Sudan University of Science and Technology

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Prevalence and Risk Factors of Tick Borne Blood Parasites in Sheep in Omdurman Area, Khartoum State

معدل الانتشاروعوامل الخطر لطفيليات الدم المنقولة بواسطة القراد في الضان بمنطقة امدرمان – ولاية الخرطوم

A thesis Submitted in Partial Fulfillment of the Requirement for the Master Degree of Preventive Veterinary Medicine

By

Mona Yagoub Suliman Mohammed

(B.V.M.2004,University of Khartoum)

Supervisor

Professor : Siham Elias Suliman Mohammed

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الآيــة

قال تعالى:

(وَمِــنَ الأَنعَــامِ حَمُولَــةً وَفَرْشــاً كُلُــواْ مِمَّــا رَزَقَكُــمُ اللّــهُ وَلاَ تَتَّبِعُــواْ خُطُــوَاتِ الشَّيْطَانِ إِنَّهُ لَكُمْ عَدُوٌّ مُّبِينٌ)

صدق الله العظيم

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DEDICATION

To my parent, husband,
brother , sister sons , daughter
and all my familly

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Thanks first and last to Allah who made me the resolve and made this work possible.

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ABSTRACT

This study was conducted in Omdurman area-Khartoum State to investigate the prevalence of tick borne blood parasites in sheep and to assess the association between theileriosis, babesiosis and anaplasmosis and the risk factors (locality, breed, sex, age, body condition, tick infestation and use of acaricides). A total of 150 blood samples were collected from three localities namely Ombada, Karary and Omdurman from January to February 2019. The samples were examined in laboratory microscopicly using Giemsa stain. The results showed that the prevalence of theileriosis was 11.3 %, babesiosis was 22% and anaplasmosis was 22%. Univariate analysis for the results was done using chi-square test showing no correlation between prevalence of theileriosis, babesiosis and anaplasmosis and risk factors except there was significant association between theileriosis and tick infestation(X^2 = 4.94; P = 0.026) and babesiosis with the breed (X^2= 6.506; P = 0.039) and usiage of acaricides(X^2 = 0.006). The multivariate analysis using logistic regression for the results showed no significant association between tick borne blood parasites and risk factors except significant association between usage of acaricides and Babesia infection Exp (B) =.329 ; p – value = .008) . This study confirmed that tick-borne blood parasites were endemic in Omdurman area.

الخلاصه

اجريت هذه الدراسه في منطقة ام درمان–ولاية الخرطوم لتقدير معدل انتشار مرض طفيليات الدم المنقوله بواسطة القراد في الضان ودراسة عوامل الخطر المرتبطة بها (المكان ، السلاله ، الجنس ، العمر ، حالة الجسم ، وجود القراد واستحدام مبيدات الحشرات). تم جمع 150 مسحه دمويه من ثلاث محليات هي امبده وكرري وام درمان من شهر يناير الي فبراير 2019 وتم فحصها مجهريا باستخدام صبغة جمسا. واظهرت نتائج الدراسه ان معدل الثاليريا 11.3% والبابيزيا 22% والانابلازما 22%. التحليل الفردي باستحدام مربع كاي (p-value≤ 0.05)اظهر بانه لا يوجد ارتباط بين معدل انتشار مرض الثايليريا والبابيزيا والانابلازما وعوامل الخطر ماعدا وجود ارتباط بين تكرار مرض الثاليريا ووجود القراد (X^2 = 4.94; P= 0.0261) وبين تكرار مرض البابيزيا و سلاله الحيوان (X^2 = 6.506; P=0.039) واستخدام مبيدات القراد (X^2= 7.486; P = 0.006). باستحدام التحليل بالانحدار اللوجستى لمعرفة درجة الارتباط بين معدل انتشار طفيليات الدم المنقولة بواسطة القراد وعوامل الخطر اثبتت النتائج عدم وجود ارتباط بينهم ما عدا وجود ارتباط وثيق بين استعمال مبيدات القراد ومرض البابيزيا (Exp (B) =.329; P value =.008).هذه الدراسه اكدت ان امراض طفيليات الدم المنقوله بواسطه القراد في الضان متوطنه في منطقه امدرمان .

INTRODUCTION

Tick borne diseases (T B Ds) are one of the most important constrains to livestock production in developing countries (Makala *et al*., 2003). They are responsible for high morbidity and mortality resulting in decreased production of meat, milk and other livestock by- products together with loss of draught power (Osman *et al*., 2017).

Sheep production in Sudan faces many problems including infectious diseases caused by bacterial, viral and parasitic agents .Parasitic diseases have largely been neglected primarily because they do not often cause acute fatal diseases. Blood parasites feed on blood its constitute of nutrients .They are difficult to control due to their resistant to drugs, and lack of vaccine against most of them due to several factors such as antigenic variation and difficulties in propagation of these organism in artificial media (Mohamed, 2006).The infection with blood parasites can be suspected from general symptoms of the diseases such as decrease in production, emaciation, loss of appetite and jaundice (OIE,2018). Sheep can be affected with blood parasites as *Babesia*, Theileria and *Anaplasma* and the two most important species of *Babesia* are *B.ovis* and *B.motasi* transmitted by *Rhipiciphalus bursa* and *Haemaphysalis spp* respectively. *Theileria Lestiquaradi* cause disease in sheep and goat and transmitted by tick of genus *Hyalomma*. Anaplasmosis also affect Sheep in tropical and sub-tropical regions and transmitted by up to 17 different tick vector species.(Alfaki ,2004). In Sudan, tick and tick borne diseases are wide spread, they represent a threats to domesticated animals specially sheep which estimated as in spite of the largest number of the livestock, the outcome is reduction in production and productivity. The most important tick –borne diseases (T B Ds) agents present in Sudan are *Theileria annulata* ,*T.parva* ,*T.Lestoquardi* ,*Babesia bovis* ,*B.bigemina* ,*B.ovis*

,*Cowderia ruminantum* ,*Anaplasma marginale* ,*A.centrale* and *Borrelia anserine*(EL Hussein *et al.*, 2002).

