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**A Study of Bovine Babesiosis in El-fasher - North
North Darfur State - Sudan**

دراسة داء البابيزيا في الأبقار في محلية الفاشر – ولاية شمال دارفور - السودان

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Dedication

To :

Unlimited Support Parents.

My brothers and my sisters.

My sincere husband and lovely nephews.

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Abstract

This study was conducted from September 2017 to March 2018 in Elfasher locality –North Darfur State, to identify *Babesia* species and tick infected cattle and to measure some hematological and biochemical parameters associated with bovine babesiosis.

A total of 227 whole blood were collected from Elfasher locality [Elfasher veterinary hospital, Elfasher slaughter house, and cattle farms in Elfasher]. For identification of *Babesia* using thin smear Giemsa stain. Packed cell volume was measured in 100 blood samples using hematocrit technique. Biochemical parameters were determined in 100 serum samples were total protein, albumin, bilirubin, calcium, sodium and potassium. One hundred and fifty five ticks were collected from 23 animals for identification using Estrada standerd. The result revealed that 133 smear samples were positive to *Babesia* spp. [58.5%]. Packed cell volume was statistically significant decreased when compared with non-infected [$p < 0.01$] animals.

All biochemical parameters showed no statistical significant decrease when compared with non infected animals [$p < 0.01$].There were 2 genera of tick were identified and these were *Hyalomma* and *Rhipicephalus*. These genera included 7 species and these were *Rhipicephalus eversi* [37%], *Hyalomma anatolicum* [21.9%], *H. impeltatum* [16.2%], *H. dromedarii*[12.9%], *H. rufipes* [1.2%] and *H. truncatum* [9.0%],*H. predixtatus* [1.2%]. In conclusion, bovine babesiosis the most important tick –borne disease in Elfasher locality leading to death of the animal due to anemia.

مستخلص

أجريت هذه الدراسة من سبتمبر 2017م إلى مارس 2018م في محلية الفاشر - ولاية شمال دارفور لمعرفة أنواع الباييزيا والقراد والذي يصيب الأبقار، وقياس بعض معالم وبيان الدم والكيميائي الحيوي المرتبط بمرض الباييزيا. تم جمع عدد 227 عينة دم كلي من [المستشفى البيطري، السلخانات ومزارع الأبقار بمحلية الفاشر] لمعرفة طفيليات الباييزيا، وذلك بعمل الشريحة الخفيفة بصبغة جيمسا. كما تم قياس حجم الخلايا المكس بتقنية مكداس الدم لعدد 100 عينة دم. كذلك تحديد المعلم الكيميائي الحيوي للبروتين الكلي، الزلال (البومين)، حمرة المرة، الكالسيوم، الصوديوم والبوتاسيوم في 100 عينة مصل، تم جمع 155 عينة قراد من 23 حيوان لمعرفة أنواعها بواسطة عياري أسترادا. لقد أظهرت النتائج أن 133 عينة شريحة موجبة لطفيل الباييزيا البقرية (58.5%)، ووجد بالإحصاء المعنوي لحجم الخلايا المكس إنخفاض مقارنة بالحيوانات السليمة ($P < 0.01$). كل معالم الكيميائي الحيوي أظهرت عدم وجود إختلاف معنوي، لكن الكالسيوم المصلي أظهر فرق معنوي مقارنة بالحيوانات السليمة ($P < 0.01$). لقد وجد عدد 2 جنس من القراد هما رايبيسفالس وهالوما. هذان الجنسان يحتويان على 7 أنواع وهي، رايبيسفالس ايفنزاي (37.5%)، هالوما اناتوليكم (21.9%)، هالوما أمبلتاتم (16.2%)، هالوما دورميداري (12.9%)، هالوما مارقيناتم (1.2%)، هايوما ترانكاتم (9.0%) وهالوما بريدكستاتس (1.2%). وفي الختام، إن مرض الباييزيا البقرية من أهم وأكثر الأمراض التي يحملها القراد في محلية الفاشر، والذي يؤدي إلى نفوق الحيوانات نسبة لفقر الدم.

