بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Master of Preventive Medicine (Batch 9)

Prevalence of the Theileriosis Infection in Animals Dairy Cattle Farms in Khartoum Bahri, Khartoum State, Sudan

نسبة انتشارعدوى مرض الثاليريا في حيوانات مزارع ابقار اللبن في الخرطوم بحري، ولاية

الخرطوم، السودان

A Thesis Submitted to the College of Graduate Studies in Partial Fulfillment of Requirements for the Degree of Master of Preventive Veterinary Medicine (MPVM)

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قال تعالي :

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ * خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ * اقْرَأْ وَرَبُّكَ الْأَكْرَمُ * الَّذِي عَلَّمَ بِالْقَلَمِ * عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ)

(سورة العلق: الآيات 1-5)

(صدق الله العظيم).

Dedication

To my father and mother with great love.

And for

great supports and encouragement

For all my family.

and to all those who made this work possible.

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First of all, thanks and praise to Allah the compassionate, and the most Merciful for giving me health and strength throughout the period of study.

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Abstract

This study was conducted from February to March 2020 in Khartoum Bahri, Khartoum, State to determine the prevalence of bovine theileriosis and its associated risk factors of the disease. A total of 150 blood samples were collected randomly from dairy cattle farm in Bahri (kuku, Alkadru, Soba, Alselat) for detection of theileria blood parasite using direct Geimsa stain techniqueand for hematological parameters using complete blood counts machine.

Questionnaire was filled by farms owners to identify possible risk factors associated with theileria infection. These including; locality, age, sex, body condition, tick's infestation and use of acaricides. Results showed that the overall prevalence of theileria in Khartoum Bahri was (28.6%). The high prevalence rate was recorded in Alselat (34.4%) compared with other Alkadaru (25%), Soba (26.1%) and Kuku (32.3%). The high prevalence was record in age >4years (44%), <2(24%) and 2-4years (27.2%). The high prevalence in male (30.5%) followed by female (28%) and high prevalence recorded in animals with poor body condition (30.5%) compared with good body condition one (%27.4).

The univariate Chi square analysis showed that there was no relationship between ages, sex, body condition, ticks infestation and use of acaricides but there was significant (P<0.05) association of tick's infestation and body condition.

Independent t, test of hematological parameters the result showed that there was significant different of RBCs and HGB (P-value=0.02 and 0.01) respectively.

The ccurrent study concluded that dairy cattle were highly infected with theileria due to poor management and ticks control of the diseases. Also the disease affected some of hematological parameters in blood component of infected animals.

ملخص الاطروحة

اجريت هذة الدراسة في الفترة من فبراير الي مارس 2020 في بحرى ولاية الخرطوم بهدف تقصي مدي انتشار مرض الثاليريا البقري وعوامل الخطر المرتبطة بة. تم جمع 150 عينة دم عشوائية من بعض مزارع الابقار في الخرطوم بحري (الكدرو، سوبا، كوكو و السليت) لفحصها بحثا عن طفيلي الدم باستخدام تقنية صبغة جيمسا المباشرة وفحص معايير الدم باستخدام جهاز تعداد الدم الكامل.

تم تعبئة الاستبيان عن طريق اصحاب المزراع للتحقق من عوامل الخطر المرتبطة بالاصابة بالثاليريا وتشمل :

المكان، العمر، ، الجنس، حالة الجسم، الاصابة بالقراد و استخدام مبيدات القراد. هذة النتائج اظهرت معدل انتشار الكلي لمرض الثاليريا في الخرطوم بحرى 28.6%. سجل معدل انتشار مرتفع في السليت (34.4%) مقارنة بالمناطق الاخرى، الكدرو (25%)، سوبا (26.1) و كوكو (32.3). سجل معدل انتشار عالي في العمر اكبر من 4 سنة (44%)، اقل من 2سنة (24%) و من 2-4سنة (27.2%). سجل معدل انتشار علي في الذكور (30.5%) ويليلة الاناث (28%). سجل معدل انتشار مرتفع في حالة الجسم السيء(35.5%) مقارنة بحالة الجسم الجيدة (27.4%).

تحليل مربع كاي احادي المتقير اظهر عدم وجود علاقة بين العمر، الجنس، حالة الجسم، الاصابة بالقراد واستخدام المبيدات الحشرية.

الاختبار المستقل تي لمعلمات الدم اظهرت النتائج ان هنالك اختلاف لكرويات الدم الحمراء والهيموغلوبين الدم (القيمة المعونية =0.02 و 0.01) علي التوالي.

تبينت من الدراسة ان الابقار المزارع الحلوب مصابة بشدة بالثاليريا نتيجة لسوء الادارة والسيطرة علي امراض القراد. وكذالك المرض اثر علي بعض معلمات الدم في مكونات الدم للحيوان المصاب.

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List of Abbreviations

Abbreviation	Definition
TBDS	Tick Borne Diseases
CSESP	Committee on Systematic and Evolution of the Society of Protozoologists
PCR	Polymerase Chain Reaction
SPSS	Statistical Package for the Social
CBC	Complete Blood Counts
RBC	red blood cells)
WBC	white blood cells
HGB	hemoglobin concentration
MCV	mean corpuscular volume
HCT	Hemocrit
MCHC:	mean corpuscular hemoglobin concentration
µl	Microliter
EDTA	Ethylene Diamine Tetra-acetic Acid
Spp	Several species

Introduction

Piroplasmosis is one of the most common complex diseases since it affects wide range of ruminants and causes harmful impacts on the health including productivity and reproductively (Mousa *et al*., 2017).

The theileriosis is one of the Trans-Boundaries Disease of cattle, which are economically important protozoan disease in tropical and subtropical regions; they are transmitted by ixoded ticks and have complex life cycle in both vertebrate and invertebrate host (OIE, 2018). The most marked clinical signs of theileriosis in cattle are 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 discharges, dyspnea, and generalized lymph node enlargement. Severe cases may be associated with diarrhea and dysentery (Radostits *et al*., 2000). Diarrhea, lacrimation which may lead to corneal opacity or complete blindness and losses of appetite are often present (Norval *et al*., 1992). Tropical theilerosis has been reported to cause considerable damage to livestock development in the Sudan. For this economic evolution is very useful in animal health management, including prevention measures base on financial data and records and data analysis (Lopes *et al.*, 2015). The theileria species infect a wide range of both domestic and wild animals and are transmitted by ixoded ticks of genera Amblyomma, Haemophysalis, Hyaloma, and Rhipicephalus (FAO, 1983). *T. annulata* is transmitted in Sudan mainly by tick species *Hayalomma anatolicum* which is abundant in central, Northern Sudan and Western Sudan (Abaker *et al*., 2017). Small ruminant theileriosis is mainly caused by *Theileria lestoquardi*, *Theileria ovis*, and *Theileria separata*. *T. lestoquardi* is the most virulent specie and occasionally causes death while *T. ovis* and *T. separata* are benign and cause subclinical infections in small ruminants (Alessandra and Santo, 2012).

The distribution of 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). *Theileria annulata* infection in cattle is the most important tick borne disease in Northern Sudan –Rive Nile State (Elhussein *et al*., 2004). Earlier studies base on Geimsa stained blood and lymph node biopsy smear have shown high prevalence and wide spread nature of *T. annulata* infection in Northern Sudan (El Hussein *et al*., 2012).

