض القراد في الحيوان(قيمة0.000) . كان هناك اثنين من أجناس القراد التي تم تحديدها والتي كانت هيالوما ورايبوسيفالوس. أنواع الجنس من هذين النوعين هي *H.rufipes* و *H.dromadarii* و *H.impltatum* و *H.anatolicum* و H.trancatum و *R.evansi*. في الختام ، فإن المرض منتشر في الولاية الشمالية.

Table of Contents

List of Tables

List of Figures

Introduction

Theileriosis is group of diseases caused by the protozoan parasite of genus *Theileria* and transmitted only after cyclical development in ixodid ticks the signs of illness in cattle include enlargement of super facial lymph nodes namely parotid and pre scapular (Rbison, 1982). Rise in body temperature, difficult breathing and frothy exudates from nostril s are reported (Boulter and Hall, 2000). Diarrhea, lacrimation which may lead to corneal opacity or complete blindness and loss of appetite are often present (Norval *et al.*, 1992). *Theileria annulata* infection causes severe economic losses including losses due to expensive anti-theilerial drugs ,cost of prevention and control measurement ,losses due mortality and drop of milk productivity of infection . pregnant cow may also abort and remain infertile for long time (latif, 1994). The causative agent of tropical theileriosis is maintained exclusively in cattle with rang of distribution of vector *Hyalomma anatolicum* (FAO,1983). Theilerial infection wide spreading in Sudanese cattle (Shommein, 1976; Morzaria *et al*.,1981; FAO, 1983). *Theileria annulata* infection in cattle is the most important Tick born disease in Northern Sudan -River Nile State (Elhussein *et al*., 2004).

Tick born disease constitute major constrain of livestock production and have considerable economic impact (Jongejan and Ulineberg,2004). The theileriosis occurs in wide zone of the Africa, southern Europe and large part of Asia (Dolan,1989). Tropical theileriosis is the most important. In the Sudan (Salih *et al* ., 2007) reported that 14%of cattle in South Darfur were positive for *T.annulata* antibodies.*Theileria annulata* as major consider to development of dairy industry in Northern Sudan (Osman,1990).Mohammed (1992) reported prevalence of 38.5% of theileriosis in pure exotic dairy breed and 38.7 - %-45% in crossbreed in Khartoum State. Tropical theileriosis represented 15-18% of all bovine cases admitted for treatment at Atbara vet hospital during 1991-1993 (Elghali, Elhussein (1995).More than 70 species of ticks representing the Sudanese fauna had been identified (Hoogstraal 1956). Morzaria et al.,(1981) they found that *Hyalomma anatolicum* to be chief vector ,*H .dromedarii , H.rufipus , H.impletatum* , were experimentally shown to transmit the parasite . They transmit a large number of pathogens than any other vector group. The

most economically important ticks of livestock in Sudan belong to the genera *Hyalomma anatolicum*, *Rhipicephalus, Rhipocephalus (Boophilus*) and *Amblyomma* (El Hussein *et al*., 2012). Salih, et *al*., (2004) found 4tick genera and 11speceis distribution on cattle in Northern Sudan the identified ticks included *Amplyomma, Heloma, Rhibocephalus* groups.

The picture of tick infestation and theileriosis in cattle in Northern State is not yet clear.(General Director of Animal Resources, Fisheries Northern State 2017)

Objective

The objective of this study

- To determine the prevalence of theileriosis in cattle in Northern State.

-To Study risk factor associated with the disease.

-To identify ticks infesting in cattle in study area.

Chapter One

Literature Review

1.1-Pathogenic and effect of Bovine Theileriosis: -

Theileria annulata, the cause of tropical theileriosis, occurs in large parts of the Mediterranean coast of North Africa extending to northern Sudan, and South-eastern Europe, the Near and Middle East, India, China and Central Asia are also affected. Endemic regions of T.*annulata* and *T. parva* do not overlap (OIE,2009).*Theileria. taurotragi* generally cause no disease or mild disease and *T.mutans*non-pathogenic. These latter three 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*, occurring in the East, and *T. buffeli T. orientalis(referred* to as *T.buffeli*) with a global distribution (OIE,2009).

1.2-Vector (Ticks infestation and identification)

Tick and tick born disease are wide spread in the Sudan constituting serious on production of sound milk and development of meat industry (Dolan,1989). More than 63 species of ticks representing the Sudanese fauna had been identified (Hoogstraal, 1956).

Morzaria and Penderson.,(1981) found that *Hyalomma anatolicum* to be chief vector *T.annulata* ,*H .dromedarii* , *H.rufipus* , *H.impletatium* , were experimentally shown to transmit the parasite. Theileriosis is caused by *Theileria annulata* and transmitted by *H. anatolicum* in Sudan and by *H. scupense* in North Africa (Gharbi *et al*., 2014). Salih *et al.*, (2004) found 4tick genera and 11speceis infesting in Northern Sudan the identified genera included *Amplyomma, Hayalomma, Rhibocephalus* groups.

Shommein *et al*., (1976) reported the mean vector of cattle *T.annulata* Sudan common ticks transiting are *H. anatolicum*.

In Sudan ticks are responsible for much of the economic losses that occur in animals especially foreign breeds (Elhaj et al,(2003) . El Ghali and Hassan (2012) found ticks threaten livestock industry not only through their role in transmitting fatal diseases but also by direct damages including loss of body decrease in weight gain and milk production damage of hides and udders and injection of toxins (Jongejan and Uilenberg 2004). Elhussein *et al*., (2015) found three genra and eleven species of ticks in White Nile-Sudan.

1.3-Classification of the causative agent: -

Phylum *Apicomplexa,* Genus *Theileria,* Family *Theileriidae* Order *Piroplasmida,* Subclass Piroplasmia . *Theileria* are obligate intracellular protozoan parasites that infect both wild and domestic Bovidae throughout much of the world Some species also infected small ruminants.*T. lestoquardi(T.hirci*) is the only species of economic significance infecting small ruminants and it occurs in the Mediterranean . Most *theileriae* are confined to Asia or Africa corresponding to the geographical distribution of their vector ticks except for the worldwide distribution of the pathogenic. *Buffeli* (*OIE,* 2011).

1.4-life cycle and transmission: -

Ordinarily, *T.parva* and *T. annulata* only mature and enter the saliva after the tick attaches to a host usually a tick must be attached for 48–72 hours before it becomes infective however if environmental temperatures are high infective sporozoites can develop in ticks on the ground and may enter the host within hours of attachment. Transovarial transmission does not occur with either *T.parva or T. annulata*. Inside the host, *Theileria* sporozoites undergo a complex life cycle involving the replication of schizonts in leukocytes and piroplasms in erythrocytes Cattle that recover from *Theileria* infections usually become carriers. Then Both *T. parva* and *T.annulata* are spread by ticks (OIE, 2009).

The most important vector for *T. parvais 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* .

Theileria porozoites are transmitted to susceptible animals in the saliva of the feeding tick (OIE scientific and technical department,2009) then mentioned 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 epithelial cells where it remains during the tick molt cycle and develops into a single motile kinetic. The motile kinetic egresses the gut cell and subsequently invades the salivary glands where another round of a sexual multiplication. Sporangia occurs, producing many thousands of sporozoites. These are injected into a mammalian host when the tick feed figure (1) (OIE, 2009).