Introduction

Babesiosis is widespread throughout the tropics causing heavy losses in non-resistant livestock. Babesiosis is caused by protozoan parasites transmitted by ticks of genus *Boophilus*. Without treatment mortality rates are very high. Infection with *Babesia spp* is characterized by hemoglobinuria which colours the urine dark brown and which gives the disease the common name of (red water). Infection associated with *Babesia bovis* are acute or subacute, rapidly leading to death. Acute disease can cause nervous symptoms agalactia (reduction or losses of milk) are early signs of infection. In zebu cattle young animals less than nine months old, are more resistant to the disease (Soulsby, 1982)

The disease caused by intraerythrocytic protozoal parasite of the order Piroplasmidae, phylum Apicomplexa of the genus *Babesia* (Alonso *et al.*, 1992). This disease is transmitted by tick and distributed worldwide affecting many species of mammals with a major impact in cattle and man (Bock *et al.*, 2004; Schorn *et al.*, 2011; Zanet *et al.*, 2014). *Babesia* is causing high morbidity and mortality in livestock of tropical and subtropical regions of the world, however, the major impact occurs in the cattle industry and the species affecting bovine are *Babesia bovis*, *B. bigemina*, and *B. divergens* (Bock *et al.*, 2004). Bovine babesiosis is the most important second common blood-borne parasite of cattle after trypanosomiasis (Hamsho *et al.*, 2015).

Clinically, babesiosis is characterized by hemoglobinemia and hemoglobinuria. The disease is diagnosed by blood smears, but the parasite of carrier animals is difficult to detect. Other methods for detection that molecular methods and serology are the most important to demonstrate specific antibodies that are needed. Antiprotozoal agents and vector control methods are main methods which prevent and control bovine babesiosis with early diagnosis (Enbiyale *et al.*; 2018).

In Cameroon tick-borne diseases (TBDs) continue to be a major threat to the animal industry especially in Adamaoua region. The species of ticks

identified are *Rhipicephalus [Boophilus] decoloratus*, *Hyalomma truncatum*, *Hyalomma marginatum rufipes*, *Rhipicephalus sanguinius* and *Hemaphysalis laevis*. *Babesiosis* and *Anaplasmosis* seems to be the prevailing disease in vna and naturally wired by tick. The report of [Adamaoua *et al.*, 2016] . Within the European cattle, all ages groups are highly susceptible to babesiosis [De Vos and Potigator, 1994].

Objectives

- To identify *Babesia* species and ticks in cattle in El-fasher locality.
- To determine packed cell volume, serum total protein, serum albumin, serum bilirubin, serum calcium, serum sodium and serum potassium.

CHAPTER ONE

Literature Review

1.1. Classification of *Babesia* in cattle

Phylum	- Apicomplexa
Class	- Sporozidae
Order	- Eucoccidiorida
Suborder	- Piroplasmorina
Family	- <i>Babesidae</i>
Genus	- <i>Babesia</i>
Species	- <i>bovis</i> <i>bigemina</i> <i>divergens</i>

Source: Soulsby (1982) and Bock *et al* (2004)

1.2. Morphology of *babesia* species:

The structure of these intra - erythrocytic Protozoan parasites is Pire form in shape and surround by two peripheral membranes which in host cytoplasm [Potigator and Els, 1979; Homer *et al.*, 2000].

These parasite after entering red blood cells become cyclial and develop into Trophozoite ring. The Trophozoites moult into Merozoites which have tetrad structure coined a maltase –crossform [Herwald *et al.*,2003]. Three major organells [microtubules, rhoptries and micronemes] are concentrated in the anterior polar ring – apical complex [Potigater and Els, 1979 ; Homer *et al.* , 2000]. The protozoan *Babesia bovis* [up to 2 im in length] smaller than *Babesia bigemina* [2-5 im in length]. These parasites are often found in pairs, single forms are often found within infected erythrocytes [Wanger *et al.*, 2002].

1.3 Life cycle:

The stage of the life cycle of *Babesia* species are three these are gametogony in the tick gut in which gametes fusion then sporogony in the tick salivary gands (Fig 1) an asexual reproduction occur. [Potigator and Els 1977].