Cattle affected with theileriosis, showed normocytic hypochromic anemia which may be due to their toxic metabolites which cause impairment in bone marrow and hinder erythropoiesis. Constant blood loss owing to tick infestation is accountable for anemia. Leucogram revealed significant increase in monocytes and lymphocytes whereas neutrophils and total leukocytic count was dropped off. Such alterations might also be due to Theileria toxic metabolites which affect haemopoietic organs (Bishop *et al.*, 2007). Progressive anemia and related maladies are the primary cause of pathological changes in case of theileriosis. Anemia is developed due to oxidative damages to erythrocytes, their increased fragility, and destruction of erythrocytes by Theileria in reticuloendothelial system, intraerythrocytic piroplasms and autoimmune reactions. Biochemical and clinical variations seen in the disease showed relationship to degree of parasitemia, degree of anemia and severity of hypoxia (Temiz *et al.*, 2014) and (Shiono *et al.*, 2001). Haemoglobin levels were adversely affected in positive cases

and reduced up to 8mg/dl. In severely infected cases haemoglobin level was reduced to 3mg/dl. Packed cell volume percentage was decreased to 9% and total erythrocytic count was reduced to 2.3 million per cm2. This was due to destruction caused by the organism inside the RBCs during parasite multiplication (Ananda *et al.,* 2009).

Main Objective:

To determine the prevalence of theileriosis infection in dairy cattle farms in Khartoum Bahri, Khartoum State.

Specific Objectives:

- ✓ To investigate some of the potential risk factors this could be associated with Theileria infection.
- ✓ To investigate the effects of the theileria infection on haematological parameters of the infected animals.

CHAPTER I

1. Literature Review

1.1 Definition:

The Theileria are protozoan parasites infecting wild and domestic animals throughout much of the world and they have a schizogonous reproductive cycle, usually in lymphocytes of the vertebrate host, and a piroplasm stage in erythrocyte (Dolan, 1989).

1.2 Etiology of the disease:

Theileriosis caused by Theileria parasites infect a vast number of wild and domestic animals and are transmitted trans-stadially by various members of tick vectors of the family Ixodidae. *Theileria annulata* causes tropical Theileriosis, which is transmitted by ticks of the genus Hyalomma (Uilenberg, 1981). It has currently been established that the causative agents of bovine theileriosis include *Theileria parva*, *Theileria annulata*, *Theileria mutans*, and *Theileria velifera* (Onuma *et al.*, 1998).

1.3 Taxonomy:

Ultrastructural studies and description of sexual form of theileria and babesia led to the following classification according to their vision of committee on systemic and evolution of Society of Protozoologists (CSESP) which was published by (Levine *et al*., 1980).

Phylam: Apicomlexa

Class: Sporozoea

Subclass: Piroplasmia

Order: Piroplasmida

Family: Theileridae

Genus: Theileria

The most important species affecting cattle are *T. annulata* and *T. parva*, which cause widespread death in tropical and subtropical areas of the Old World including Africa, South of Caribbean and Island (OIE, 2018).

1.4 The life cycle of the theileria *spp*:

Two stages in the life cycle of the parasite are responsible for the pathogenesis of the disease. These are schizont in mononuclear cells of the reticuloendothelial system and intraerythrocytic piroplasm (Tretina *et al.,* 2015). The incubation period varies from 4 to 14 days after attachment of the infected ticks to the host (Boulter and Hall, 2000).

1.4.1The life cycle of theileria in cattle:

The bovine host is infected by inoculation of sporozoites by infected ticks during feeding and sporozoites invade leukocytes, both monocytes and B-cells with high efficiency.

After enter to the trophozoite which after neuclear division develops in to the macroschizont, inducing the host cell to become larger lymphoplastoid cells which divide in synchrony with the macrochizonts. The parasite induces lymphoproliferation, a large population of parasitized cells develop in to infected animals. These further develop in to microchizonts and ultimately merozoite which are released from the lymphocytes, the merozoites invade erythrocyte and develop in to piroplasm, this stage completing the life cycle within the bovine host (Tait and Hall, 1990).

1.4.2 The life of theileria in tick's vector:

The clean tick (larva and nymph) feeds on an infected vertebrate host, Piroplasms, infected erythrocytes are ingested in the gut. Sexual reproduction of the parasite begins with release of piroplasms in the gut of the tick resulting in the fusion of macro and mircogametes (gametogony). The fusion of two gametes forms a zygote, which is the only diploid stage in the parasite life cycle. Subsequently, the parasite invades epithelial cell of tick gut and undergoes differentiation into motile kinetes which migrate through the haemolymph till they reach and invade the tick salivary gland (Mehlhorn and Schein, 1984).

The sporogony stage begins when a kinete invades the salivary gland acini and become rounded (sporont in shape). The sporont then differentiate into a sporoplast, when the tick moutls into the next stage (nymph and adult), and starts feeding on vertebrate host, the sporoplasts become mature and form sporozoites (infective stage). The sporozoites are injected into the host found in the saliva of the tick during the feeding process (Mehlhorn and Schein, 1984).

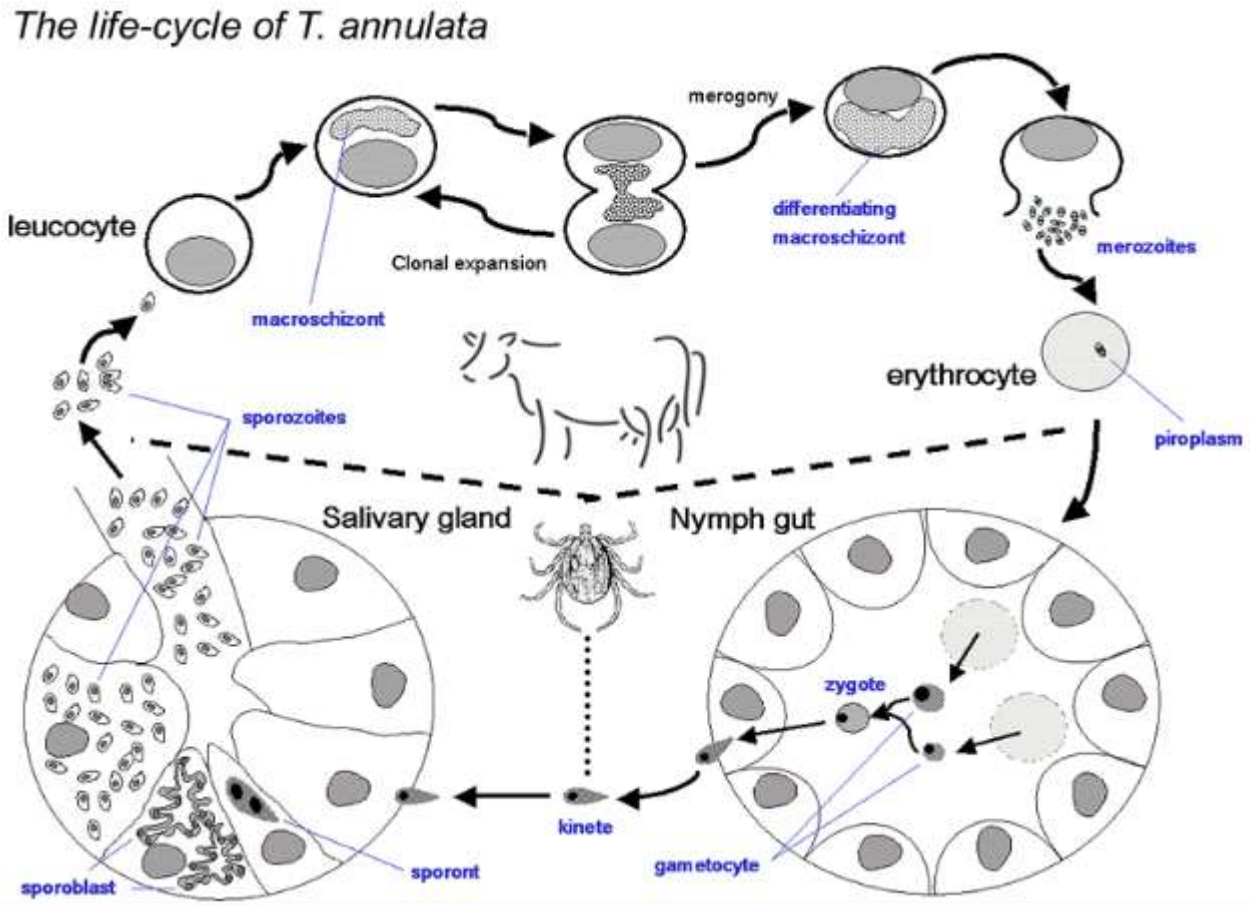


Figure (1.1): The life cycle of Theileriosis in ticks and animals (OIE,2009)