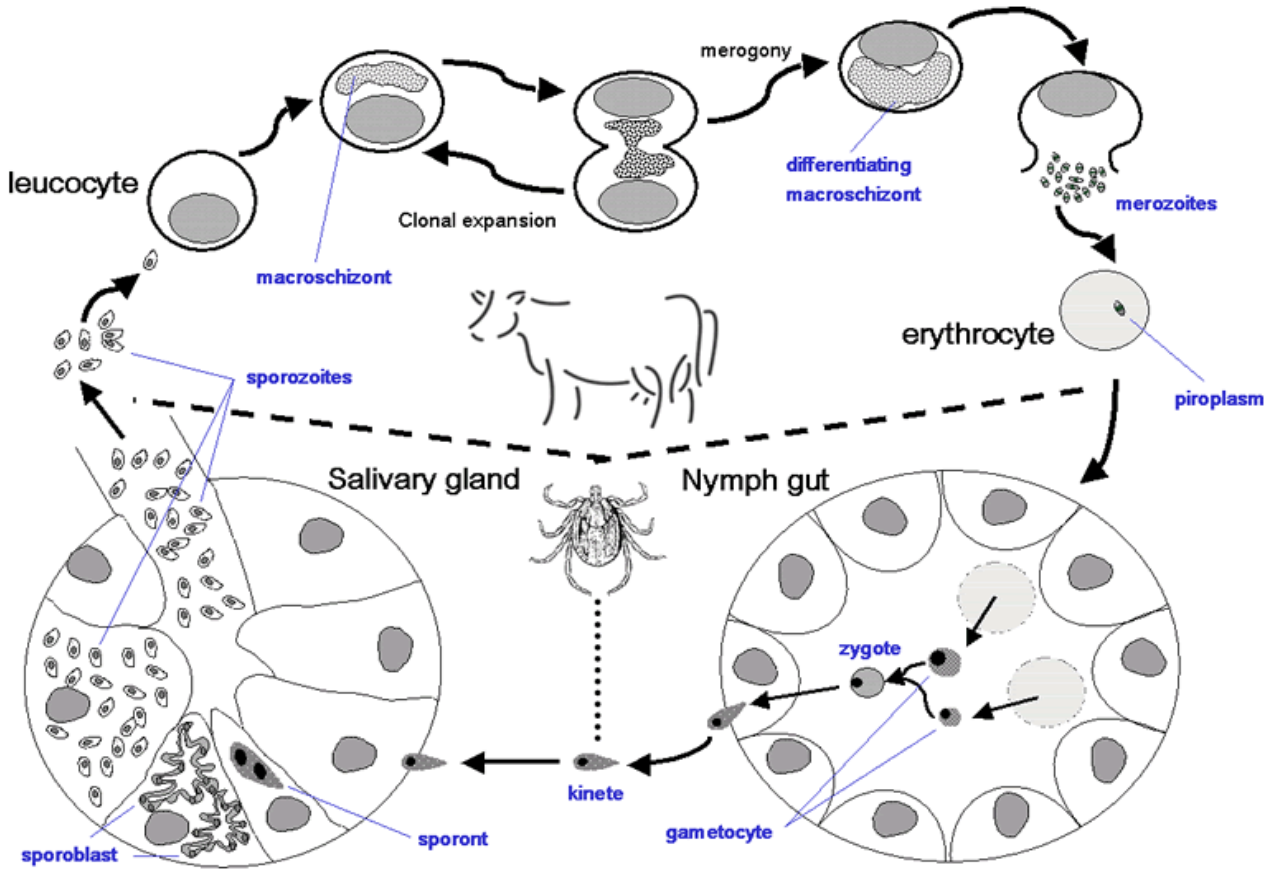

Figure (1): life cycle of *Theileria* (OIE,2009)

1.5-The clinical signs and clinical diagnosis of theileriosis: -

Theileria diagnosis includes case history clinical sign ,postmortem finding and knowledge of disease vector distribution (OIE,2000).In case of *Theileria* include enlargement of superficial lymph nodes namely parotid and pre scapular (Rbison,1982).Rise in body temperature, difficult breathing and frothy exudates from nostril s are reported (Boulter and Hall, 2000) .Diarrhea lacrimation which may lead to corneal opacity or complete blindness (figures(5) and (6). And loss appetite are often present (Norval *et al*.,1992). Mansuer(1996) recorded that clinical symptoms of theileriosis in cattle were enlargement of superficial lymph node, in appetence and intermittent fever. 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, and severe cases may be associated with diarrhea.

1.5.1-Laboratory diagnosis: -

In live animals, theileriosis is diagnosed by the identification of schizonts in thin smears from blood, lymph node. Thin blood films from each cattle were prepared, fixed by methanol stained with Giemsa stain (1:10) and wash by tap water and upright air dried and then examined microscopically (The erythrocyte form of *Theileria* were rod rounded and ring shaped. Also lab is full diagnosis of theileriosis is more accurate and confirmed .*Theileria* parasite can be easy detected in mammalian host and the vector ticks (FAO,1984) .The method used to detect the parasite in mammalian host be preparation of blood smear ,lymph node biopsy postmortem impression smear that are stained by Giemsa stain (Norval *et al*., 1992-Frosyth *et al.*, 1999). Microscopic examination shows *Theileria* schizont in the lymph node , smear, piroplasms in blood smear morphological characteristic of the

parasite may help to distinguish the spp however the defecation of piroplasmas in blood smear with absent of clinical assessment and lymph node biopsy are difficult to interpret since piroplasmas of *T.annulata* or *T. mutans* can be found in clinical normal carriers (Barnett,1977), (Norval *et al*.,1992).

Elhussein *et al*., (2012) reported current situation of tropical theileriosis in Sudan explain that the disease routinely diagnosed using microscopic examination of stained blood smear.

Salih *et al*., (2005) using ELISA and IFA test in Sudan included Northern Sudan Blue Nile, western, eastern Sudan and found 33.3% prevalence *T.annulata* .

Elhussein *et al*, (2007) compared two serological test (ELIZA&IFAT) and microscopic examination for detecting *theileria annulata*.

The first PCR application for *T.annulata* diagnosis in bovine host was based on the tams I gene (Oliveria *et al*., 1995) . They also detection Theileria *anulata Hayalomma* ticks using the same technique (olivera *et al.*, 1997). . Serological tests may not be sensitive enough to detect all infected an cross cattle re actions can occur with other species of *Theileria* (OIE, 2009).

1.5.2-Distribution of bovine theileriosis: -

Kuttler and Craig (1975) founds That cow had clinical signs suggesting intra erythrocyte parasitism and, accordingly, was treated for anaplasmosis. There was no improvement, and the animal died. A methanol-fixed blood film sent to Texas A&M University (College Station, Tex.) for Giemsa staining and microscopic examination confirmed the presence of pleomorphic piroplasms, with as many as four *T.annulata* within some erythrocytes.

The level of parasitemia was 21% and consisted predominantly of round dot and ring forms is more accurate and confirm *Theileria* parasite 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.

1.5.3-Samples and procedure: -

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.

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 ischemic necrosis throughout the brain. Serum for antibody detection. (OIE,2009)

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 (OIE2009).* 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 the 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 ribosomal RNA gene sequences.

A number of PCR methods (targeting sequences TpR, p104, p67, PIM) can be used to detect *T. T.annulata.T.parva (OIE2009).*Serological tests 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 parasite specific antibodies . ELISA 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. parvaand 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 (OIE,2011).

1.6-Prevention and control: -

1.6.1-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 newe 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 (OIE 2008).

1.6.2-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* (OIE,2008).

1.6.3-vaccination:

Live attenuated vaccines. Reliable vaccines of known efficacy have been developed for *T. annulata* and *T. parva*. 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 long acting tetracycline formulation .This treatment results in a mild or in apparent East Coast fever reaction followed by recovery .Recovered animals demonstrate a robust immunity to homologous challenge, which usually lasts for the lifetime of the animal(OIE,2012). .

Immunization 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. Immunized animals usually become carriers of the immunizing 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 (OIE,2008). Recombinant vaccines. Experimental subunit vaccines are being developed for ECF will contain antigens from both sporozoite (as the p67 protein) and schizont stages. An improved p67 vaccine has been tested in the field and might be available soon (OIE 2008).