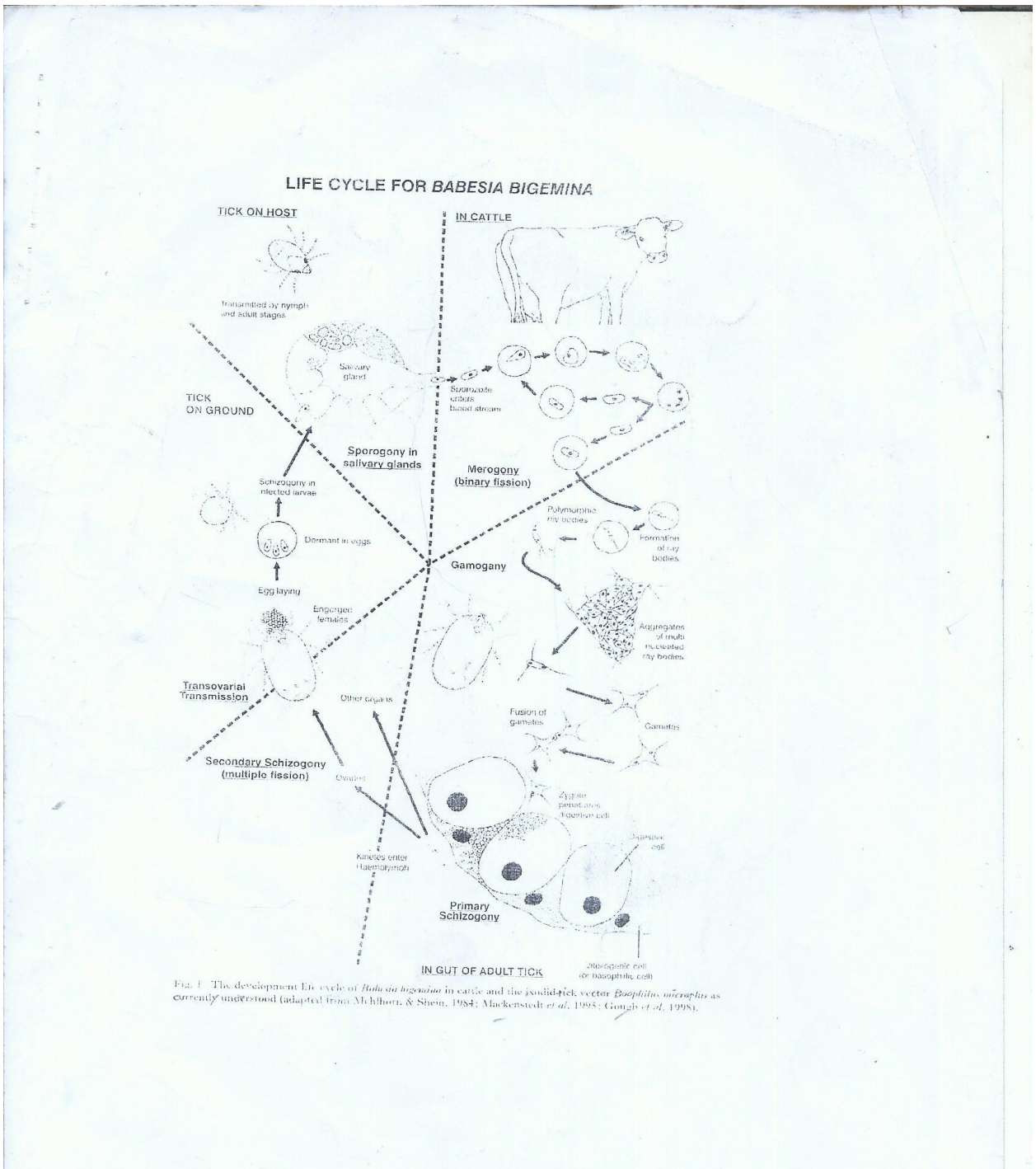


Fig. 1: The development of life cycle of *Babesia bigemina* in cattle and *Ixodes* tick vector *Boophilus microplus*

Source: [Mehlhorn and Shien, 1984; Machenstedt et al., 1995; Gough et al., 1998]

The third stage develops after cattle are infected by feeding ticks which inoculate sporozoites that invade erythrocytes and transform into trophozoites that divide by binary fission. Merozoites are released after erythrocyte membrane breaks down and invade new cells resulting in an intra – erythrocytic cycle. When ticks are feeding on infected blood gametocyte develop in the tick gut which fuse to form diploid zygotes are invade the gut cells and multiplied before emerging as haploid kinetes Figure (2). Then the kinetes migrate to many organs including ovaries and further division occurs. After eggs hatching, the kinetes migrate to the salivary gland and transform to sporogony stage [Simuunza, 2009].