1.5 Epidemiology and distributions of theileriosis:

1.5.1 Geographic distribution of tropical theileriosis in the world:

The disease occurs from Morocco and Portugal in the west through the Mediterranean basin and the Middle East to India and China in the east. *T. annulata* affects cattle and is transmitted trans -stadially by the three-host tick *Hyalomma anatolicum* in central-western Asia and north -eastern Africa, and by the two-host tick *H. detritum* in the Mediterranean basin. The extent of its distribution may overlap with that of *T. parva* in Sudan and Eritrea and with *T. sergenti* in the Far East. In endemic areas, virtually all adult animals are infected, but case fatality is about 10-20% and is confined mainly to calves. Exotic animals recently introduced may have 20-90% mortality. The disease occurs when there is much tick activity, mainly in summer and the rainy seasons, and in crossbred animals. A single tick can cause fatal infection since its salivary glands usually contain numerous sporozoites (Rodistis *et al.,* 2006).

Tropical theileriosis is an economically important protozoan disease of cattle in tropical and subtropical regions (Robinson, 1982). Tropical theileriosis was reported prevalent in the South – Eastern Europe Southern Europe (Portugal, Spain, Italy, Bulgaria, Greece, and Turkey) the near and Middle East, India, China, Central Asia and former USSR (OIE, 2004). It is also distributed in a wide belt of tropical and subtropical zones, Northern Africa including Mauritania and surroundings of the Nile into the central Sudan and Middle East to South of the Caucasus splitting round the Himalaya into south branch (Pakistan and India) and a north branch (Afghanistan, Southern Russia, North- Eastern China) (Dolan, 1989; d'Oliverira, 1997; El Metenawy, 2000).

1.5.2 Geographic distribution of tropical theileriosis and prevalence in the Sudan:

T. annulata has been reported along with *T. mutans* in the Sudan since the 1920s. Earlier surveys based on Giemsa stained blood and lymph node biopsy smears have shown high prevalence and widespread nature of *T. annulata* infection in Northern Sudan (Shommein, 1976; El Hussein *et al.,* 1991; Mohammed, 1992; Zakia *et al*., 2003).

In the Sudan *T. annulata* is endemic in northern Sudan and The disease occurs across a wide zone of the Sudan that extends from Northern Sudan, particularly in Khartoum, Gezira, Kassala up to Sennar in the South (FAO, 1983). Tropical *T. annulata* infection in cattle is the most important TBD in Northern Sudan and *T. parva* in southern Sudan (Elhussein *et al*., 2004). Elhussien *et al*., (1991) reported that 37% of examined animal showed Theileria in North Sudan. Mohammed, (1992) conducted a survey in Khartoum state and found that the prevalence rate was 43% at Soba and 41.3% in Kuku. Serological detection of *T. annulata* antibodies was reported in various parts of the country, Kurdofan, Darfour and Gadarif (Kheir *et al*., 1994). Who found the disease occurs within the range of distribution of the vector *H. anatolicum* (FAO, 1983; El Neima, 1983).

Salih *et al*., (2005) recorded prevalence rate (72.8%) in Northern Sudan, (90.5 %) in Central Sudan, 18.6 % in Eastern Sudan, 20.2 % in western Sudan, (33.3 %) in Blue Nile, and (33.5 %) in White Nile. El Haj., (2010) who reported prevalence rate (55 %) in dairy cattle using IFA in Khartoum State. Using enzyme-linked immunosorbent assay (ELISA), seroprevalence of tropical theileriosis was shown to be prevalent all over the Sudan ranging between (6.3%) in South Darfur State and (86.5%) in Khartoum State.

1.6 The Clinical signs of the disease in cattle:

The occurrence of the disease varies depending on the parasite strain, the host's susceptibility and the quantity of sporozoites inoculated (Boulter and Hall, 2000).

Bovine theileriosis, which primarily causes fever, anaemia, jaundice, and superficial lymph node enlargement in infected animals, is a tick-borne haemoprotozoan disease caused by parasites of the genus Theileria, which invades bovine erythrocytes and leukocytes (Liu *et al.,* 2009). *T. annulata* infection (tropical theileriosis) is characterized by high fever, weakness, weight loss, inappropriate appetite, conjunctival petechial, enlarged lymph node, anemia, lateral decumbency, diarrhea (Rodistid *et al*., 2007). Marked clinical signs of theileriosis include lymph nodes enlargement, anorexia, depression, listlessness, dullness, and dyspnoea, pyrexia upto 107oF, and suppressed cough, ocular and nasal discharges. In severe cases, diarrhoea or dysentery and systemic changes are seen (Rodistis *et al.*, 2000). Icteric and pale mucous membranes (Mira and Ralph, 1989). Anaemia, jaundice and diarrhoea occur in the later stages of the disease. Yellow or Black colour loose faeces and haemoglobinuria may be observed in some cases (Gill *et al*., 1977; Uilenberg, 1981; Hashemi – Fesharki, 1988; Sandhu *et al*., 1998). The animal becomes dull, recumbent and death may follow within two to three weeks of infection (Gill *et al*., 1977; Uilenberg, 1981).

1.7 Pathogenicity:

The severity of infection depends upon virulence of the causative strain, the quantum of infection, the susceptibility status, age and health of the host (Gill *et al*., 1980; Irvin and Mawamchi, 1983; Preston *et al*., 1992). The parasite replicates in both lymphocytes and erythrocytes causing severe lymphocytopenia, anaemia and jaundice. Norval *et al*., (1992) stated that *T. annulata* had a lymphoproliferative and a lymphodestructive phase as well as a haemolytic phase. The severity of the disease does not necessarily correspond with the extent of the parasitaemia. An animal may be seriously ill when less than 25% of the blood cells are infected with piroplasm stages, and less severe reactions may occur where 45% of the red blood cells are infected (Soulsby, 1982).

1.8 Morbidity and mortality:

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

Mortality rate in exotic breeds of cattle may increase up to 90%, while in indigenous breeds, it ranges up to 5% or less (Durrani *et al.,* 2010) and (Iriminoiu, 2000)

1.9 Theileriosis in other animals:

1.9.1 In sheep and goats:

The important pathogen of sheep and goats is *T. hirci* (synonym *T. lestoquardi*), the cause of malignant ovine theileriosis. The disease is enzootic from North Africa through the Middle East to India and China, approximately the same geographical region as bovine tropical theileriosis.