1.7-Public Health: -

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

1.8- Incidence of tropical theileriosis and tick infestation in the world and Sudan: -

The disease reported in 1908 in the Sudan as piroplasma (anon,1908) and since it has been assumed considerable economic importance (Shommein and Obeid1973). (Shommein and Hagir ,1988).(Latif 1994).

The disease occurs in a wide zone of Africa,Southern Europe and a large part of Asia (Dolan, 1989) . Texas bovine *Theileria* isolate originally described by Kuttler and Craig (1975). Geimsa-stained blood films confirmed the presence of numerous *Theileria* parasites. The Texas isolate reported by Kuttler and Craig, (1975) was found during a study of anaplasma –sero positive cattle and was described as only mildly pathogenic in splenectomized calves with no evidence of pathogen city seen in the infected host cattle in which it was originally founds .Elhussein *et al.,(* 2015) found sero prevalence of *T.annulata* anti bodies in white Nile state-Sudan 6.7% prevalence serum 61% it is high prevalence *Theileria annulata* antibodies indicated disease endemic. Salih and Hassan, (2005) using ELIZA and IFA test in The Sudan then found 33.3%prevelance *T.annulata* with regard to localities .*T annulata in* cross breed calf in India .And theileria infections are wide spread in Sudanese cattle (shommein 1976 Morzaria *et al.,* 1981,FAO1983).*Theileria anuulata* in particular is considered as major obstacle to development dairy industry in northern Sudan (Osman1990) . *Theileria annulata* infection were detected in37% of apparently healthy cattle in River Nile state northern Sudan. The prevalence rate of infection was higher in adult cross breed than indigenous cattle (Elhussein *et al.,* 1991). Tropical theileriosis is characterized by fever , in appetence nasal and ocular discharge s ,lymphadenitis , anemia , ictrus and diarrhea in the later stage s of the disease however infection of other spp of bovine theileriosis could also result in rare syndromes sign involvement development of skin lesion s(Losses1986)or ocular lesion (Zakia *et al*., 1997).

The present of *T. annulata* in the Sudan was firstly confirmed (1939). there after it was reported from several districts of the country (anon1908-1939). The presence of *T. annulata* in cross-breed animals and the local breeds in the same farm is a

relatively high prevalence in cross bred animals. According to Osman (1989) upgrading of cattle in the way practiced now in Sudan, lowers tolerance of herds to ticks and tick-borne diseases. The presence of low-grade parasitaemia unaccompanied by clinical signs is indicative of a carrier state, though *T. mutans* could not be excluded as it is reported by the FAO (1983). However, these results agree with the findings of Bansal *et al*., (1987) who found that animals of all age groups were carrier showing sub-clinical rather than clinical infection The incident disease low among exotic breed also Mohammed, (1992)reported *Theileria* prevalence 38.5%in pure exotic dairy breed and 38.7 In Northern State –Sudan the tick and TBD (theileriosis) . Then Tick-borne diseases (TBDs) constitute a major constraint of livestock production and have a considerable livestock production and have a considerable economic impact (Jongejan and (Uilenberg, 2004).

TBDs are widespread, causing substantial economic losses and threat to the development of animal wealth in Sudan (FAO, 1983). The disease occurs in a wide zone of Africa, Southern Europe and a large part of Asia (Dolan, 1989). Tropical theileriosis is the most important TBD in the Sudan. Salih *et al.,* (2007) reported that 14% of cattle in South Darfur were positive for *T.annulata* antibodies. The prevalence of tropical theileriosis and the diagnosis clinically in Northern State (Vet. authorize Northern State).

1.9-Prevelance of bovine theileriosis in Sudan

Tropical *T.annulata* infection in cattle is the most important TBD in Northern Sudan and *T.parva* in southern Sudan (Elhussein *et a.,l* 2004).

Sero prevalence of *Theileria* in Khartoum State86.5% and 17.9% in the western part of the Sudan (Salih *et al*., 2005).Bothina *et al.,* (2015) found prevalence 3.6% of theileriosis ((base on geimsa stains) and 31% positive to *T.annulata* anti bodies (base on IFA test) in Nyala dairy farm in South Darfur state Sudan .

Safieldin *et al.,* (2011) **.**Factor affecting seasonal prevalence of blood parasite in dairy cattle in Omdurman locality.

Walker.A.R;Morzoria and Penderson, (1983) reported prevalence rate 38.9% for *T.mutans* and 74.6% for *T.annulata* in Northern Sudan.

Elhussein, *et al*., (2012) about current situation of tropical theileriosis in Sudan explain that the disease routinely diagnosed using microscopic examination of stained blood smear .

Salih, *et al.*, (2005). Using ELISA and IFA test in Sudan included Northern Sudan, Blue Nile, western, eastern Sudan, found 33.3% prevalence T.*annulata* with regard to localities ,the highest prevalence 85.5%was found in central Sudan while 12.5% in eastern Sudan. Shommein *et al.,* (1976) found in Khartoum north rate of infection of *Theileria T.annulata* is approximately 10%.

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 at cold season and 49◦C at hot season and the humidity less than 20%. The mean annual rainfall of the State is about 250mm/year.

2.2- Study design and sampling:

Across-sectional study design was conducted in Northern State in different localities,(figure2). Which include, Northern area included six sites from Halfa and Dalgo, Middle area consist of nine sites from Dongula, Alburgag and Alogoled and southern area included nine sites from Merowe and Aldbh, for determination of theileriosis in cattle (total of sites twenty four area). Information regarding age, sex, origin of the animals was recorded during collection of sample from animals were determined based on owners information. Sampling technique was applied to select animals at the study area. (base no random sample methods and population of herds cattle's).

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)

After calculation. The sample size result was 329 head of cattle (Martin and Willeberg 1987). Select of 329 head of cattle randomly base on24sites of herd cattle from study area.