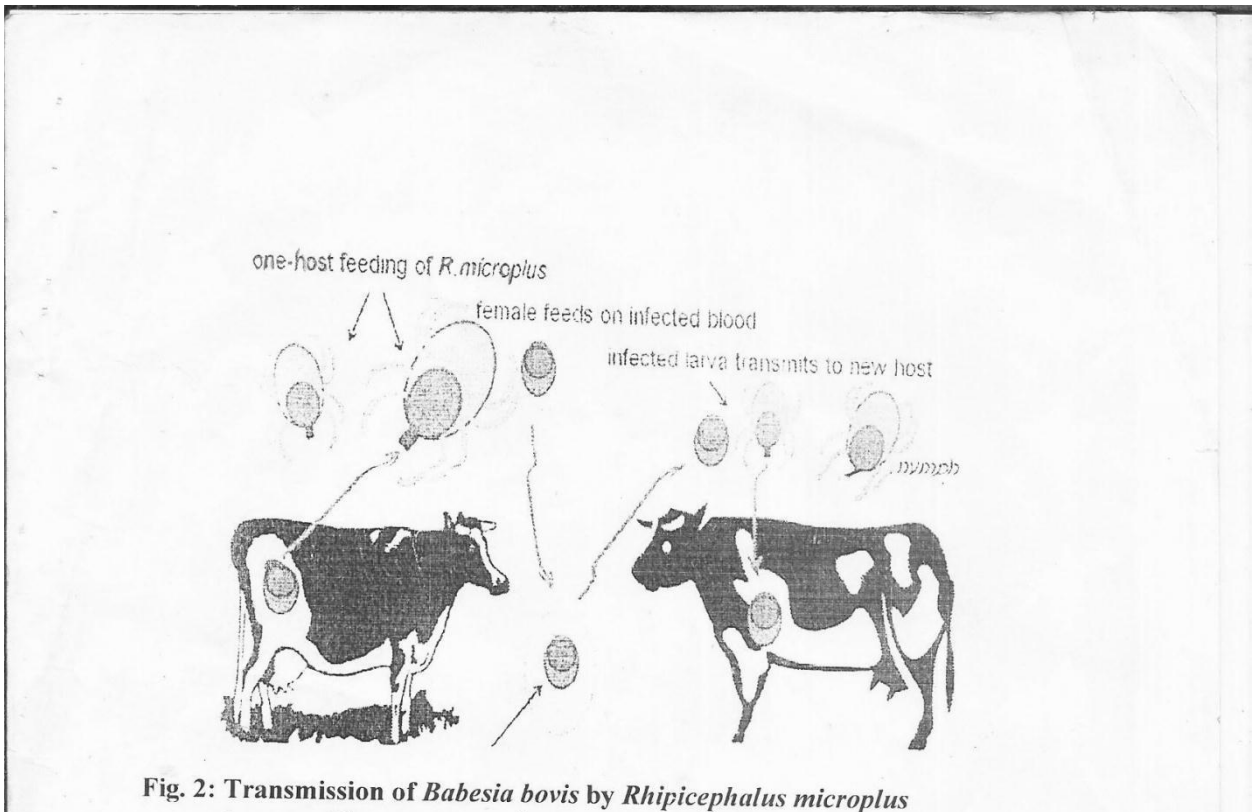


Fig. 2: Transmission of *Babesia bovis* by *Rhipicephalus microplus*

Source: [Joan Kleyhan, 2015]

1.4 Bovine babesiosis:

1.4.1. Etiology

According to Radostits *et al.*,(2007)there are 4 species infected cattle and these are *Babesia bovis* includes *B. argentina*, *B. berbera*, *B.colchica* , *B. bigemina*, *B. divergene* *B. cauxsica*, *B. occidentalis*, *B. karelica* and *B. major*.

1.4.2. Epidemiology

The distribution of *Bovine babesiosis* associated with *B. bovis* and *B.bigemina* is an important disease in tropical and subtropical regions in the world including America. Thus, *B. bigemina* occurs in south America, Australia, and Africa, but *B. argentina* in the tropics including south and central America, Australia, Asia and Southern Europe. While *B. divergens* found in north west Europe. The species *B.berbera* occurs in Mediterranean an Europe and Africa, *B. major* found in United Kingdom and Europe [Rodestitis *et al.*, 2007].Ticks are vector of a wide rang of disease -causing agent such as virus, parasites and bacteria [Mansfield *et al.*, 2017].

The transmission of the disease by ticks and the principal of vectors of *B. bovis* and *B. bigemina* are *Rhipicephlaus* formly *Boophilus* species [Enbiyale *et al.*, 2018]. Also *B.divergens* transmitted by *Ixodes ricinus* [Radostits *et al.*, 2007]. Transculi *et al.*,(2013) recorded that *R. sanguineus* *R.decoloratus*, *R.geigi*, *R. annulatus*, *R. eversi*,*R. bursa*, *Ixodes ricinus* and *I. presulcatus* are transmitted *Babesia* spp generally. The epidemiology of babesiosis associated with several parameters such as distribution of tick, availability of the host, presence of the parasites within the vectors and the environmental conditions when the absence of any one of the parameters will discontinue the spread of infection [Perry *et al.*, 1998].