Objectives of this study:

The research aims were to achieve the following objectives:

1- To investigate the prevalence of theileriosis, babesiosis and anaplasmosis in sheep in Omdurman area.

2- To determine the risk factors associated with the mentioned tick borne protozoan disease.

CHAPTER ONE

Literature Review

1-1 Sheep Breeds and their Distribution in Sudan:

The estimated Sudanese national Sheep flock is 40612000 head (CIU, 2017). Sudan Sheep are conventionally classified on the basis of morphology and distribution into four main groups: Sudan desert, Sudan Nilotic, Sudan Arid Upland and Sudan Equatorial Upland (Macleory, 1961; Wilson and Clarke, 1975). Fused ecotypes from non-systematic crossbreeding at the boundaries of the ecozones have also been recognized. More than 65% of the sheep in Sudan are the Sudan Desert type *Ovis aries* (Sulieman *et al* .,1990) ,which is believed to be distributed north of latitude 18 N, extending east ward into Eritrea and west ward into Chad (Wilson,1991) and are raised under pastoral system in the eastern and western regions of the country .Sudan Desert Sheep are further classified into tribal sub types ,e.g Hamari ,Kabashi , Shenbali in North and West Kordofan state (Mukhtar,1985), Shgor ,Dubasi and Watish in the central states (Sulieman *et al*., 1990) and Bourug in the Butana area of eastern Sudan.

1-2 The Ticks :

Ticks are members of the class Arachnida which parasitize mammals, birds and reptiles .All ticks are blood sucking parasites .Ticks are found in most parts of the world but are generally limited to those habitats frequented by their hosts. Namely to woods, tall grasses, crevices and shrubby vegetation where they climb and wait to cling on a passing host (Oleg kozhukhov, 2007).

There are two families of ticks ,the family *Argasidae* (soft ticks) , which lacks scutum and the dorsum is covered by leathery integument and the family *Ixodidae* (hard ticks)whose scutum or dorsal shield covers the

entire upper surface of male and relatively a small area just behind the head in the female (Soulsbly,1982).

1-2-1 Tick BIOLOGY:

There are four stages in the life cycle of ticks which are egg, larva, nymph and adult (Hoogstraal ,1956). According to the number of host they require during their life cycle, ticks can be classified into three groups .These are One host ticks in which three developmental stages engorge on the same host. The two ecdyses take place on the host (Boophilus *decoloratus* and *B-annulatus).* Two host ticks in which the larvae engorge and moult on the host and the nymph engorges on the same host and drops to the ground to moult .The resultant adult feeds on a second host (*Rhipiciphalus evertsi evertsi* and *Hyalomma marginatum rufip).* Three host ticks a these require a new host for every developmental stage. The engorged larvae and engorged nymph moult on the ground (*Ixodes ricinus ,Amblyomma lepidum)* (Alfaki , 2004) .

1-3 Tick borne blood parasites :

1.3.1 Historical background:

Theileria were first seen in cattle by Koch in (1893) and those of *Babesia* by Babes in (1888). In fact *B.ovis* was discovered in Romania by Babes in (1892) .Sheep *Theileria* species, *T.hirci* and *T.ovis* were discovered by Rodhain in (1916) and Dschunkowsky and Urodschevich in (1924). After 7 years of the first record of *Theileria* sp. Cliver in (1905) reported *Theileria* from cattle in Sudan. Then piroplasms of both *Babesia* and *Theileria* have been regularly reported from cattle, horse and dogs .Ten years after the discovery of bovine theileriosis sheep theileriosis has been reported (Nagwa ,1986) .

1-3-2 Theileriosis:

Theileria are obligate intracellular protozoan parasite that infect both wild and domestic Bovidae throughout much of the world .Some species also infect small ruminants .They are transmitted by ixodid ticks ,and have complex life cycle in both vertebrate and invertebrate host .There are six identified *Theileria* spp .that infect cattle ; the two most pathogenic and economically important are *T.parva* and *T.annulata* .*T. lestoquardi* (*T.hirci*) is the only species of economic significance infecting small ruminants (OIE,2009) .

1.3-2-1. Classification:

The classification of the genus *Theileria* according to the revision of the Committee on systematic and Evolution of the society of protozoologists (CSESP) which was published by Levine *et al* .(1980)and confirmed by DNA sequencing (ELLis *et al* .,1992)is :

Phylum : Apicomplexa

Class : Sporozoea

Sub class : Piroplasmia

Family : Theileridae

Genus : *Theileria*

1.3.2.2 Species, Host and Distribution:

Theileria are widely distributed in cattle and Sheep in Africa ,Asia ,Europe and Australia and have a variety of tick vectors and are associated with infections which range from clinically in apparent to rapidly fatal .Although the specification of many *Theileria* are still controversial largely because of their morphological similarity and there are three species of major veterinary importance. Minor and mildly pathogenic species infecting cattle include *T.mutans* and *T.taurotragi* and *T.sergenti* in Asia; the identities of the European and Australian bovine species are uncertain.

In sheep, the non – pathogenic *T. ovis* occurs in Europe , Africa and Asia (Urquhart *et al*., 1996).

1.3.2.3 Life cycle:

Safieldin and Elmalik (2005) mentioned the life cycle of *Theileria* species which involves two components, the life cycle in vertebrate host and life cycle in the tick vector.