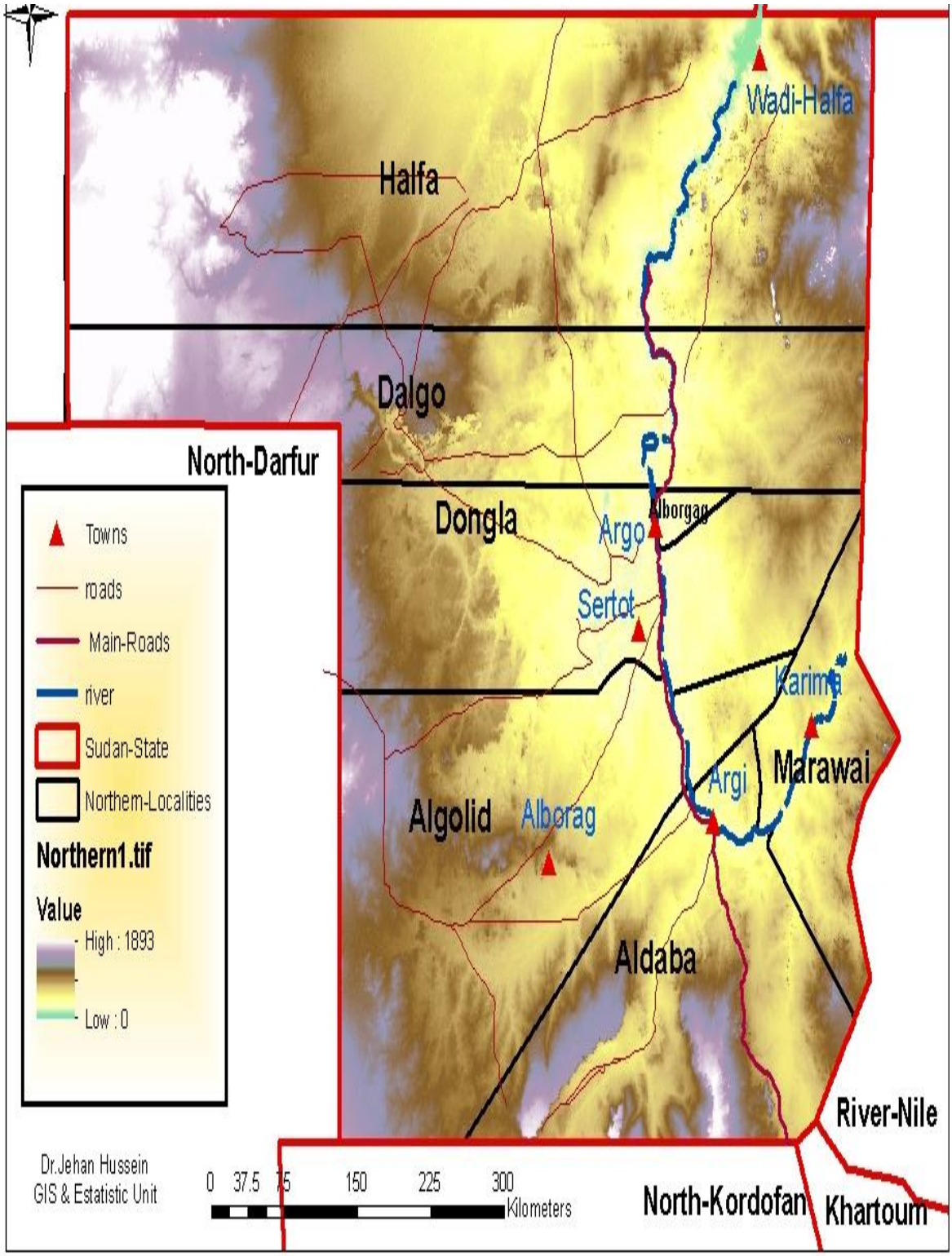

Figure (2): Map Study area, Northern State Localities.(sample collection sites).

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 were 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 Dongula Veterinary Parasitological Laboratory for examination of the parasites.

2.5-Examination of cattle: -

Three hundred and thirteen head cattle were clinically inspected for the presence of ticks. Sample Ticks from animals by thumb forceps and put ticks in tube content 70% ethanol according to Köhler-Rollefson *et al*., (2001), tick was identified under dissecting microscope according to Hoogstraal (1956) in Central Veterinary Laboratory (CVRL-Soba). Blood samples were collected from 329 cattle's into clean and dry sterile tubes containing Ethylene Diamine Tetra-acetic Acid (EDTA) as an anticoagulant.

2.6- Laboratory investigation procedures:

Geimsa staining procedures and microscopic examination of slides was conducted according to OIE (2012) and Burgdorfer (1970). The slides were immersed in Giemsa stain (1:10 solution) in staining rack for 30 minutes. Then the slides were washed with distilled water to remove excess stain and made air dry. The stained blood smears were examined under oil immersion lens of microscope (100X) for appreciation and identification of different *Theileria* species according to their morphological characteristics,(figures 3,4).

2.7-Data analysis: -

The data collected were analyzed by the statistical software called SPSSvirsion22(2013) for Windows (Stata Corp. College Station, USA). The prevalence was calculated by dividing the number of cattle found to be positive for

Theileria by the total number of cattle examined for *Theileria* spp. The association of risk factors for theileriosis was assessed using Chi-square test.(table2).

Chapter Three

Results

3.1- The Prevalence of cattle theileriosis: -

Thirty-eight animals were found positive (11.6%) and 291 animals were negative (88.4%) to cattle theileriosis (Table 1). Therefore, the overall prevalence of cattle theileriosis in Northern state was 11.6%.(base on result of blood smears stained by Geimsa, Figures (3),(4),(5),(6).

Table 1: The Prevalence of cattle examined (n=329) for theileriosis in Northern State-Sudan.

	Frequency	**Percent**
Positive	38	11.6
Negative	291	88.4
Total	329	100.0

3.2-Risk factors associated with bovine theileriosis: -

3.2.1-Sex of animal.

From225cattle examined females,27were found infected and the rate of infection was12%. Out of104examedmale,11were found positive and the infection rate was10.6%.

The Chi-square test, showed that there was no association between theileriosis and sex of animals (p-value >0.432), (Table 2).

3.2.2-Age of animal.

One hundred and seventy-six cattle examined the age less than one year or equal, 30 of them were found positive for theileriosis infection and rate of infection17%. While infection rate among cattle that more than 2 years old was5.2%. (8/153). Chi-square test, showed that there was association between theileriosis and sex of animals (p-value <0.001) (Table 2).

3.2.3- Previous history of disease.

Table (2) , showed that 92animals had previous history of diseasebut35 of them were found infected (38.04%). The rate of theileriosis infection was 1.3%among animals with no previous history of disease. But 237 animals were found without previous history of disease among these 3 animals was found infected and the rate of infection was 1.3%. The Chi square test showed that their association between theileriosis infection and previous history of disease (p-value<0.005) (Table 2).

3.2.4- Breeding of animals

Total number of females examined was160cross breed animals. Among these, 34 animals were found infected. The rate of infection was 21.3%. Total number of local breeds examined was 169 local animals. Among these, 4 animals were found infected. The rate of infection was 2.4% .

The Chi-square test, showed that there was significant association between theileriosis and breed of animals (p-value =0.000) (Table2).

3.2.5- Present of ticks on animals.

About 313 animals were found infested by ticks and among these 38 animals were infected by theileriosis. (12.1%), while 16 animals were found free from ticks, The Chi square test showed that there was highly significant association between theileriosis infection and presence of ticks in exam cattle. (p-value=0.000). table (3,4).

3.3-Environement factors

3.3.1-Localities:-

According to result the prevalence of bovine theileriosis was 15.6% (26/167) in Dongula , in Marawi 11.1% (8/72) , in Aldabah 7.3% (3/41), and 2% (1/49) in Halfa Locality. No significant association between theileriosis infection, (p-value=0.692) (Table 2).

3.3.2-Seasonalty: -

The number of animal examined in summer 189 animals among these animals were found infected 36 and the rate of infection was19 % .In winter the animals examined 140 among these found 2 infected and the rate of infection was 1.4% no significant association between theileriosis infection (p-value=0.283), (Table 2).

Table 2: Summary of Risk factors frequency for the distribution of 329 cattle examined for theileriosis in Northern State – Sudan.

Risk factor	Animal tested	Animal positive	Affect percentage%	Significant Difference	p-value %
Sex of animal:					
Female	225	27	12	NS	0.432
Male	104	11	10.6		
Age of animal:					
≤year	176	30	17	*	0.001
>year	153	8	5.2		
Breed of animal:					
crossbreed	169	34	21.3		0.000
local breed	160	4	2.4	*	
Previous history of disease of animal:					
Present	92	35	38.04	*	
Not present	237	3	1.3		0.005
Present of ticks in animal:					
Yes	313	36	12.1	*	0.000
No	16	2	0.0		
Localities					
Dongla	167	26	15.6	NS	0.692
Merwoe	72	8	11.1		
Aldbh	41	3	7.3		
Halfa	49	1	2		
Seasons	189	36	19		
summer	140	2	1.4	NS	0.283
winter					

*P<0.05 Significant Difference, NS P>0.05 No Significant Difference

Table (3): Ticks infestation of examined cattle(n=329) of Northern State-Sudan.