1.4.3. Pathogenesis

When the animal become infected multiplication of the parasite in peripheral vessels [*B.bigemina*] or in visceral vessels [*B. bovis*]. The intravascular mechanism is hemolysis leading to hemoglobinemia and

hemoglobinuria, anemia and icterus [Radostits *et al.*, 2007]. A fatal outcome due to anemia and anorexia, also hypoxia and secondary inflammatory lesions in various organs specially liver and kidneys. In survival animals there are ischemia in skeletal and heart muscle. Pharmacologically, active substances resulting in vascular malfunctions and hypotensive shock. Also these active substances kinins and catecholamines lead to increase vascular permeability and dilatation of the blood vessels resulting to edema and hypovolemic shock. Hypoxia leading to centrilobular liver and kidney tubule epithelium degeneration with immune pathologic reactions. Damage of kidney tubule epithelium impairs ion exchange resulting in hydrogen ion retention leading to acidosis [Bock *et al.*, 2004]. The infection by *B.bovis* can be due to over production of pro –inflammatory cytokines and the direct effect of red blood cells damage by the parasite. Macrophages activated by the protozoan parasite and pro- inflammatory cytokines. Parasitocidal molecule are produced [Wadhwa *et al.* , 2008].

1.4.4.Diagnosis

1.4.4.2.Clinical findings

Generally, the infection by *B.bovis* is more pathogenic than *B.bigemina* [Brown *et al.*, 2006].The incubation period usually appears 2-3 weeks after tick infestation. A direct inoculation of the blood, the period can be a short as 4 to 5 days for *B. bigemina* and 10 to 12 days for *B. bovis* [ANON,2009]. This disease associated with age, breed and immune status. The common feature of acute infections are fever, lathery, diarrhea, hemolytic anemia, tachycardia, hemoglobinuria and icterus. While chronic infection characterized by absence of hemoglobinuria. Fatal cerebral babesiosis with hyperaesthesia, convulsions and paralysis due to aggregation of red blood cells in the cerebral capillaries and extravascular with endothelial damage in acute *B. bovis*. Recovered animals become carriers[Figueroa *et al* 2010].

Variation in ruminant status, jaundice, change from paleness in mild cases to severe yellow coloration, breathing is labored and rapid and the heart beat is fast and loud [Moghazy *et al.* , 2014].

Hyper excitability, impaired vision with red - tinged color of the urine [red water] are the sign of the disease [Nyindo,1992]. Other manifestations stated by Hall, [1977] included salivation, lacrimation diarrhea or constipation, delirium and in coordination of gate.

1.4.4.2.Hematology

Erythrocyte count and hemoglobin levels are due to anemia occurs in the peak of anemia resulting invasions for parasites to red blood cells and the peak of anemia occurs at 9- 26 day s after infection. Also a platelets count and fibrinogen content are depressed [Radostits *et al.*, 2007].

Examination of blood and organ smears stained with Giemsa are used for diagnosis of *Babesia spp* [Callow *et al* 1993; Bose *et al.*,1995]. For the best result, blood collected after prinking tip of tail or margin of an ear. Blood from general circulation may contain up to 20 times fewer *B.bovis* than capillary blood [Callow *et al.* , 1993]. In *B.bigemina* infection parasitized cells are evenly distributed throughout the blood circulation. Thick blood films are 10 times more sensitive and useful for the detection of low level *B.bovis* [Bose *et al.* , 1995].

1.4.4.3. Biochemical findings

Serum changes due to babesiosis resulting in significant increase of aspartate trnsaminase [AST], GT [glytamyle transaminase] , Hypoproteinemia hypoalbuminemia and decreased A/G ratio. These result indicated that the parasites causing harmful effect of toxic metabolites on the liver cells In babesiosis there is significant increase of serum globulins may due to the immune response against *Babesia spp*[Stockham *et al.*,2000; Singh *et al.* , 2001]

1.4.4.4. Serology

Serological diagnostic procedures or molecular detection methods to demonstrate specific antibodies of *babesia* species [Pohl, 2013], but these test are not of values the clinical stage of the disease, but are used for the purposes of research, epidemiological studies, export vaccine certification

or where vaccine break down are suspected [Bose *et al.*, 1995;De Vos *et al.* ,Molloy,2000]. These immunological /serological methods such as indirect fluorescent antibody test which based for detection of parasite antigens by serum antibodies using Fluorochrome –labeled antibody anti-Ig [secondary antibody]. Enzyme linked immunosorbent Assays, complement fixation test [Salih *et al.* ,2015]and molecular methods such as DNA probes, polymerase chain reaction reverse line blood hybridization and real time PCR [Mosqueda *et al.* , 2012]. Bose *et al.* (1995) reviewed the relative sensitivity of DNA probes and sub inoculation into susceptible, usually splenectomised calves to provide a diagnosis.

1.4.4.5. Necropsy

Smears can be also taken from heart muscles, kidney, liver, lung, brain and blood vessels of extremities [lower leg] at necropsy [Merck and Merck, 2016].

The main pathological changes of *B. bovis* infection in animals is develop hypotensive shock syndrome. Dead animals were anemic with jaundice, hemoglobinuria, excess thick granular bile echymotic hemorrhages of epicardium and congestion of the brain and visceral organs.