1.3.2.3.1 Life cycle of theileria spp in Vertebrate host:

The schizogony stage begins when *Theileria* sporozoites (infective stages) are injected into the vertebrate host in the saliva of infected ticks (Nymphs or adults) during the feeding process. The injection of the parasites commences from day 4 to day 5 of ticks attachment on the host. It is considered to be a period of matureatim of the parasites within the tick salivary gland (Walker, 1990) and 10 – 30 minutes after injection, sporozoites invade different leukocytes sub – types depending on the *Theileria* species.

Inside the leukocyte the parasite develops into macroschizont and induces transformation and proliferation of the host cells (William and Dobblaer, 1993).

Cell cycles of parasites leading to the formation of multinucleated parasites , the microschizonts (Shiels *et al* ., 1997) ,which are equally divided to daughter cells upon cell host division (Irvin *et a*l ., 1982) .The microschizonts differentiate into merozoites which increase in number , leading to lymphocyte ruptures and release of merozoites .The trigger for differentiation are not well known .The free merozoites penetrates erythrocytes 8 – 10 days post infection with *T. annulata* (Mehlhorn and Schein , 1984) . Inside the red blood cells the parasites develop into the piroplasm stage, , depending on the species,which appear as rod ,comma or round shaped organisms .Piroplasms replicate inside red blood cells and newly formed merozoites proceed to infect other blood cells .Some very

small merozoites change into ovoid forms . Only these ovoid forms are able to develop within the gut of a feeding tick (Mehlhoen and Schein ,1984)

1.3.2.3.2 Life cycle of *Theileria* spp .in the tick:

The life cycle of various *Theileria* species in the tick is probably similar larvae and nymph ingest millions infected erythrocytes even from an animal with low parasitaemia .The majority of these ingested piroplasms are rapidly destroyed within the gut lumen due to the secretion of the acid phosphatase by the gut epithelial cells during feeding (Walker ,1990) . At or just after engorgement, the free parasites, which have variety of shapes and size become micro and macrogametes. Zygote formation takes place in the gut lumen (Mehlhorn and Schein, 1984), resulting of spherical zygote which invades a gut epithelial cell and develops into a kinete. Only single motile kinete is formed from each zygote (Melhoorn *et al*., 1978).The formation of kinetes and their appearance in the hemolymph take place prior to moulting of the tick (Mehlhorn and Schein, 1984). kinetes enter the salivary glands and develop in the cells of type 111 acini (Fawcett *et al* ., 1982 ; Fawcett *et al* ., 1982 ; Fawcett *et al* ., 1985) .This entrance into the salivary glands appears possible only after the glands have redeveloped following moulting (Fawcett *et al* ;1981 ;Fawcett *et al* .; 1981 ;Fawcett *et al* ., 1982) . Once the parasite enters the salivary gland cells, it begins to develop and the host cell undergoes marked hyper trophy. This development and hyper trophy varies from tick to tick and from acinus to acinus in the same tick. With the onset of tick attachment and feeding on new host , there is a rapid increase in acinus hypertrophy and the parasite becomes multinucleated (sporogony) and hence formation of sporozoite and their release into the tick saliva takes place (Fawcett *et al* ., 1982 ;Fawcett *et al* .,1985) .

1.3.2.4: Transmission of Theileriosis :

Theileria spp are transmitted by ticks acting as a biological vectors .*Hyaloma spp*.are the vector for *T.lestoquardi*, *T. ovis* and *T.separata*. *Theileria* sporozoites are transmitted to animals in the saliva of the feeding tick. (IICAB, 2009).

1.3.2.5 Clinical signs of Theileriosis:

Malignant ovine Theileriosis, (*Theileria lestoquardi* infection) may be acute, sub-acute or chronic. In the acute form, fever is followed by rapid death. the animal may develop in appetence , depression , respiratory distress , paler of visible mucous membrance , oedema of the throat and swelling of the superficial lymph nodes (Tageldin *et al* ., 1992;El-Hussein *et al* ., 1993 ;latif *et al* ., 1994). In sub-acute and chronic forms, the symptoms are comparable to those found in the acute form except that they are less marked (losos, 1986) . Malignant ovine Theileriosis is reported to cause high morbidity and mortality rates that may reach up to 90% (Hooshmand –Rad and Hawa ,1973 ; Tageldin *et al* ., 1992 ; latif *et al* ., 1994) In Nahr ElNeil State (Northern Sudan) the disease represented 21% and 12% of the total diagnosed diseases of sheep during the years 1981-1992and 1992 -1993 ,respectively (EL Ghali and El-Hussein ,1995) , and Nagwa (1986) found that 68% (96 of 152) of sheep examined in Khartoum State were infected with *Theileria* species . The severity of the disease depends on the susceptibility of the animal and the virulence of the strain. It is also dose dependents. The more sporozoites injected by the tick cause severe infection El Hussein *et al* .,(1993) reported that there was no correlation between severity of the disease and the levels of parasitaemia in sheep .They concluded that it might be related to strains rather than number of parasites .

1.3.2.6: Treatment of Theileriosis:

1- Chloretetracyclin and Oxytetracycline are effective in different dose aschedules as per different status of disease.

2- Buparvaquone 2.5 mg / kg bwt.

3- Halofuginone 1-2 mg / kg bwt is effective.

4- Menoctone – Asingle dose of 10 mg / kg (IV or IM) (Mandal , 2012).

1.3.3: Babesiosis:

Babesia are intra erythrocytic parasites of domestic animals and are the cause of anaemia and haemoglobinuria .They are transmmitted by ticks in which the protozoan passes transovarially, via the egg, from one tick generation to the next .The disease, babesiosis is particularly severe in naive animals introduced into endemic areas and is a considerable constraint on livestock development in many parts of the world. (Urquhart *et al*., 1996).