Malignant theileriosis**:** in sheep and goats is similar to bovine tropical theileriosis due to *T. annulata*. Like the latter, it is also transmitted by Hyalommn spp. but in China, the main vector is Haemaphysalis spp. The disease can be acute, subacute, or chronic, depending on the resistance of the sheep or goats, and is seasonal, depending on availability of ticks. The acute disease is characterized by fever and very high mortality in 3-6 days. (Tageldin *et al.*, 1992). Anemia, jaundice, and enlargement of lymph nodes are characteristic, and both piroplasms and schizonts can be demonstrated in smears of blood and tissues, respectively. In subacute and chronic cases, signs are generally less marked except for anemia and emaciation. An indirect fluorescent antibody test is available. Parvaquone and buparvaquone may be used to treat early cases (Rodistis *et al*., 2006), it was first described in Sudan by (Mason, 1914). And then reported in Khartoum State by (Tageldin *et al*., 1992).

1.9.2 In equine:

Equine theileriosis, an OIE list disease, caused by tick borne *Theileria equi*, is responsible for important economic losses in the equine industry. The disease is endemic in tropical and subtropical regions of the world, including Southern Europe, Africa, The Middle East, Asia, South America and Central America. Disease can occur in per acute, acute and chronic forms. In animals there are: fever, anaemia, jaundice, haemoglobinuria and in some cases death can occur (OIE, 2008b).

In the Sudan Abdoon (1984) reported that *H. anatolicum* was responsible for equine piroplasmosis transmission. *Babesia caballi* and *Theileria equi* have been identified as the causative agents of acute, sub acute and chronic equine piroplasmosis in equids (de Waal, 1992).

1.9.3 In dogs:

There have been no reports of pathogenic Theileria species in dogs. The only species associated with a hemolytic disease of dogs is the *Babesia microti*-like, controversially named parasite, *Theileria annae* (Zahler *et al*., 2000; Camacho *et al*., 2001, 2004; Camacho Garcia, 2006).

Other Theileria *spp*. that has been reported from dogs are *Theileria annulata* (Criado *et al*., 2006).

1.10 Laboratory diagnosis of the disease:

1.10.1 Parasitological test (Microscopic examination)

Diagnosis is usually achieved by finding Theileria parasite in Geimsa stained blood smears and lymph node needle biopsy smears, but species specific diagnosis is difficult as most theileria piroplasm are morphological identical except for *T. velifera*. Schizons are not always present in the superficial lymph node during the disease course (OIE, 2009). (Elhussein, *et al*., 2012) the current situation of tropical theileriosis in Sudan explains that the disease routinely diagnosed using microscopic examination of stained blood smear.

In the live animals, theileriosis is diagnosis by the identification of schizonts in thin blood smears from blood, lymph node. Thin blood films from each cattle were prepared and fixed by methanol, stained with Geimsa stain (1:10) and wash by tap water and up right air dried and then examined microscopically (the erythrocyte form of theileria were rod rounded and ring shaped. Also lab is full diagnosis of theileiosis is more accurate and confirmed. The theileria parasite can be easy detected in mammalian host and the vector ticks (FAO, 1984)

1.10.2 Serological tests:

Antibody detection depends on antigen antibody reaction. Serum antibodies appear 2 to 4 weeks after infection, reach high titres during and shortly after infection subsides. They stabilize and decline to variable levels after 24 to 26 weeks. Antibodies against TBDs can be detected by different serological tests (Burridge and Kimber, 1973). Serological test based on ELIZA have been developed for the detection of antibodies to *T. annulata*, but tests used for *T. parva* and *T. mutans* are indirect ELIZAs based on parasite specific antigens, these ELIZAs provide higher sensitivity than IFA test (over 95%) (OIE, 2018).

1.10.3 Molecular Biology Test:

Development of molecular biology has made accurate tools available for detection of parasite molecules. These techniques are important in veterinary diagnostic parasitology. Since antibodies can remain circulating for some time after the parasite has been cleared from the animals, serological assays do not always provide information about the actual presence of the parasite (Zarlenga and Higgins, 2000). The polymerase chain reaction (PCR) is the most commonly used tool. Target sequences are DNA and ribosomal RNA as well as fragments derived from random amplified polymorphic DNA (RAPD) (Comes *et al*., 1996).

1.11 Control and Treatment of the disease in animals:

1.11.1 Ticks control with acaricides:

The most practical and widely used method for controlling of theileriosis is the chemical control of tick with acaricides (OIE, 2018). 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 new 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, 2008a).

1.11.1.1 The Methods of Ticks control:

Tick control is one of the most important factors influencing the epidemiology of bovine theileriosis. It has been achieved mainly by application of acaricides:

1) Dipping
2) Spraying.

Dipping is considered the most effective method for acaricide application (Norval, 1989).

1.11.2 Vaccination of animals:

Indigenous cattle live with the disease and do not require any intensive tick control or treatment. For valuable exotic stock or their crossbreeds, vaccination and strategic tick control are recommended. Vaccines can be made from either the sporozoite or the schizont.

- **The sporozoite vaccine:** is based on the infection-and-treatment method using schizont-infected cell lines and simultaneous tetracycline treatment as for *T. parua*. It has been suggested that the most economical way to control theileriosis in India is to vaccinate calves and to reserve buparvaquone for treating clinical cases (Singh *et al*., 1993).

- **The schizont vaccine** was formerly blood containing a mild strain of the parasite. The newer vaccines are prepared from live schizonts grown in lymphoid cell culture and attenuated by prolonged passage. They cause virtually no adverse reactions and vaccinated cattle show good resistance to the disease for at least 3.5 years. Therefore, it is necessary to revaccinate, preferably with a different cell line vaccine, if tick population is too low to establish endemic stability. The risk for spread of the vaccine strains in the field is very low (Gubbels, *et al*., 2000).

1.11.2 Treatment of infected animals:

Chemotherapeutic agents such as buparvaquone are available to treat *T. parva* and *T. annulata*. Infections treatment of these agents does not completely eradicate theileria infection 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). Synthetic naphthquinine (Clexon, Parvaxon) are effective against schizonts and Buprvaquone (Butalex) is effective against both scizonts and piroplasm stage (Salih and Hassan, 2003).

Many drugs have been used for the control of *T. annulata* infection in cattle. Tetracycline drugs are effective only when they concurrently are administered with the infection (Brown *et al*., 1977).

1.12 Economic impact of Theileriosis:

Theileria annulata infection causes severe economic losses due to expensive antitheilerial drugs, cost of prevention and control measurement, losses due to mortality and drop of milk productivity of infection. Pregnant cow may be also abort and remain infertile for long time (latif, 1994).

Theileria parva and *Theileria annulata* cause a higher mortality rate in cattle, and represent two of the most virulent species compared with the other reported Theileria species. *Theileria annulata* is widely distributed throughout Europe, the Middle East, Russia, China, and Africa (Uilenberg, 1981).

Latif, (1994). Reported that 85 % of dairy farms investigated in Khartoum State experienced clinical theileriosis. Annual total production losses were established to be between US$ 4 and 6 million, mostly due to deaths in calves (22 %) and heifers (30 %). later Gamal and El Hussein, (2003) reported that 1.5 million Sudanese dinars (approximately US$ 6000) were lost in one farm due to an outbreak of tropical theileriosis. The authors reported that the disease had reduced the expected profitability by 30 %. The disease is highly fatal especially in high producing improved cattle (El Hussein *et al*., 1991).

CHAPTER II

Materials and Methods

2.1 Study area:

The present study was conducted in Khartoum Bahri Khartoum, State, Sudan. Khartoum Bahri Located at latitude 15^{O} 38.19 N and longitude, 32^{O} 318199 E. Khartoum Bahri which divided in to: (Alkadru, Kuku, Alselat and Soba). Which the blood samples collected.