The characteristic lesion of acute *B. bovis* is a cherry pink discoloration of the cerebral cortex is of infections. All changes are the same when the animals are infected by *B. bigemina*. But cardiac hemorrhage and splenic enlargement are not as marked as in *B. bovis* infection. Also pulmonary edema is more regular feature [Sharma *et al.*, 2013].

1.4.5. Treatment:

Diamidine derivatives are either aromatic such as diminazene diacetate, pentamidine isethionate and phenamidine isethionate or carbanilide and these are anicarbalide, imidocarb dipropionate. Diamidine derivatives are binding to DNA and interfere with parasite replication [Pilck *et al.* ,1995 ; Patrick *et al.*;1997].

Currently, diminazine acetate [Berenil]and imidocarb dipropionate [imidocarb] are the most widely used. Diminazine is effective against *B. bovis* and *B. bigemina* at a dose rate 3.5 mg/kg intramuscularly [De Vos, 1979]. Imidocarb is used subcutaneously at the dose of 1.2 mg/kg for treatment, while 3mg/kg provide protection from *B. bovis* for 4 weeks and *B. Bigemina* for at least 2 months [Tylor and MC Hardy, 1979]. The high dose of imidocarb dipropionate can provide short term protection from clinical disease caused by *B. bovis* [4weeks] *B.bigemina* [8weeks] as babesiacide [De Waal and Comb rink, 2006 ; Mosqueda *et al.*, 2012].

1.4.6. Control and prevention :

For prevention and biosecurity the prevention of introduction of the disease into a non- enzootic area depends on quarantine to [prevent the introduction of the vector tick and laboratory examination ensure freedom of these animals from the infection with pathogen [Radostits *et al.*,2007]. Difficulties of using acaricides for eradication of ticks because the arthropods develop a resistance against these drugs. For limitation of prevalence requires different solution in different circumstances it is largely depend on tick control by the frequent application of acaricides. Chemotherapy to kill the parasites in cattle host and to a lesser degree by immunization of host cattle. The mechanism of immunity to babesiosis is the lack of knowledge on how to immunity to this disease work. Cattle develop a durable long-lasting immunity after a single infection with *B. divergens*, *B. bovis* or *B. bigemina*. Application of these feature in some countries to immunise cattle from babesiosis [Callow, 1984;Gray *et al.* , 1989; De Vos and Jorgensen, 1992].

1.4.7. Bovine babesiosis in Sudan

Abdalla, [1984] studied babesiosis caused by *Babesia. bigemina* in cattle in Northern Sudan during an outbreak the disease and the results showed that *Boophilus annulatus*, *B. decoloratus* and *Hyalomma. a.anatolium* transmitted *Babesia bigemina* and *B.bovis*, using microscopic examination and serology. But also *Thileria annulata* and *Thieleria mutans* were identified

Awad *et al.*, (2011) recorded that in study conducted in prevalence and genetic diversity of *Babesia* species in cattle in Sudan, the prevalence *Babesia bigemina* and *Babesia bovis* was 4.0% and 1.9% respectively.

An epidemiological studies used by El Emam, (1999) on ticks and tick-borne diseases in Kosti province, Sudan. Eleven different tick species were identified in five localities these include *A. lepidum*, *A. vargatum*, *B.annulatus*, *B.decoloratus*, *H.a.anatolicum*, *H. a. excavatum*, *H. dromidarii*, *H.impeltatum*, *H. m. rufipes*, *H. truncatum* and *R. e. eversi*. The highest tick population per animal per locatin per year was at Ummhani (1096.2) followed by Tendelti (963.6, Karmal(928.1), Jadeed(614.5), and Kost(304.3). *H. impeltatum* (32.8%) followed by *A. lepidum*(23.5%) *B. annulatus* (14.6%) *B. decoloratus* (10.9%) and *H.m.rufipes* (10.3%) ,also *Babesia bigemina* was detected in blood smears.

In epidemiological study carried out at Equatoria region, Sudan. *Thieleria parva*, *Thieleria annulata*, *T.mutans*, *Babesia bigemina*, *Babesia bovis* and *Anaplasma marginale* were detected by using indirect flourescent antibody test. Tick identification showed five genera and twelve species. Ibrahim, (1994).