1.3.3 .1: Hosts and Intermediate host

Babesiosis infected all domestic animals .The intermediate hosts are hard ticks of the family Ixodidae in which transovarian infection ensures that *Babesia* are transmitted by stage of the next generation of ticks. Depending on the species of *Babesia*, this may be the larval, nymphal or adult stages or even all the three. When infection persists from one stage to the next in two–or three – host ticks feeding on different hosts, transmission is said to be transtadid. The organisms lie singly or in pairs inside the red blood cells. (Urquhart *et al.,* 1996).

1.3.3.2: Classiffication:

According to Levine *et al*. (1980), the parasite confirmed by DNA sequencing (Ellis *et al.,* 1992) abrief classification of *Babesia* is given below.

Phylum: Apicomplexa

Subclass: Sporozoea

Family : Babesiidae

Genus : *Babesia*

1.3.3.3: Life cycle of Babesiosis:

Babesia multiplies in erythrocytes by asynchronous binary fission, resulting in considerable pleomorphism .This replication eventually gives rise to gametocytes that are ingested by the vector tick .Conjugation of gametocytes occurs in the tick gut followed by multiplication by multiple fission and migration to various tissues including the salivary glands. Further development occurs in the salivary glands before transmission .The ovaries are also invaded, which leads to transovarial transmission (Mandal, 2012). The host gets the infection when the larva sucks blood. After one moulting the larvae transform into adult and they transmit the infection in a similar way (Gray *et al*., 2010). The infective stage of *Babesia*, sporozoite inters the host when the tick sucks blood (Lefevre *et al*., 2010).

1.3.3.4: Small ruminant Babesiosis:

Two species of *Babesia* , the smaller *B.ovis* and the larger *B.motasi* , were known to infect sheep and goats in tropical and subtropical areas , including south Europe and were transmitted by various tick genera ,such *as Rhipicephalus Haemaphysalis* ,*Dermacentor* and *Ixodes* species (Urquhart *et al* ., 1996) .

1.3.3.5: Clinical Signs of Babesiosis :

Typicaly the acute disease occurs 1-2 weeks after the tick commence to feed and is characterized by fever and haemoglobinuria (red water).The mucous membrane first congested and then become jaundiced , the respiratory and pulse rates are increased , the heart beat is usually very audible ,and in cattle ruminal movements ceased and abortion may occur. If untreated, death commonly occurs in this phase. Otherwise convalescence is prolonged. There is loss of weight, milk production and diarrhea followed by constipation is common .In animals previously exposed to

infection, or infected with a *Babesia* species of low pathogenicity, clinical signs may be mild or even in apparent (Urquhart *et al.*, 1996).

1.3.3.6 Treatment of Babesiosis:

The success of the treatment depends on early diagnosis and the prompt administration of effective drug. The first specific drug used against bovine babesiosis was Trypan blue, which is a very effective against *B. bigmina* infections, however, it did not have any effect on *B. bovis* and it had the disadvantage of producing discoloration of animal's flesh, so it is rarely used. Diminazene aceturate, which is widely used currently in the tropics as a babesiaci de, was withdrawn from Europe for marketing reasons (Demessie and Dersos, 2015). Imidocarb is the principal babesiacide used in animals, the only one that consistently clear the host of parasites and for over 20 years, it has been used in the treatment and prophylaxis of babesiosis and anaplasmosis, imidocarb remained in edible tissues of ruminants for long periods after treatment (Mosqueda *et al*., 2012) .The combination of imidocarb dipropionate and oxytetracycline is the most effective treatment of babesiosis in sheep and goats (IJaz *et al*., 2013). In addition , supportive therapy such as blood transfusion , anti – inflammatory drugs , tick removal , iron preparations , dextrose , vitamin B complex , purgatives and fluid replacements , may be necessary in severe cases of babesiosis (Mosqueda *et al* ., 2012) .Vitamin E also act as supportive therapy as vitamin E ameliorates the oxidative effect of *Babesia* by increase antioxidant effect (AbdelHamid *et al* ., 2014) .

1. 3.4: Anaplasmosis:

Anaplasmosis is a disease caused by rickettsial parasite of ruminants, *Anaplasma* spp. The microorganisms are gram negative, and infect red blood cells. They are transmitted by natural means through a number of haematophagous species of ticks. (Hartelt *et al*., 2004).Twenty species of ticks have been shown to transmit *Anaplasma*. Transovarial transmission

occurs and insects (blood sucking flies e. g deer flies, stable flies) play a significant role in mechanical transmission (Alfaki, 2004). Anaplasmosis can also be transmitted by use of surgical , dehorning , castration and tattoo instruments and hypodermic needles that are not disinfected between using (Hartelt *et al;*, 2004) .

1.3.4.1: Species:

Anaplasma in cattle**:**

Anaplasma marginale **-** found worldwide.

Anaplasma centrale - found mainly in south America,Africa and the Middle East .

Anaplasma in sheep and goat **:**

Anaplasma ovis – found worldwide (Boes and Durham, 2017).

1.3.4.2 Life Cycle of *Anaplasma*:

Once *Anaplasma* in the blood , the organism enters the red cell by invagination the cell membrane so that a vacuole is formed ; thereafter it divides to form an inclusion body containing up to eight (initial bodies) packed together . The inclustion bodies are most numerous during the acute phase of the infection, but some persist for years afterwards. (Urquhart *et al.*, 1996).