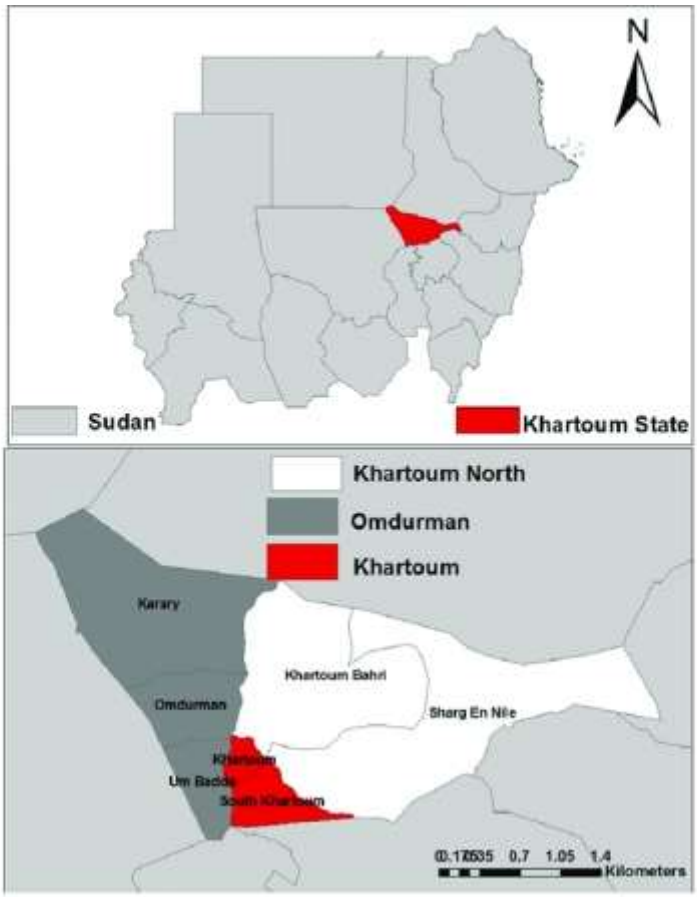


Figure (2.2): Location of Khartoum Bahri.

2.2 Study design:

The study was carried out from February to March 2020 to estimate the prevalence of Bovine theileriosis infection in dairy cattle farms and the potential risk factors could be associated with the disease.

2.3 Sampling method:

A total of 150 blood samples collected from animals of dairy cattle farms with clinically heath and infected animals of different age, sex, body condition. Samples were taken from four different areas the blood samples randomly collected from Kuku (32 animals), Alkadro (48 animals), Alselat (29 animals) and Soba (42 animals).

Blood samples were collected most commonly from coccygeal vein in adult animal and from jugular vein in young animal with Ethylene Diamine Tetra-acetic Acid (EDTA). in a clean sterile tube from all 150 animals of dairy cattle. The sample storage under -4 C^{o} and then immediately transfer to University of Bahri Veterinary Medicine (Educational Veterinary Hospital of Alkadaro), laprotory parasitological. The samples examined for Geimsa stain and complete blood counts. The hematological examination parameters such as hemoglobin (HGB), total red blood cells (RBC, S), total white blood cells (WBC, s), haemocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration(MCHC), were also analyzed using hematological analyzer(SHINIVA).

2.4 Diagnostic procedure of the blood samples:

2.4.1 Smear Preparation and Geimsa staining for blood samples:

The thin blood smear prepared from peripheral blood and apply one drop of blood on to a microscopic slide near to edge. At an angle of 45º another side, place and then the blood spread by gently moving forward of the slide. Then dry after fixation with methanol for minutes (Salih *et al.,* 2007).

The slide stained with 4%Giemsa stain for 30 minutes and then wash with distilled water. One hundred microscopic field examined under oil immersion lens oil (100 xmagnification). The presence of one piroplasm will be considered as a positive case (Salih *et al.,* 2007).

2.5 Haematological Analyzer:

A total of 150 animals blood samples collected and storage under -4c^0 and then directly send to University of Bahri –Department of veterinary Parasitology (Educational Veterinary Hospital of Alkadaro) which examine within 24 hours for complete blood counts by:

- ✓ Haematological analyzer (SHINOVA), Appendix A and B.

2.6 Questionnaire: Appendix E.

Questionnaire was filled in farm during the collection of samples from animals which including the following information:

- ✓ General information about the herd and farms.
- ✓ Risk factors associated with the disease.

The risk factors including in the 2-3 aspect of questionnaire for herd. The age which divided in to three categories less than 2years, 2-4years and over 4years and all other risk factors including two categories such as: Sex to male and female. Body condition to good and poor. Ticks infestation: yes and no. The use of acaricides: yes and no

2.7 Statistical analysis

The data was firstly entering in excel sheets, coded and save. Then export to Statistical Package for Social Science for statistical analysis.

Collected data were analysed by SPSS version 16. and Cross tabulation of Chi-Square test was used to evaluate association between hypothesized risk factors and theileria infection and level of significance was set as *p*-value <0.05.

CHAPTER III

Results

3.1 Prevalence of Bovine theileriosis:

Out of 150 animals examined for blood parasite, 43 animals tested positive for theileriosis, the overall prevalence was 28.6% and 107 animals was negative for theileriosis table (3.1).

Table 3.1

No of examined animals	No of negative animals	No of positive animals	Prevalence %
150	107	43	28.6

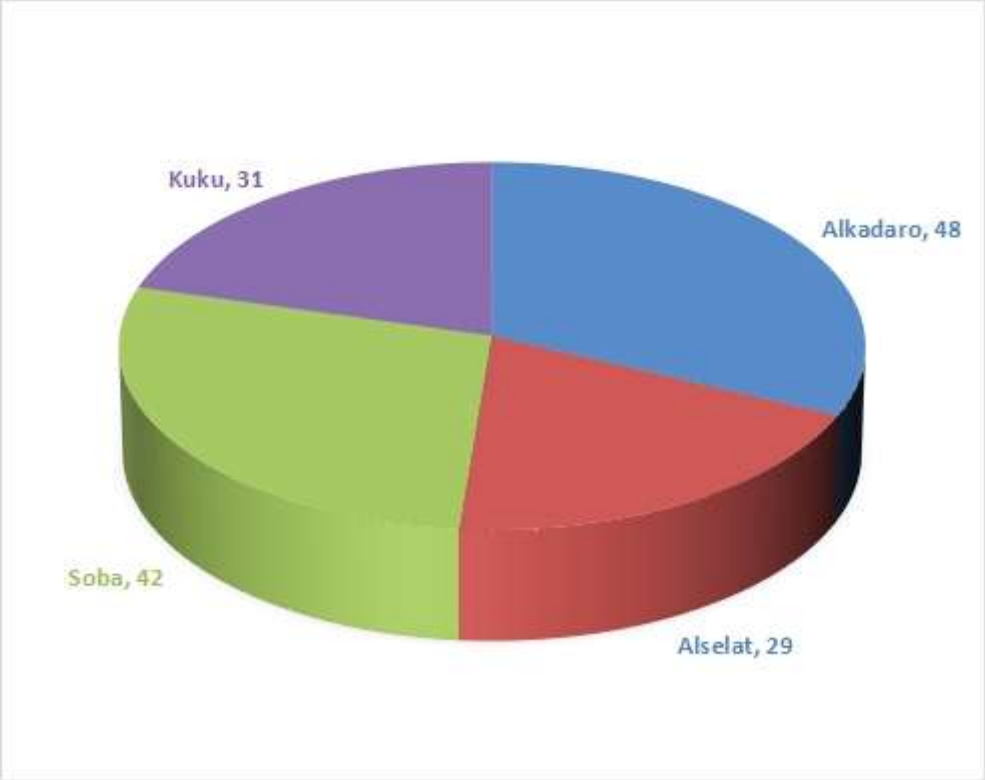
3.2 Risk factors analysis:

3.2.1 Locality:

The highest prevalence was recorded in Alselat (34.4%), followed by Kuku (32.3%), Soba (26.1%) and Alkadaru (25%) Table (3.2) and figure 2.3.