Across sectional study was conducted between September and October 2010 in five states of south Sudan on the basis of the percieved risk of tick-borne disease. The indirect Enzyme –Linked Immunosorbent Assay (ELISA) was used *Thieleria parva*, *T. mutans*. *Anaplasma marginale* and *Babesia bigemina* were detected . The prevalence rate of *T. parva* and *T. mutans* 27.3% and *B. bigemina* 31.3%, respectively. The risk was 57.6%

and 52.8% for *A.marginale* and *B. bigemina*. Tick identification were included *Rhipicephalus appendiculatus*, *Rhipicephalus decoloratus*, *Rhipicephalus e. eversi* *Rhipicephalus microplus*, and *Amplyomma varigatum*. There was great variation ($P < 0.001$) [Kivaria *et al.*, 2012]

Mohammed *et al.*, (1990) recorded that in outbreaks of Babesiosis in domestic livestock in Estran Region of the Sudan. In autumn(July, August and September) in 1988 extremely high rainfall and environmental condition became suitable for tick survival, the disease locally known as “bloody urine” killed substantial number of animal in Khashm El Girba (South-west of Kassala), the causative agent is *Babesia bigemina* and ticks identified were *Boophilus annulatus*, *Rhipicephalus e.eveti*, *R. sanguineus*. *Hyalomma excavatum*, *H. rufipes*, *H. marginatum* and *H. dromidarii*. Losses due to babesiosis in Nigerian cattle were estimated as 360 million Nigerian Naira but Losses among Sudanese animals were greater,.

In study of Morzaria and Penderson, (1983) in Northern sudan, *Thieleria mutans*, *T. annulata*, *T. verlifera*, *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *A.centrale*, *Cowderia ruminantium* and *Trypanosoma and thieleria* were identified. Also four species of ticks were identified these are : *Hyalomma. a.anatolicum*, *H. dromedarii*, *H.m. rufipes* and *H. impeltatum*. respectively, with the prevalence rate of 74.6% for *T. mutans*, 38.9% for *T.annuata*, 58.5% for *B.bigemina* and 16.3% for *B. bovis*, 14.4% for *T. annulata*, 25.8% for *T. parva*, 62.7% for *B. bigemina* and 29.9% for *B. bovis*.

A study conducted by Omer *et al.*, (2015), and prevalence of *Babesia bigemina* and tick infesting cattle in Kassala state, during January 2009 to December 2010. Blood smear and blood spot collected in filter paper from 334 heads, while ticks were collected from 105 heads. The prevalence of *Babesia bigemina* was 0.90% in blood smears , Three genera of ticks, *Hyalomma*, *Rhipicephalus* and *Amplyomma* were identified including eight species ; *Hyalomma anatolicum*, *H. dromidarii*, *H.rufipes*, *H. impeltatum*, *Rhipicephalus evertsi evertsi*, *R. sanguineus*, *R. decoloratus* and *Amblyomma lepidum*.

Salih *et al.*, (2008) studied epidemiologically study on Tick-borne in cattle Central Equatoria State, Southern Sudan, the result showed 69(11.5%) of animals were positive to piroplasms using Giemsa stained blood smear .But by detection using polymerase chain reaction the infection rate was increased to 297(49.5%) animals .The species of piroplasms identified were eight ; *Thieleria parva* (71.2%), *Thieleria mutans* (73%), *Thieleria velifera* (45.3%), *Thieleria taurotragi* (2,7%), *Thieleria buffeli* (0.5%). *Thieleria annulata* (0.2%), *Babesia bovis* (1.7%), and *Babesia bigemina* (0.3%).

While the over all seroprevalence rates by using indirect enzyme-linked immunosorbent assay (ELISA) to *T. parva*, *T. mutans* *B. bigemina* and *A. marginale* were found to be 58.2%, 88.9%, 52.1%, and 37.8% respectively. Salih et al, (2008)

CHAPTER TWO

Materials and method

2.1. Study area :

The study area is located in Northern Darfur State (El –fasher town). Annual rainfall in NDS is generally low and very variable ranging from 20 to more than 50mm. Unlike in south of the state soil type and soil infertility are of two types sandy and clay soils. the first type covers 90% of the area. Various type of livestock including cattle, sheep, goats, camels in addition to equines and poultry are raised under traditional husbandry system characterized by poor husbandry practices with low productivity.

Animal population North Darfur State is estimated as 41221 cattle,7229959 sheep,5109406 goats,1425473camels and considerable numbers of donkeys and horses[Reported from Ministry of Animal Resources North Darfur State, 2016].

The majority of these livestock are indigenous(local) breeds owned by agro-pastoralists and sedentary cultivators. In El-fasher, natural grazing takes place except in limited areas where crop residues and cut forages and seasonal fluctuation. Livestock is a major source of livelihood in El-fasher as in many parts of Darfur. They are owned by a large segment of community and attempts to increase their productivity are an important means to improving the standard living of the people.