Tick-spp	Dongla	Merwoe	Aldbh	Halfa	Total
H.rufipus	130/82	65/35	35/32	28/21	258/170
H.impletatum	80/52	34/20	10/2	20/6	144/80
H.anatolicum	77/29	40/21	4/36	10/5	131/91
H.droedarii	60/39	20/5	24/22	23/4	127/70
H.trancatum	35/30	13/16	0/4	8/2	56/52
R.evansi	17/29	8/19	0/0	0/0	25/48
Total	399/261	180/116	73/96	89/38	741/511

H=hyalomma/R=Rhipociphalus

Table 4: Summary of the tick's survey

Tick-spp	Total number of ticks	Percentage%
H.rufipus	428	34.2
H.impletatum	224	17.9
H.anatolicum	222	17.7
H.droedarii	197	15.7
H.trancatum	108	8.6
R.evansi	73	5.9
Total	1252	100

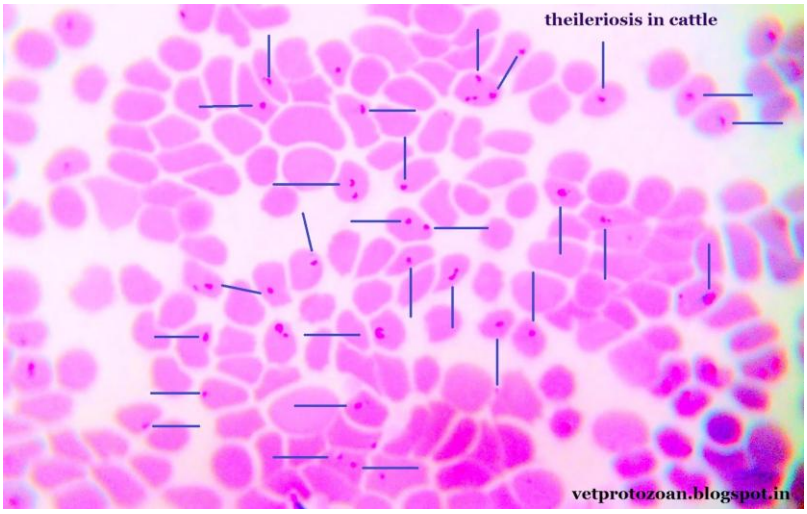

Figure (3): *Theileria.spp* seen during surveillance in cattle by ,Geimsa stain method .

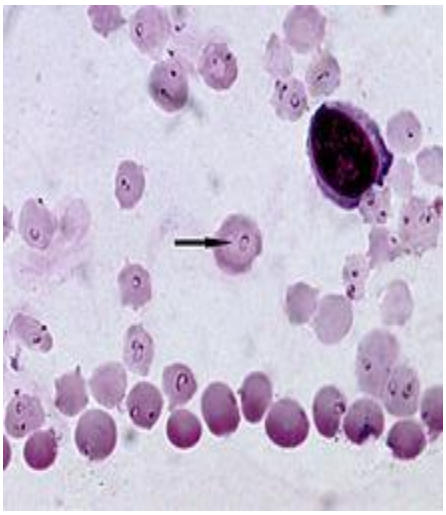

Figure (4): blood smear in cattle stained by Geimsa stain and showed schizont of cattle *Theileria spp.* during surveillance.

Figure (5): lacrimation, and enlargement prescapular lymph nodes found in Northern State Dongla, Mragha area in positive case during surveillance of theileriosis in cross breed calf

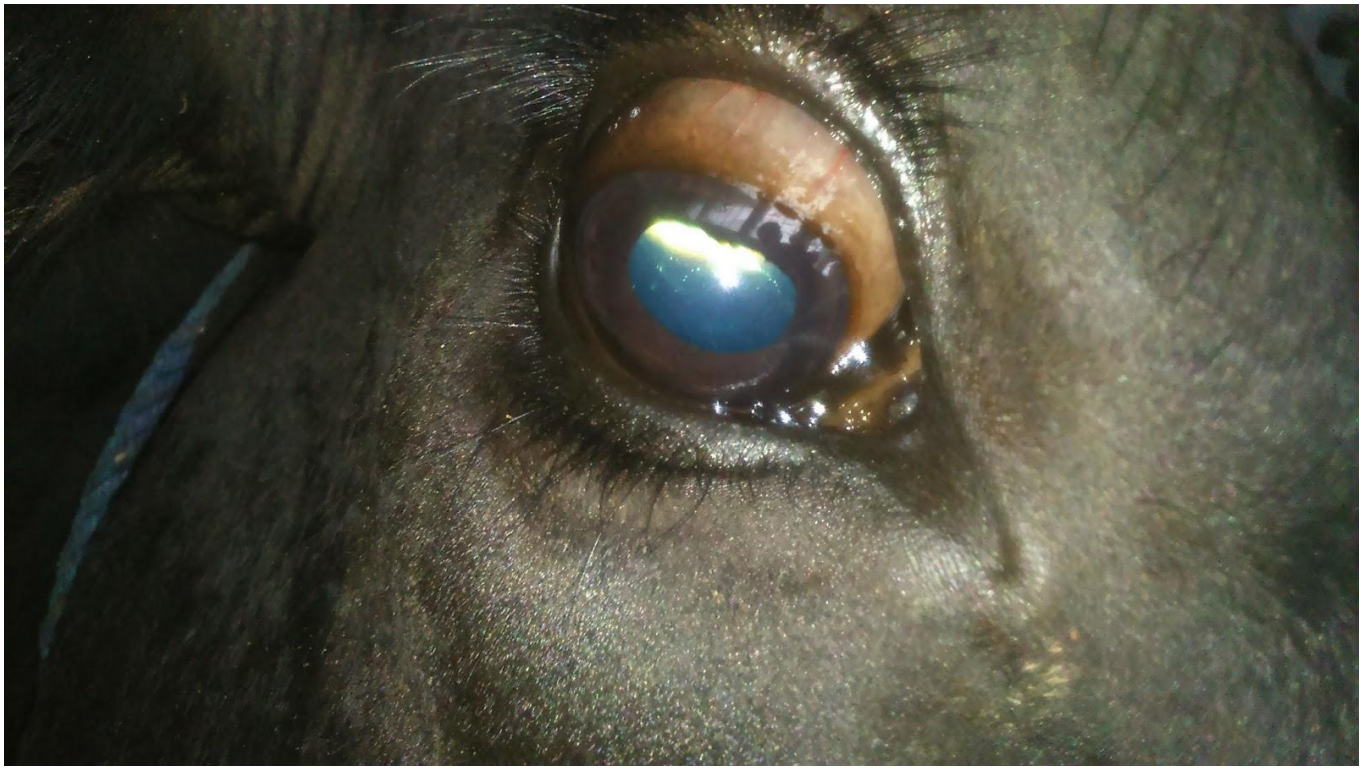

Figure (6): Corneal opacity, blindness in Northern State Dongla, Alslame area in positive case showed during Theiriosis surveillance in the cross-breed calf

Chapter Four

Discussion

In this study fined the prevalence of the theleria, Spp. disease were 11.6% in Northern State. Similar fined, Elghali , Elhussein, (1995) reported prevalence rate was 15%and18% during period (1991/1992/1993) in River Nile. And Shommein (1976) fined the incidence of disease in single farm was 10.46% and sero prevalence 86.5%in Khartoum ,17.9%in western part of Sudan .

In this study fined the prevalence rate of infection higher in adult cross breed (21.2%) than indigenous cattle (2.4%) similar (Elhussein,1991) ,(Elhussein *et al.*,2004), Osman (1976), Shommein and Hagir (1988) (Gharbi *et al*., 2014), found exotic cattle are more susceptible to *theileria* infection than local cattle. Elhussein et al., (2012). Multivariate logistic regression analysis of breed and age of cattle were identified as potential risk factor for the disease in Khartoum. Crossbreed cattle have high infection than indigenous cattle.

In my study prevalence of disease among age group less than one year is very high 17%(calves), comparison by animals equal or more than one year were 5.2%. Like Elhussein *et al.,* (1991) and Latif *et al*., (1994) found the disease higher in younger animals.