1.3.4.3 Clinical signs of Anaplasmosis:

The first clinical signs are typically fever, ranging from 103^0 F to 106^0 F and lasting12-24 hours. Most other clinical signs are manifestation of acute anemia, including mucosal pallor, muscle weakness, tachycardia, tachypnea, exercise intolerance, and behavioral changes. Additional signs that may be present include depression, anorexia, ptyalism, dehydration, constipation, and frequent urination with dark yellow urine. Hemoglobinuria does not occur because the anemia result from the destruction of parasitized erythrocytes in the spleen, not from intravascular hemolysis. Jaundice and weight loss may occur later in the disease. Milk

production declines rapidly in dairy cows .*A .ovis* infection in sheep and goat is typically asymptomatic (Tucker, 2001)

1.3.4.4 Treatment of Anaplasmosis:

Tetracycline compounds are effective in treatment if given early when the diseases occurred and especially before the parasitaemia has reached its peak .Tetracycline is effective when injected at 5 to 10 mg / kg, providing treatment is repeated twice at 24 hours interval. The treated animals are not free of *Anaplasma* after treatment, but can be eliminated completely by a longer treatment (lefevre *et al*., 2010). More recently imidocarb has been shown to be effective and may also use to sterilize carrier animals (Taylor *et al*.,2007).

Symptomatic treatment such as blood transfusion, drugs that stimulates erythropiosis, drugs which protects liver cells may help in recovery (lefevre *et al*., 2010).

1.3.5 Diagnosis of tick –borne blood parasites:

Tick – borne diseases are major economic constraint to livestock production. Identification of these haemoprotozan and rickettsial infection is essential in understanding the epidemiology and it is important to distinguish between species and sup species involved.

Converntional techniques including serological and microscopic examinations do not always meet these requirments. Clinical examination and surveillance tools, such as the complement fixation test (CFT) and the enzyme linked immmunosrbent assay (ELISA) have been successfully used over decades . In addition, DNA-based testes for diagnosis, differentiation and characterization of different haemoparasites have been developed. Molecular diagnostic techniques, such as DNA hybridization and polymerase chain reaction (PCR) allow detection of parasites in blood tissue or tick with high levels of sensitivity (Salih , 2015)

1.3.6 Control of tick borne blood parasites:

Control of tick borne hemoparasitic diseases of ruminant by using effective methods such as vector control, chemoprophylaxis and immunization (Demessie and Derso, 2015).

1.3.6.1 Tick control:

1-3.6.1.1 Acaricids control:

The use of acaricide has been a major component of integrated tick control methods. Acaricides are often inappropriately used, have residual effects in milk and meat subproducts, and are not environmentally friendly, being responsible for the increase of acaricide – resistant ticks. Resistance is associated with mutations in genes related to drug susceptibility. The appearance of acaricides, resistance leads to the rise of individuals for which the lethal dose is higher than the one for the majority of determining species. Noadays, combination of powerful acaricides are being used worldwide; products combining different active components are available in an attempt to include a diverse number of mechanisms of action to reduce the emergence of insecticide resistance (Jonejan and Uilenberg, 1994).

1.3.6.1.2 Biological control:

For many years, acquired resistance to ixodid tick, has been recognized as possible biological control method. Such resistance acquired after repeated infestation by ticks, is immunologically mediated. Acquired immunity is expressed by reduction in the number of ticks which attach to the host, reduced ingorgement weight, and reduced egg and larval production resulting in significantly reduced tick population. The occarence of this type of resistance varies with the tick species and the type of breed and between indviduals. Probably depending on natural selection of animals exposed to the tick in question over many generations (Jongegon and Uilenberg, 1994). Recently particular attention has been focused on the

development of entomopathogenic fungi as biocontrol agents arrange of several ticks under laboratory and field conditions.

Biocontrol agents usually favor especially comparison to the use of acaricides. But few bio pesticides have been used in spite of their potential. The inability to successfully adopt biocontrol strategies includes factors like environmental stability (e.g Uv resistance, temperature tolerance), ability to initiate infection at low humidity, and potential unspecific damage to non target invertebrates. (Domingos *et al*., 2013).

1.3.6.1.3 Vaccines:

Research on alternative to the use of acaricides considered a more cost effective and environmentally safe strategy. Vaccines based on the BM86 tick antigen are used in the first commercially available cattle tick vaccines and showed good result in reducing tick numbers, affecting weight and reproductive performance of female ticks which resulted in reduction of cattle tick populations over time and consequently lower reduction of the pathogen agents they carry (Domingos *etal*.,2013).

Chapter Tow

Material and Methods

2.1. Study area:

Omdurman area was selected for this study. It is located in central Sudan in Khartoum State on the west bank of the white Nile and River Nile, around 12 Kilometers of aradius latitude 15^0 - 38^0 N and longitude $32^{0\,-}$ 26^0 E .Omdurman is containing three localities ,Omdurman in 895 Km , Karary in 4646 Km and Ombada in 2667 Km . The count of sheep is 91,601 in Omdurman, 48.256 in Karary and 38,840 in Ombada. The total was 178.697 sheep of local breed (Khartoum state, 2017).The climate of Omdurman is a semi – dessert arid type characterized by a wide range of changing in daily and seasonal temperatures. Sheep was the main target in this study. Samples were selected randomily in some sheep centers located in the three localities of Omdurman area. , These centers were Alfiteihab ,Almuailih and Alarda in Omdurman , Gandahar and Albaraka slaughter house in Ombada and Alahamda center in Karary .

2.2 Sample size:

This study was conducted during February and March 2019. Atotal of 150 blood samples were collected according to formula of Thrusfield (1995)

This formula is: $n = 1.96^2 . p_{exp}(1-p_{exp})/d^2$

Where:

N = sample size

1.96^2 = constant

P_{exp} = expiated prevalence

d = desired accuracy level at 95% confidence interval

The expected prevalence was calculated depending on previous study by Elnoor (2017), who found the prevalence of anaplasmosis in sheep in Khartoum state was 11.3%

N = 148 animals .The sample size was completed to 150 samples.

2.3 Questionnaire:

Samples were collected from different age groups and sexs at time of visiting .Information of sheep examined including: date ,owner name ,telephone, locality , , breed , sex , age ,body condition , presence of ticks and usage of acaricides were recorded using serial numbers .