Table 3.2: The prevalence of theileriosis according to locality:

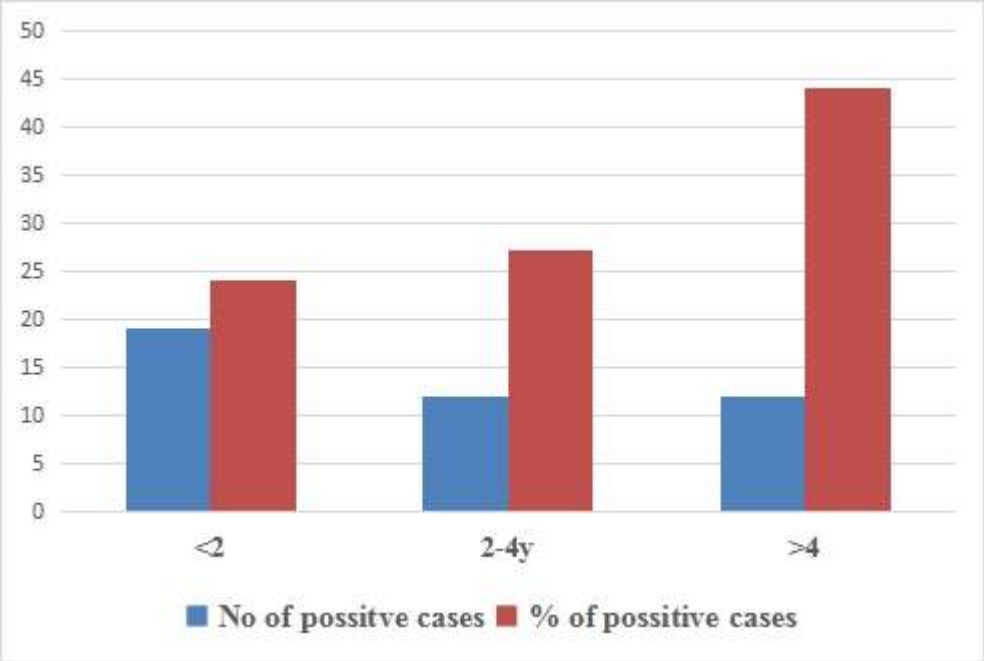
Area	No of examined animals	Positive (%)
Alkadaru	48	12 (25)
Alselat	29	10 (34.4)
Soba	42	11 (26.1)
Kuku	31	10 (32.3)
Total	**150**	**43 (28.6)**



Figures 3.3 Prevalence of bovine theileriosis in the study areas.

3.2.2 Age of animals:

The age of cattle the older animals (>4 years old) with high prevalence (44%) of theileriosis. In animals less than 2years and 2-4years the prevalence was (24 % and 27.2%) respectively. Figure 3.3



Figures 3.4 Age (years) of animals and percentage of the theileriosis infection in dairy cattle farms.

3.2.3 Sex:

The prevalence revealed that males were highly infected (30.5%) and female (28%).

Table (3.3): Prevalence of theileriosis according to sex

Sex	No of animals examined	Positive (%)
Male	36	11 (30.5)
Female	114	32 (28)
Total	**150**	**43 (28.6)**

3.2.4 Body condition:

The animals associated with poor body condition was high infected, the prevalence was (30.5%) and (27.4%) for good body condition Table (3.4).

Table (3.4): Prevalence of theileriosis according to the body condition

Body condition	No of examined animals	Positive cases (%)
Good	91	25 (27.4)
Poor	59	18 (30.5)
Total	**150**	**43 (28.6)**

3.2.5 Ticks infestation:

The theileria was high prevalence in cattle with ticks infestation (30.5%) compared with 26.9% in cattle without tick's infestation Figure (3.4).

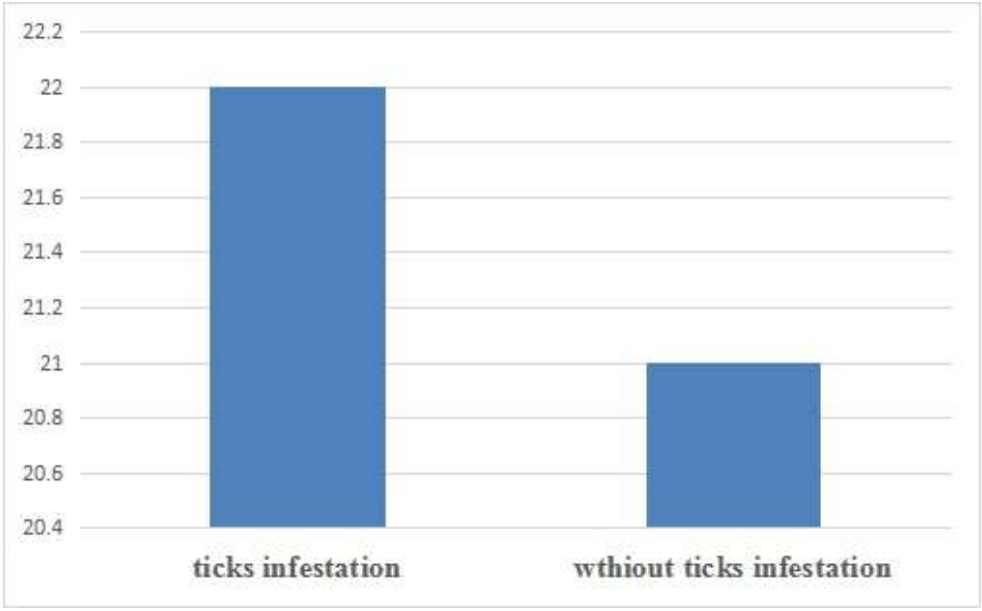


Figure 3.5: Effect of ticks infestation on bovine theileriosis.

3.2.6 Use of accaricides:

The theileria prevalence (36.3%) was high in animals that were not treated with accaricides followed by treated animals (24.2%). Table (3.5).

Table (3.5): Prevalence of theileriosis and the use of acaricides in dairy farms.

Use of acaricide	No of examined animals	Positive (%)
Yes	95	23(24.2)
No	55	20 (36.3)
Total	**150**	**43 (28.6)**

3.3 Hematological parameters analysis of animal's blood samples:

Mean values of hematological parameters of infected animals with Theileria infection and healthy animals are shown below in Table (3.6) and Figure (3.6)

The significant different (P<0.05) reduction in red blood cells and haemoglobin concentration for positive animals compared with negative animals Table (3.6) and Figure (3.5).