In these studies, were found prevalence in summer 19% and in winter 1.4%. Agreement result Elghali and Elhusein (1995) reported that *Theileria* in cattle is a serious problem in River Nile State during summer resulting of heat stress breakdown of immunity and agreement with Safieldin *et al.*, (2011) factor affecting seasonal prevalence of blood parasite in dairy cattle in Omdurman locality.

In this study were fined two genera of ticks, *Hyalomma* and *Rhibicephalus.* Also found Six species *H.rufipes H.anatolicum, H. dromedarii , H.impletatum , H.trancatum R. evertsi*. Similar, species of tick had been identified in Sudan (Hoogstraal,1956). Shommein, (1976) found that Theileriosis in Sudan transmitted by the *Hyalomma anatolicum, H. rufipes , H .deteritum ,H. margnatum ,Rhibicephalus evertsi* .

The result in showed increase in tick activities and infestation during summer season, and decreased in winter season. Similar Walker *et al*., (1983) found tick breeding activities increase in dry hot season (August) could have resulted from stress by sudden change in the macro climate from wet to cool dry. Walker *et al.,* (1983) they found increase in tick activity during end of rainy season and cool dry period.

In this majority of the cattle owners revealed that economic impact of disease by loss of milk, growth, death and cost of tick control and disease treatment. Like (latif,1994) found bovine tropical theileriosis infection cause severe economic losses due to expensive anti theilerial drugs, cost prevention and control measurement losses due to mortality, drop of milk productivity of affected animal pregnant cow may also abort and remain infertile for long time.

It was concluded that theileriosis is prevalent in Northern State. There is need for further investigation using more advance technique for identification of the carriers' cattle of theileriosis.

Conclusions and Recommendations

Conclusions

The Bovine theileriosis diseases is prevalent in Northern state (11.6%).The disease is high significant with a Associated risk factors like host factors (age, breed, presence of tick in animals ,previous history of disease) ,that mean disease high prevalence in young age of cattle ,crossbreed than local, in addition to presence of ticks , previous history of disease high prevalence. There is slight significant associated with environment and risk factor like seasonality and localities. Ticks survey In this study were fined two genera of ticks, the result of common species identified (*H.rufipus*34.1%), *H.impletatum* (17.9%), *H .anatolicum*(17.1%), *H.trancatum* (8.6), *H.droedari (15.7%). R.evertsi* (5.8%).

Recommendations

- Control of theileriosis in Nothern State.

- Further investigation using more advance technique for identification of the carriers' cattle of theileriosis.

- Help owner cattle to control the tick and avoid risk factors associated with the disease.

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Appendix

Frequency table

Result of the disease

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	+ve	38	11.6	11.6	11.6
	-ve	291	88.4	88.4	100.0
	Total	329	100.0	100.0	

Sexof animals

		Frequency	Percent	Valid Percent	Cumulative Percent
valid	Male	104	31.6	31.6	31.6
	Female	225	68.4	68.4	100.0
	Total	329	100.0	100.0	

Age of animal

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	less one year	176	53.5	53.5	53.5
	more thanye	153	46.5	46.5	100.0
	Total	329	100.0	100.0	

Previuos history of disease

		Frequency	Percent	Valid Percent	Cumulative Percent
valid	yes	92	28	28	28
	No	237	72		100.0

Breeding of animal

		Frequency	*Percent*	*Valid Percent*	*Cumulative Percent*
Valid	Local	169	51.4	51.4	51.4
	Cross	160	48.6	48.6	100.0
	Total	329	100.0	100.0	

Present of ticks

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	169	51.4	51.4	51.4
	No	160	48.6	48.6	100.0
	Total	329	100.0	100.0	

Data analysis

Case processing Summary

Cases

	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
age * result of disease	**329**	**100.0%**	**0**	**0.0%**	**329**	**100.0%**
***sex* * result of disease**	**329**	**100.0%**	**0**	**0.0%**	**329**	**100.0%**
***breeding* * result of disease**	**329**	**100.0%**	**0**	**0.0%**	**329**	**100.0%**
Present of tick* result of disease	**329**	**100.0%**	**0**	**0.0%**	**329**	**100.0%**
Previous histry * result of disease	**329**	**100.0%**	**0**	**0.0%**	**329**	**100.0%**

Result of Disease *age of animals

Crosstab

		Age of animals		
Result of the disease		**Less than or equal one year**	**More than one year**	**Total**
Result +ve	**Count**	**30**	**8**	**38**
	Within result of disease	**79.9**	**21**	**100.0%**
	Within age of animals	**9.1**	**2.6**	**11.6%**
-ve	**count**	**146**	**145**	**291**
	within result of disease	**50.2**	**49.8**	**100.0%**
	Within age of animals	**83**	**94.8**	**88.5%**
total	**count**	**176**	**153**	**329**
		53.5	**46.5**	**100.0**
		100.0	**100.0**	**100.0**

Result of Disease *sex of animals

Crosstab

		Sex of animals		
Result of disease		**Female**	**Male**	**Total**
Result	**+ve**	**count**	**27**	**13**	**38**
		within result of disease	**71**	**29**	**100.0%**
		Within sex of animal	**12**	**10.6**	**11.6%**
	-ve	**count**	**198**	**93**	**291**
		within result of disease	**68**	**32**	**100.0%**
		Within sex of animal	**88**	**89.4**	**89.4%**
	Total	**count**	**225**	**104**	**329**
		Total	**68.4**	**31.6**	**100.0**
			100.0	**100.0**	**100.0**

Result of Disease *breed of animals

Crosstab

		Breed of animals		
Result of disease		**Less than or equal one year**	**More than one year**	**Total**
Result	**+ve**	**count**	**34**	**4**	**38**
		withinresult of disease	**89.5**	**10.5**	**100.0%**
		Withinbreed of animal	**10.3**	**1.2**	**11.6%**
	-ve	**count**	**126**	**165**	**291**
		within result of disease	**43.3**	**56.7**	**100.0%**
		Within breed of animal	**78.8**	**97.6**	**78.8%**
	Total	**count**	**160**	**169**	**329**
			48.6	**51.4**	**100.0**
			100.0	**100**	**100.0**

Result of Disease * of previous history of disease

Crosstab

		previous history of disease		
Result of disease		Less than or equal one year	More than one year	total
+ve	count	35	3	38
	Withinresultof disease	92.1	7.9	100.0%
	Withinprevious history	10.7	0.9	11.6%
-ve	count	102	189	291
	withinresultof disease	35.3	64.9	100.0%
	Withinprevious history	74.5	98.4	74.5%
Total	count	137	192	329
		41.6	58.4	100.0
		100	100	100.0

Result of Disease *present of ticks

Crosstab

		Present ofticks		
Result of disease		**Yes**	**No**	**Total**
+ve	**count**	**38**	**0**	**38**
	with in result of disease	**100.0**	**0.0**	**100.0%**
	Within present of ticks	**11.6**	**0.0**	**11.6%**
-ve	**count**	**275**	**16**	**291**
	within result of disease	**94.5**	**5.5**	**100.0%**
	within present of ticks	**87.9**	**100**	**98.5**
Total	**count**	**313**	**16**	**329**
	Total	**95.1**	**4.9**	**100.0**
		100	**100**	**100**

Table (3): Ticks infestation of examined cattle(n=329) of Northern State-Sudan.