2.4 Diagnosis of tick borne blood parasites:

2.4.1 Preparation of blood smears:

Blood smears were prepared from the blood collected from the jugular vein .As spreader kept at an acute angle in order to obtain one thin layer smear. The slides were air dried and immediately fixed in absolute methyle alcohol for 2-3 minutes. .

2.4.2 Giemsas Staining procedure:

One ml of Giemsa stock solution was diluted in 9 ml. The slides were then flooded with the stain for 45 minutes. They were washed with distilled water and allowed to air dry at room temperature and scanned under x 100 magnification using oil immersion lens for presence of piroplasms.

2.5 Data Analysis:

Chi. Square (x) test and Logistic Regression were used for assessing the statistical association of various factors for presence of tick borne blood parasites using Microsoft of Excel and computer application SPSS version 16 for data analysis .

CHAPTER THREE

Results

3.1 Overall prevalence rate of tick borne blood parasites in sheep in Omdurman area:

A total of 150 blood samples of sheep were collected and examined microscopically examination for the presence of tick born blood parasites (Table 1). The overall prevalence of theileriosis was 11.3% (17/150), babesiosis was 22% (33/150) and anaplasmosis was 22% (33/150).

3.2 The prevalence rate of tick borne blood parasites in sheep based on localities of Omdurman area

In this study 56 blood samples were collected from Omdurman, 14 from Karary and 80 blood samples from Ombada. The chi- square test showed no significant association between localities and infection with *Theileria* , *Babesia* and *Anaplasma* (Table 2) .

3.3 Risk factors analysis:

3.3.1 Risk factors analysis with *Theileria spp :*

3.3.1.1 Sex of animals:

According to the sex of sheep examined (Table 3). The result showed 1 out of 35 males (2.9%) and 16 out of 115 females (13.9%) were infected with *Theileria*. There was no significant variation between *Theileria* infection of males and females (x^2 =3.264; p-value =0.071).

3.3.1.2 Breed of animals:

Considering breed of sheep examined, the result showed 7 out of 93 (7.5 %) Hamary, 8 out of 42 (19 %) Kabashy and 2 out of 15 (13.3 %) Balady were infected with *Theileria.* (Table 3) There was no significant difference between *Theileria* infection and breeds (X^2 = 3.888; p-value =.143).

3.3.1.3 Age of animals

According to the age of animals examined, the animals were classified based on age (Table 3) into three groups, < 1 year, 1-2 year and > 2 year. The result showed that 1 out of 24 (4.2 %), 4 out of 42(9.5 %) and 12 out of 84 were infected with *Theileria* respectively. No association between *Theileria* infection and the age of animals (X^2 = 2.092 ; p-value =.351).

3.3.1.4 Body condition of animals:

According to the body condition, the animals examined were classified as good, moderate, and poor body condition, the animals in good body condition were 12.6 % , 8.9 % in moderate and 14.3 % in poor body condition (Table 3) . The chi –square test showed there was no significant association between body condition and *Theileria* infection (X^2 =.532; p-value = .767).

3.3.1..5 Presence of ticks:

The animals in this study were examined for the presence of tick on their bodies (Table 3). Ten animals out of 52 (19.2%) were showed ticks on their bodies were positive to *Theileria* and 7 animals out of 98 (7.1%) were showed no ticks on their bodies were positive to *Theileria* . There was a significant association between tick on animal bodies and *Theileria* infection (X^2 =4.94 ; p-value = .026) .

3.3.1.3.6 Usage of acaricides:

Regarding use of acaricides , there was no significant association(Table 3) between using of acaricides and *Theileria* infection (X^2=2.9 ; p-value =.088) . However, sheep were sprayed by acaricide are more likely to be healthy (8.5%) than that sheep had not sprayed (18.2 %).

3.3.2 Risk factors analysis with *Babesia* spp in sheep :

3.3.2.1 Sex of animals:

Considering sex of sheep examined, the result showed 5 out of 35 males (14.3) and 28 out of 115 females(24.3) were infected with *Babesia* (Table 4).There was no significant association between sex and *Babesia* infection (X^2 = 1.583 ; p-value = .208) .

3.3.2.2 Breed of animals:

According to the breed , the result showed 16 out of 93 Hamary (17.2%) , 15 out of 42 Kabashy (35.7 %)and 2 out of 15 Balady (13.3%)were infected with *Babesia* (Table 4).The statistical analysis showed significant association between breed and *Babesia* infection (X^2 =6.506; p-value =.039).

3.3.2.3 Age of animals:

According to the age , 5 animals out of 24 (20.8 %) < 1 years, 4 animals out of 42 (9.5 %) 1-2 years and 24 animals out of 84 (28.6 %) 2 years were positive to *Babesia* (Table 4) . In the chi-square test, the result showed no significant association between age and *Babesia* infection (X^2 = 5.943; p-value =.051).

3.3.2.4 Body condition of animals:

According to the body condition, the infection rate was higher (Table 4) in animals that had poor body condition (28.6 %) compared with those had good and moderate body condition (23.0 % and 19.6 % respectively). There was no significant association between body condition and *Babesia* infection (X^2 =.407; p – value = .816).

3.3.2.5 Presence of ticks:

According to the presence of tick in the body of sheep (Table 4), the result showed sheep had a tick on their bodies were high infected with *Babesia* (26.9%) than sheep had no ticks on their bodies (19.4%). The chi-squared

test showed no significant association between tick infested in animals and *Babesia i*nfection (x^2= 1.124; p value =.289).

3.3.2.6 Usage of acaricides:

In this study, the used of acaricides (Table 4) was highly statistical significant association with *Babesia i*nfection (X^2=.020; p-value =.006).