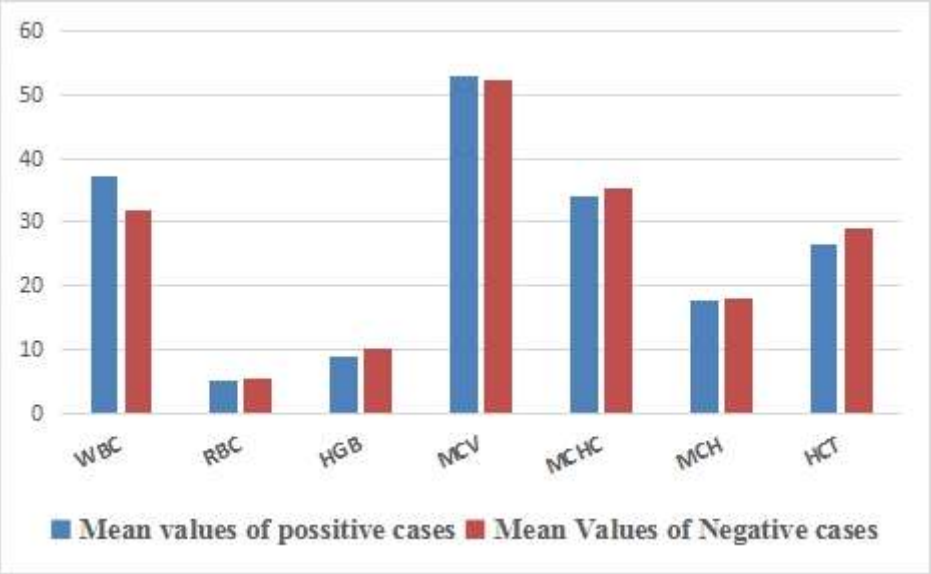


Figure 3.5: The mean values of hematological parameters affected by theileria infection in animals.

Table (3.6): Haematological parameters Vs Infection (N=150 animals).

CBC	Theileriosis	Total No of animals	Mean ± Std	*P* - value
WBC	Negative	107	31.8±14.9	0.5
	Positive	43	37.2±56.1	
RBC	Negative	107	5.5±1.3	0.02*
	Positive	43	5.0±1.0	
HGB	Negative	107	10.1±3.2	0.01*
	Positive	43	8.8±2.2	
MCV	Negative	107	52.3±4.9	0.5
	Positive	43	52.8±6.7	
MCHC	Negative	107	35.2±6.6	0.3
	Positive	43	34.1±6.7	
MCH	Negative	107	18.1±2.4	0.3
	Positive	43	17.1±2.4	
HCT	Negative	107	28.9±8.1	0.09
	Positive	43	26.5±7.3	

*= significant at

p-value <0.05

3.1.1 White blood cells count (10^3/µl):

Total of 150 animals were 43 detected positive and 107 animals negative the mean values of the white blood cells (WBCs) count of the infected animals ($37.2x10^3$/µl) and healthy (negative) was 31.8 thousand cells/ microliter. *P*-value (> 0.05) differences are not statistically significant

. **3.1.2 Red blood cells count (10^6/µl)**:

The mean values of the red blood cells (RBCs) of the infected cattle were ($5.01x10^6$/µl) and healthy animals ($5.5 x10^6$/µl) was statistically significantly different *P*-value (<0.05).

3.1.3 Haemoglobin concentration (g/dl):

The mean values of haemoglobin of the infected animals were (8.8g/dl) and healthy animals were (10.1g/dl) and statistically significantly different *P*-value (<0.05).

3.1.4 Haemocrit (%):

The mean values of packet cell volume of the infected animals were (26.5%) and healthy animals (29.1%). Not statistically significantly different *P*-value (>0.05).

3.4 Erythrocyte indices:

3.4.1 Mean corpuscular volume (femtoliter)**:**

The mean value of the mean corpuscular value (MCV) of the infected animals was (52.8 fl) femtoliter and healthy animals was (52.3 fl). Not statistically significantly different *P*-value (>0.05).

3.4.2 Mean corpuscular haemoglobin (pg):

The mean value of Mean corpuscular haemoglobin (MCH) of infected animals recorded was (17.7pg) and healthy animals were (18.1pg). Not statistically significantly different *P*-value (>0.05).

3.4.3 Mean corpuscular haemoglobin concentration (g/dl):

The Mean corpuscular haemoglobin concentration (MCHC) value of the infected animals was recorded (34.1 g/dl). And healthy animals were reported that (35.2g/dl). Not statistically significantly different *P*-value (>0.05).

CHAPTER IV

Discussion, Conclusions and Recommendation

4.1 Discussion

Ticks-borne diseases of cattle remain an important impediment to livestock development in the Sudan (Gamal and El Hussein, 2003). The present of *T. annulata* in the Sudan was firstly confirmed (anon. 1908).

In the current study, the Giemsa stained blood smears examination revealed 28.6% prevalence of Theileria parasite in dairy cattle in Khartoum Bahri, Khartoum State Sudan. Similar result was reported by (Safa, 2019) who found the prevalence (29,8%) of theileriosis in cattle in Omdurman Localities. But lower prevalence of theileriosis was reported by Ali, (2006) who reported that the infection rate was 16.6% of bovine thieleriosis in Khartoum state. and Shommein, (1976) found the incidence of disease in single farm of dairy cattle was 10.46% and sero-prevalence 86.5% in Khartoum State, while the incidence was 17.9% in western part of Sudan.

In this study the high prevalence rate (44%) was detected in old aged group of animals examined (over 4 years); while the low prevalence rate (24%) was detected in the young age group (less than (2 years) These findings were in agreement with Flach *et al.*, (1995). and Darghouth *et al*., (1996) who conducted epidemiological investigations on tropical theileriosis in Morocco and Tunisia, respectively. Similar result was reported by (Bothina, 2008), high susceptibility of old groups of cattle's to the theileriosis than young's.

In the present study the prevalence rate (30.5%) in the males, while female prevalence rate was (28%), similar result reported by (Abaker *et al.,* 2017) who found **p**revalence of *Theileria annulata* in dairy cattle in Nyala, South Darfur State, Sudan, male prevalence was (11.1%) higher than female prevalence rate (6.8%).

In current study the animals with tick's infestation reported high prevalence of theileria infection (30.5%) compared with animals free of tick's infestation. It's could be due to irregular control of tick's infestation and poor managements in dairy cattle farms.

The prevalence of theileria infection was found to be low in farms using acaricides compared with farms do not use acaricides. This in agreement with (Cauvin *et al*., 2018) that acaricides do have measurable success at reducing the burden of tick burden disease on farms of white tailed deer during summer months.

This study reported significant (P<0.05) in haematological parameters in naturally animals infected with theileria. Low levels of RBC and Hb concentration were reported, compared with the normal rates of healthy animals examined. This finding could indicate that the disease causese anaemia. Similar findings were reported by (El Haj, 2000) who worked on young calves infected with *T. annulata* in Khartoum State. The findings also agree with those of Sharma and Gautam, (1971). The clinical Theileriosis cases of indigenous calves.

Losos, (1986) reported that *T. annulata* piroplasms have been found to cause RBCs destruction. (Maxi *et al*., 1982) showed that theilerial infection was associated with normocytic normochromic non-responsive anaemia. The decrease of the RBCs, PCV, and Hb is the results of the destruction of the myeloid tissue by *T. annulata* infected lymphoblastoid cell toxins (Wilde, 1963; Laiblin, 1978).

4.2 Conclusions:

The study concluded that

- Tropical theileriosis is widely distributed in Sudan and the prevalence rate reported in this study was (28.6%) in dairy cattle farms.
- The theileria infection reduces the red blood cells counts and hemoglobin concentration due to destruction in red blood cells and may lead to anaemia event death may occur in calves and adult animals.
- Poor management of dairy farms and irregular use of acaricides to control the tick's infestation play important role in the theileria infection**.**

4.3 Recommendations:

- ✓ Prober awareness of animal's owner's in the Sudan specially in Khartoum State about the disease effects on animals due to high density of dairy animal's production system.
- ✓ Further investigations using more advanced techniques like molecular biology to detect carrier animals.
- ✓ The proper management and regular tick's control in dairy farms should be applied.