Tick-spp	Dongla	Merwoe	Aldbh	Halfa	Total
H.rufipus	130/82	65/35	35/32	28/21	258/170
H.impletatum	80/52	34/20	10/2	20/6	144/80
H.anatolicum	77/29	40/21	4/36	10/5	131/91
H.droedarii	60/39	20/5	24/22	23/4	127/70
H.trancatum	35/30	13/16	0/4	8/2	56/52
R.evansi	17/29	8/19	0/0	0/0	25/48
Total	399/261	180/116	73/96	89/38	741/511

H=hyalomma/R=Rhipociphalus

Table 4: Summary of the tick's survey

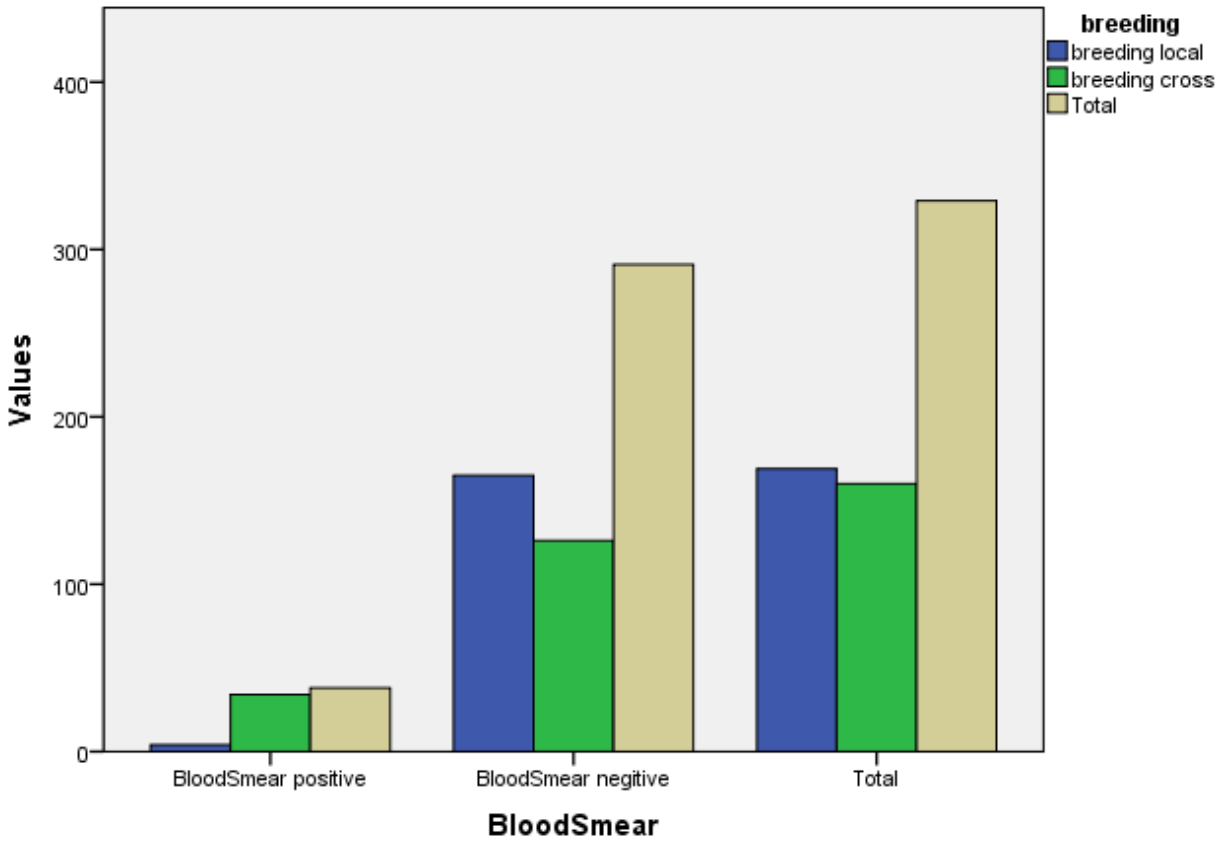
Tick-spp	Total number of ticks	Percentage%
H.rufipus	428	34.2
H.impletatum	224	17.9
H.anatolicum	222	17.7
H.droedarii	197	15.7
H.trancatum	108	8.6
R.evansi	73	5.9
Total	1252	100

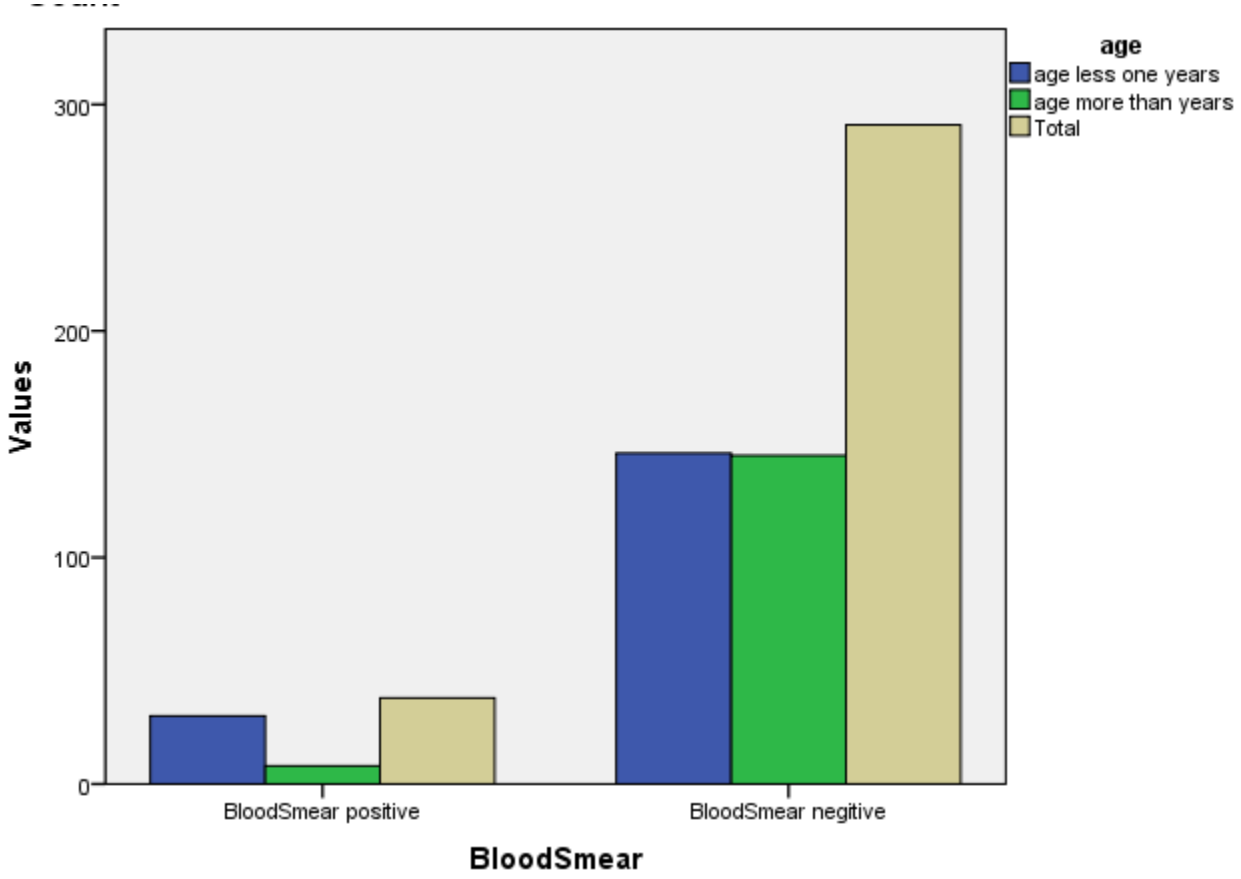
Table : summary of responses of owner cattles in Northern State Localities questionnaire survey about theileriosis impact and risk factors .