3.3.3 Risk factors analysis with *Anaplasma* spp

3.3.3.1 Sex of animals :

Considering sex of sheep examined 8 out of 35 males (22.9%) were infected with *Anaplasma* (Table 5) compared with 25 out of 115 females (21.7%) . There was no association between sex and *Anaplasma* infection (X^2 = .020; p-value =.889).

3.3.3.2 Breed of animals :

According to the breed , the result showed 21 out of 93 Hamary (22.6%) , 9 out of 42 Kabashy (21.4%) and 3 out 15 Balady (20.0%)were infected with *Anaplasma (*Table 5).There was no statistical association between breed and infected with *Anaplasma* (X^2=.061; p-value = .970) .

3.3.3.3 Age of animals :

Regarding age groups, there was no significant association (Table 5) between age and infected with *Anaplasma* (X^2= 4.208; p-value =.122**).**

3.3.3.4 Body condition of the animals:

According to the body condition. The infection rate (Table 5) was higher in poor body condition (42.9%) compared with those had moderate and good body condition (23.0% and 17.9% respectively) There was no significant association between body condition and infected with *Anaplasma* (X^2 =2.384; p value =.304).

3.3.3.5 Presence of ticks:

In the chi-squared test (Table 5), the result showed that there was no association between *Anaplasma* infection and presence of tick in the body of sheep (X^2 = .054 ; p-value = .817).

3.3.3.6 Usage of acaricides:

According of using of acaricides (Table 5), the result showed that there was no association between *Anaplasma* infection and used of acaricide (X^2 = 1.346; p value = .246).

Table 1: The Overall prevalence of tick-borne blood parasites in sheep in Omdurman area – Khartoum state

Blood parasites	No. tested	No. of positive	Prevalence%
Theileria	150	17	11.3
Babesia	150	33	22
Anablasma	150	33	22

Table 2: The prevalence of tick-borne blood parasites in sheep in Omdurman area - Khartoum state

Locality	No. of animal examined	+ve *Theileria*(%)	+ve *Babesia*(%)	+ve *Anaplasama(*%)
Omdurman	56	6 (10.7)	15 (26.8)	14 (25)
Karary	14	0 (0)	1 (7.1)	3 (21.4)
Ombada	80	11(13.8)	17 (21.2)	16(20)
		X^2 =2.276 P-value=0.320	X^2=2.575 P=0.276	X^2=.483 P=.786

Table 3: Univariate analysis of risk factors associated with Theileriosis infection (n = 150) using Chi squared test in Omdurman area-Khartoum state

Risk factors	No.tested	No.positive	Df	X^2	P-value
Locality					
Omdurman	56	6(10.7%)	2		
Karary	14	0(0%)		2.276	0.320
Ombada	80	11(13.8%)			
Sex					
Male	35	1(2.9%)	1	3.264	0.071
Female	115	16(13.9%)			
Breed					
Hamary	93	7(7.5%)	2	3.888	0.143
Kabashy	42	8(19%)			
Balady	15	2(13.3%)			
Age group					
<1years	24	1(4.2%)	2	2.092	0.351
1- 2years	42	4(9.5%)			
>2years	84	12(			
Body condition					
Good					
Moderate	87	11(12.6%)	2	0.532	0.767
Poor	56	5(8.9%)			
	7	1(14.3%)			
Presence of tick					
Yes					
No	52	10(19.2%)	1	4.94	0.026
	98	7(7.1%)			
Use of acaricide					
Use					
Not use	106	9(8.5%)	1	2.906	0.088
	44	8(18.2%)			

Table 4: Univariate analysis of risk factors associated with *Babesia* infection (n=150) in sheep using chi-square test in Omdurman area – Khartoum state

Risk factor	No. tested	No. positive	Df	X^2	P-Value
Locality:					
Omdurman	56	15 (26.8%)	2	2.575	.276
Karary	14	1 (7.1%)			
Ombada	80	17 (21.2%)			
Sex:					
Male	35	5 (14.3)	1	1.583	.208
Female	115	28 (24.3)			
Breed:					
Hamary	93	16 (17.2%)	2	6.5.6	.039
Kabashy	42	15 (35.7%)			
Balady	15	2(13.3)			
Age :					
<1 years	24	5(20.8%)	2	5.943	.051
1-2years	42	4(9.5%)			
>2years	84	24(28.6%)			
Body condition:				.407	.816
Good	87	20 (23.0%)	2		
Moderate	56	1 (19.6%)			
Poor	7	2 (28.6%)			
Presence of ticks					
Yes	52	14 (26.9%)	1	1.124	.289
No	98	19(19.4%)			
Use of acaricides					
Use	106	17(16%)	1	7.486	.006
Not use	44	16(36.4)			

Table 5: Univariate analysis of risk factors associated with anaplasmosis infection in sheep (n=150) using chi-square test in Omdurman area – Khartoum state

Risk factor	No. tested	No. positive	Df	X^2	P-Value
Locality:					
Omdurman	56	14(25.0%)	2	0.483	0.786
Karary	14	3(21.4%)			
Ombada	80	16(20%)			
Sex:					
Male	35	8(22.9%)	1	0.020	0.889
Female	115	25(21.7%)			
Breed:					
Hamary	93	21(22.6%)	2	0.061	.970
Kabashy	42	9(12.4%)			
Balady	15	3(20.0%)			
Age :					
<1 years	24	9(37.5%)	2	4.208	0.122
1-2years	42	7(16.7%)			
>2years	84	17(20.2%)			
Body condition:					
Good					
Moderate	87	20(23.0%)	2	2.384	0.304
Poor	56	10(17.9%)			
	7	3(42.9%)			
Presence of ticks					
Yes					
No	52	12(23.1%)	1	0.054	0.817
	98	21(21.4%)			
Use of acaricides					
Use					
Not use	106	26(24.5%)	1	1.346	0.246
	44	7(15.9%)			

Table 6: Summary of Multi variate analysis of risk factors associated with *Theileria* ,*Babesia* and *Anaplasma* infection in sheep (n=150) using Logistic Regression test in Omdurman area – Khartoum state

Risck factor	**Exp(p)**			**p-value**		
	Th	Ba	An	Th	Ba	An
Sex	.210	.485	-	.141	.285	-
Breed	.717	.848	-	.366	.586	-
Age	-	1.004	1.008	-	.813	.572
Presence of tick	2.325	-	-	.177	-	-
Use of acaricide	.647	.329	1.690	.485	.008	.266

*th=*theileria*

*ba=*Babesia*

*an=*Anaplasma*

Multivariate analysis showed no significant association between tick borne blood parasites and risk factors except significant association between use of acaricides and *Babesia* infection (p-value = .008) .