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Appendences

Appendix: A

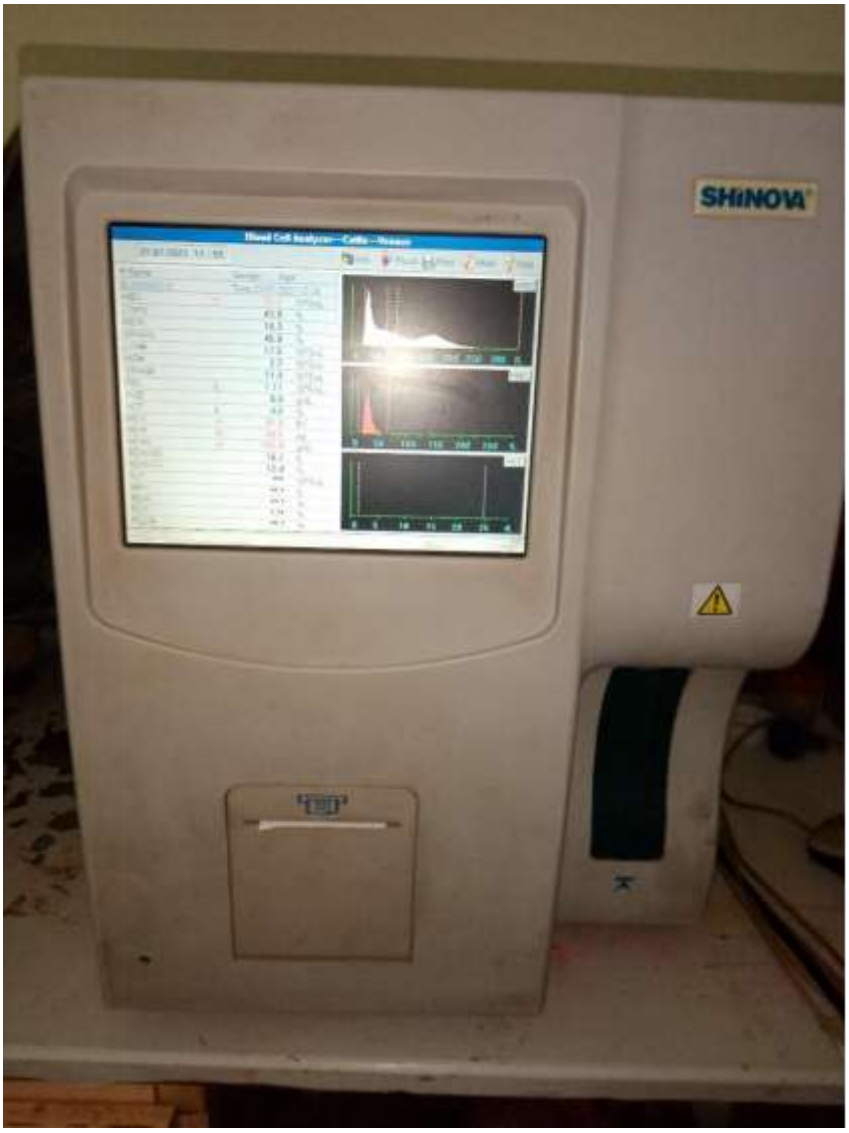
Hematological Analyzer (**SHINOVA**)

Model: Hemo 930V.

Manufacurer: Shinova Systems CO. Ltd. China

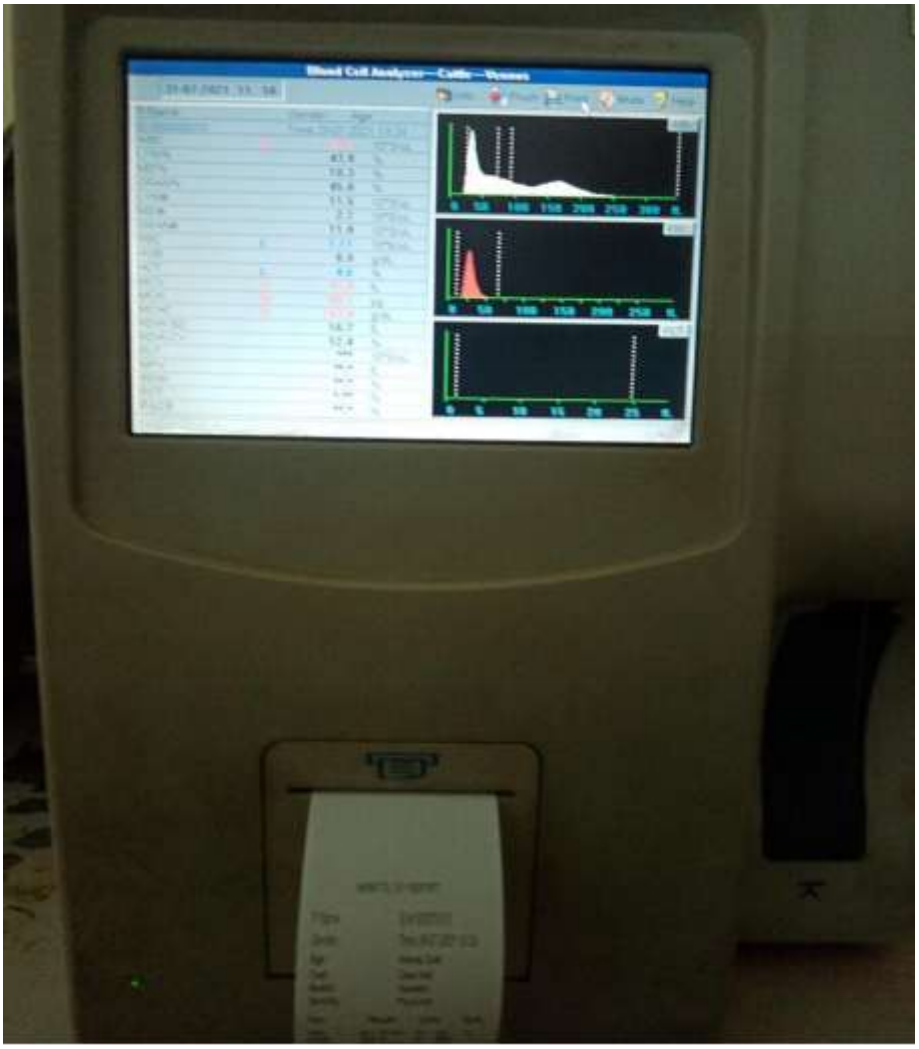
For veterinary use only

Appendix B



The hematological analyzer Machine (SHINOVA).

Appendix C



The hematological analyzer Machine with hematological report of the blood samples print after the diagnosis for complete blood counts.

Appendix D

RESCO E-Z Cleaner : Its use in the haematological analyzer of spine to clean after blood samples diagnosis for complet blood counts and shut down .

Appendix E

Questionnaire:

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنلوجيا

كلية الدراسات العليا

ماجستير الطب الوقائ

نسبة انتشارعدوى مرض الثاليريا في حيوانات مزارع ابقار اللبن في الخرطوم بحرى، ولاية الخرطوم، السودان

The investigation of theileria infection among dairy cattle (N=150 animals) farms in Khartoum Bahri, Khartoum State Sudan.

Localities ……Date……

Herd Owner……. Animal Number……

Address…….

The individual risk factors:

- ✓ **Age of animal (years):**

<2 ( ) 2-4 ( ) >4 ( )

- ✓ **Sex of animal:**

Male **( )** Female **( ).**

Body condition of animal

Good **( )** poor **( )**

Ticks infestation in animal:

Yes ( ) No **( )**

The use of acaricides in dairy cattle farms and animals:

Yes ( ) No ( )