Subject	responses	Dongola	Merowe	Aldbh	Half a	Total
No. Cattle owner responded		20(43.5)	10(22)	8(17.4)	8(17.4)	46(100.0)
No .of herd		20(43.5)	10(22)	8(17.4)	8(17.4)	46(100.0)
1/theileria most important disease	a/yes b/no c/no idea	18(39.1) 1(2.2) 1(2.2)	8(17.4) 1(2.2) 1(2.2)	4(8.7) 3(6.4) 1(2.2)	3(6.4) 4(8.7) 1(2.2)	33(71.7) 9(19.6) 4(8.7) 46(100.0)
2/know disease	a/yes	13(28)	6(13)	2(4.2)	1(2.2)	22(47)
In herd	b/no	7(15.2)	4(8.7)	6(13)	7(15.2)	24(53) 46(100.0)
3/affected of animal in herd	a/high mortality b/high morbidity c/no answer	2(4.3) 17(37) 1(2.2)	1(2.2) 8(17.4) 1(2.2)	0 7(15.2) 1(2.2)	0 2(4.3) 6(13)	3(6.4) 34(73.9) 9(19.6) 46(100.0)
4/tick infestation in cattle	a/yes b/no	14(30.4) 6(13)	6(13) 4(8.7)	5(10.4) 3(6.4)	6(13) 2(4.3)	31(67.4) 15(32.6) 46(100.0)
5/age of animal effected	a/adult b/young d/no idea	3(6.4) 15(32.6) 2(4.3)	2(4.3) 7(15.2) 1(2.2)	2(4.3) 5(10.9) 1(2.2)	2(4.3) 5(10.9) 1(2.2)	9(19.6) 32(69.6) 5(10.9) 46(100.0)

6/ coast control of tick and disease	**a/yes** **b/no**	**10(21.7)** **10(21.7)**	**5(10.9)** **5(10.8)**	**3(6.5)** **5(10.9)**	**2(2.2)** **6(13)**	**22(43.5)** **24(56.5)** **46(100.0)**
7/nutrion in herd	**a/very good** **b/good** **c/middle**	**4(8.7)** **15(33)** **1(2.2)**	**4(8.7)** **4(8.7)** **2(4.3)**	**4(8.7)** **3(6.4)** **1(2.2)**	**3(6.4)** **2(4.3)** **3(6.4)**	**15(32.6)** **24(52.2)** **7(15.2)** **46(100.0)**
8/season of disease	**a/summer** **b/winter**	**19(41.3)** **1(2.2)**	**8(17.4)** **2(4.3)**	**7(15.2)** **1(2.2)**	**6(13)** **2(4.2)**	**40(87)** **6(13)** **46(100.0)**
9/production type	**a/intensive** **b/semi intensive**	**12(26)** **8(17.4)**	**5(10.9)** **5(10.9)**	**2(4.3)** **6(13)**	**1(2.2)** **7(15.2)**	**20(43.4)** **26(56.6)** **46(100.0)**
10/economic impact	**a/death** **b/los of growth and milk production** **c/both** **d/treatment coast** **e/no comment**	**2(4.2)** **14(30.4)** **1(2.2)** **2(4.2)** **1(2.2)**	**1(2.2)** **4(8.7)** **2(4.3)** **2(4.3)** **1(2.2)**	**0** **4(8.7)** **2(4.2)** **1(2.2)** **1(2.2)**	**0** **2(4.2)** **1(2.2)** **1(2.2)** **4(8.7)**	**3(6.4)** **24(52.2)** **6(13)** **6(13)** **7(15.2)** **46(100.0)**
11/awareness theileria sign	**a/yes** **b/no**	**12(26)** **8(17.4)**	**5(10.9)** **5(10.9)**	**1(2.2)** **7(15.2)**	**1(2.2)** **7(15.2)**	**19(41.3)** **27(58/.7)** **46(100.0)**
12/disease in breed	**a/local** **b/cross**	**6(13)** **14(30.4)**	**2(4.2)** **8(17.4)**	**2(4.2)** **6(13)**	**3(6.4)** **5(10.9)**	**13(28)** **33(72)**

Result of associated between theileriosis and risk factor used graph pad prism program base on result of blood smear method:-

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age less one years
age more than years
Total
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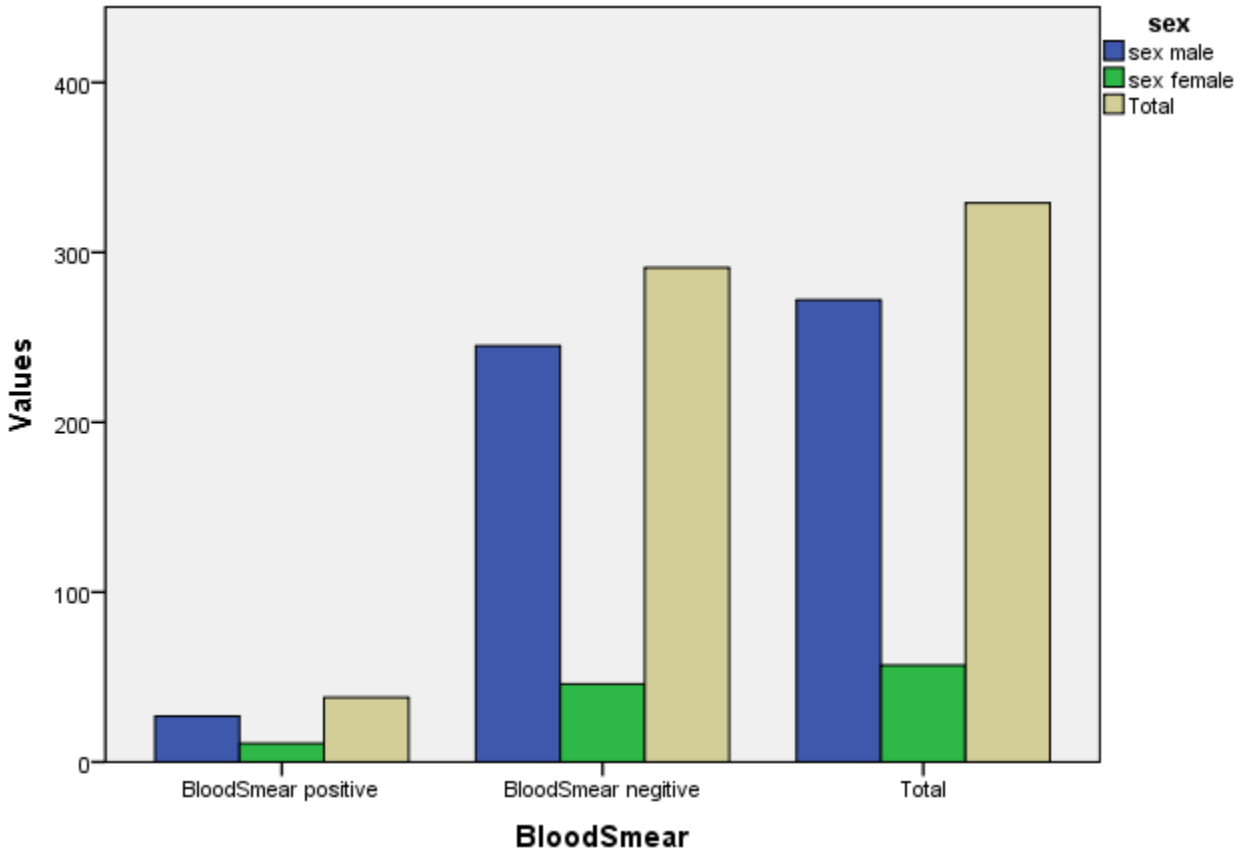
200
100
0
Values
BloodSmear positive
BloodSmear negitive
BloodSmear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sex male
sex female
Total
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100
0
Values
BloodSmear positive
BloodSmear negitive
Total
BloodSmear