CHAPTER FOUR
DISCUSSION

The target of this study was investigation of the prevalence of tick borne blood parasites in sheep in Omdurman area and studying the association between prevalence of tick borne blood parasites and risk factors.

The results of the present study showed that the prevalence of theileriosis was 11.3 %, babesiosis was 22% and anaplasmosis was 22%. The relatively high incidence of haemoparasites observed in this study could be due to the favourable environmental conditions for the survival and proliferation of ticks responsible for the transmission of the parasites since the sheep are reared under extensive and semi-intensive management systems .according to , Ibrahim (2016) and Osman *et al* (2017) the prevalence of *Theileria i*n sheep in Nyala town by microscpy was 4.9% ,also a study by Fadly (2012) showed 20% prevalence in Egypt .However the prevalence of babesiosis studied by Abbaker(2018) in sheep in Gibash locality was 12.2% ,Fadly(2012) was 17% and Adejinmi *et al* .,(2004) in Nigeria was 1.9% .Also the prevalence of anaplasmosis in sheep was 11.3% by Elnoor (2017) and Adejinmi *et al*.,(2004) was 11.2% .These variation between this studies and the present study is possibly due to variation in areas , grazing system, immunological status of animals , different ages , different breeds and sexes .

This study revealed that there was no association between the blood parasites and the difference of localities in Omdurman area .This may be attributed to the similar climatic conditions and grazing system .

Regarding the sex variable the study showed no relationship between sex and the blood parasites . which agreed with Aliimam (1995); Mohamed (2006) ; Ibrahim (2016) ; Elnoor (2017)and Abbaker (2018).

In the present study, breed was found to be the risk factor in *Babesia* infection (p- value =.039) and this may be attributed to genetic structure.

Also this study revealed no association between different age groups and prevalence of theileriosis, babesiosis and anaplasmosis, this agreed with other studies, Mohemed (2006) ; Elnoor (2017) and Abbaker (2018) .The prevalence rate of theileriosis and babesiosis in this study increase when the age of the animals increased (Fadly, 2012) .

The prevalence of tick borne blood parasites was higher in poor body condition than good and moderate .That is due to animals with poor body condition have little tolerance and lake immunity , therefore more susceptible to infection (Abbaker, 2018).

The presence of tick were examined in this study. The results showed that prevalence of tick borne blood parasites was higher in animals infested with ticks than those have not ticks. This explains the importance of tick in transmission of infection.

However, in the present study the infection was lower in animals when using acaricides than in animals when not using acricides except in *Anaplasma* .That was attributed to wrong usage of acaricides by owners.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Omdurman area is considered an important center of sheep in Sudan, the findings of this study, tick borne blood parasites was prevalent in Omdurman area and there was no relation between blood parasites and the risk factors (locality, breed, sex, age , body condition , tick infestation and use of acaricides) except the positive association between babesiosis and breed and usage of acarecide. Also there was an significant association between theileriosis and tick infestation.

Recommendations:

1- Conduction of more surveyies on tick borne blood parasites in Omdurman area by using high sensitive tests such as serological and molecular techniques.

2- Application of additional studies using large sample size for a long period to explain the seasonal effect to the diseases.

3- Controling the animal's movement to prevent spreading of the diseases by carrier animals.

4- Using of tick control programs , training owners of write usage of acaricides .

5- Treatment and vaccination of sheep.

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APPENDIXS

Appendix 1

Questionnaire:

Date : Owner name :

Telephone :

Number of blood samples : ( ) .

Locality : …….

Breed : …….

Sex : …….

Age : (1) < 1 year.

(2) 1-2 year.

(3) > 2 year .

Body condition : (1) Good.

(2) Moderate .

(3) Poor.

Presence of tick : (1) Yes .

(2) No .

Use of acaricides : (1) Use.

(2) Not use .

Appendix 2

1-Multivariate analysis of *Theileria spp*

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	sex	-1.562	1.062	2.163	1	.141	.210
	breed	-.333	.368	.816	1	.366	.717
	tickinfestation	.844	.625	1.825	1	.177	2.325
	acarecide	-.436	.624	.487	1	.485	.647
	Constant	4.775	2.649	3.250	1	.071	118.545

a. Variable(s) entered on step 1: sex, breed, tickinfestation, acarecide.

2. Multivariate analysis of *Babesia* spp

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	sex	-.723	.676	1.143	1	.285	.485
	breed	-.165	.304	.296	1	.586	.848
	acarecide	-1.111	.418	7.063	1	.008	.329
	age	.004	.017	.056	1	.813	1.004
	Constant	4.201	1.242	11.436	1	.001	66.771

a. Variable(s) entered on step 1: sex, breed, acarecide, age.

3. Multivariate analysis of *Anaplasma spp*

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	acarecide	.524	.471	1.239	1	.266	1.690
	age	.008	.014	.319	1	.572	1.008
	Constant	.387	.702	.304	1	.581	1.473

a. Variable(s) entered on step 1: acarecide, age.