2.2.collection of blood and tick samples :

A total of 227 blood samples were collected from cattle in El-fasher locality [Vet hospital, 62 farms in El-fasher region. Al mwashi market, Slaughter houses as well as Comps], from August 2017 to July 2018. These animals their age ranged between 2-4years, the number of males 58 and the females were 169 animals, while the number of local breeds were 170 and cross breeds were 57animals.

Five milliliters of blood sample was taken by vein puncture from jugular vein for hematological and chemical analysis. Whole blood (2ml) was used for smears and packed cell volume [PCV].

Blood sample for serum [100 sample] were separated and stored at 20C until used. One hundred and fifty two of tick samples were collected from 23 animals using blunt forceps and preserved in 70% ethanol.



Fig. 3: Technique of taking blood samples from jugular vein in El-fasher locality (North Darfur State)

Source: The researcher

2.3.Haematology :

2.3.1. Blood smear :

A double blood smears were made from 227 blood samples. The blood smears were made in newly labeled glass slides according to McCosker(1975) by drop of blood put on microscope slide and using another slid to spread the blood at an acute angle that makes a thin film. They were air dried and fixed in absolute methanol for 2-3 minutes.

In the laboratory, the smears stained using 10% solution of Giemsa stain for 30 minutes. The slides were washed with distilled water, air dried and scanned under X100magnification using oil immersion lens for presence of piroplasms.

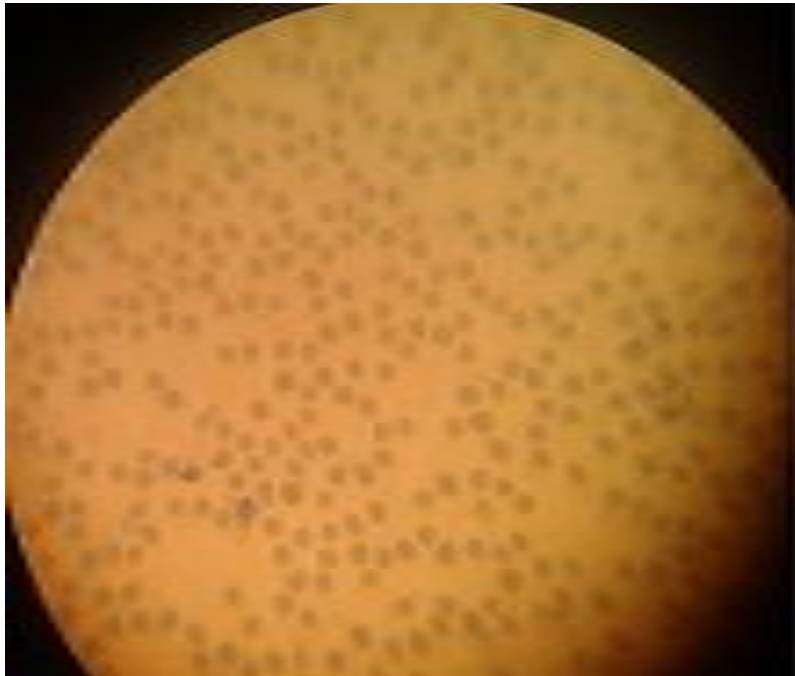


Fig. 4: *Babesia* parasite into erythrocytes of cattle

Source: The researcher

2.3.2.Packed cell volume (PCV) :

Capillary tubes with anti-coagulant and the microhematocrit centrifuge were used for determination of PCV [Sehalm and Jain, 1986]

2.4. Chemical methods :

A total of 100 serum samples were selected randomly from 227 serum samples to measure some biochemical parameters and these were serum total protein, serum albumin, serum bilirubin , serum calcium, serum sodium and serum potassium.

All biochemical parameters were analyzed by test kits [Biosystem, S. A. Spain] using spectrophotometer.

2.4.1. Serum total protein:

The protein in the serum was determined by the blue coloration resulting from the reaction with copper salts in alkaline medium and the reaction read at wave lengths 630.

2.4.2. Serum albumin:

The activity of albumin was determined by bromocresol green at PH 4.2. The reaction was read at 630nm.

2.4.3. Serum bilirubin:

Direct bilirubin in the sample react with sulfailnic acid forming colored complex that measured by spectrophotometer at length 540 nm.

2.4.4. Serum calcium:

The reaction between calcium and O-Cresolphthalin in alkaline medium produced color which was directly related to the concentration of calcium in the sample, the reading of the reaction at the weave length 560nm.

2.4.5.Serum sodium :

Sodium was reacted with chromogen to produce chromophore and the colour that referred to sodium concentration in the samples. The result was read at the length 560nm.

2.4.6. Serum potassium:

The concentration of potassium in the serum was measured by using tetraphenylboron which produced turbid colloid suspension. The turbidity was read by 560nm.

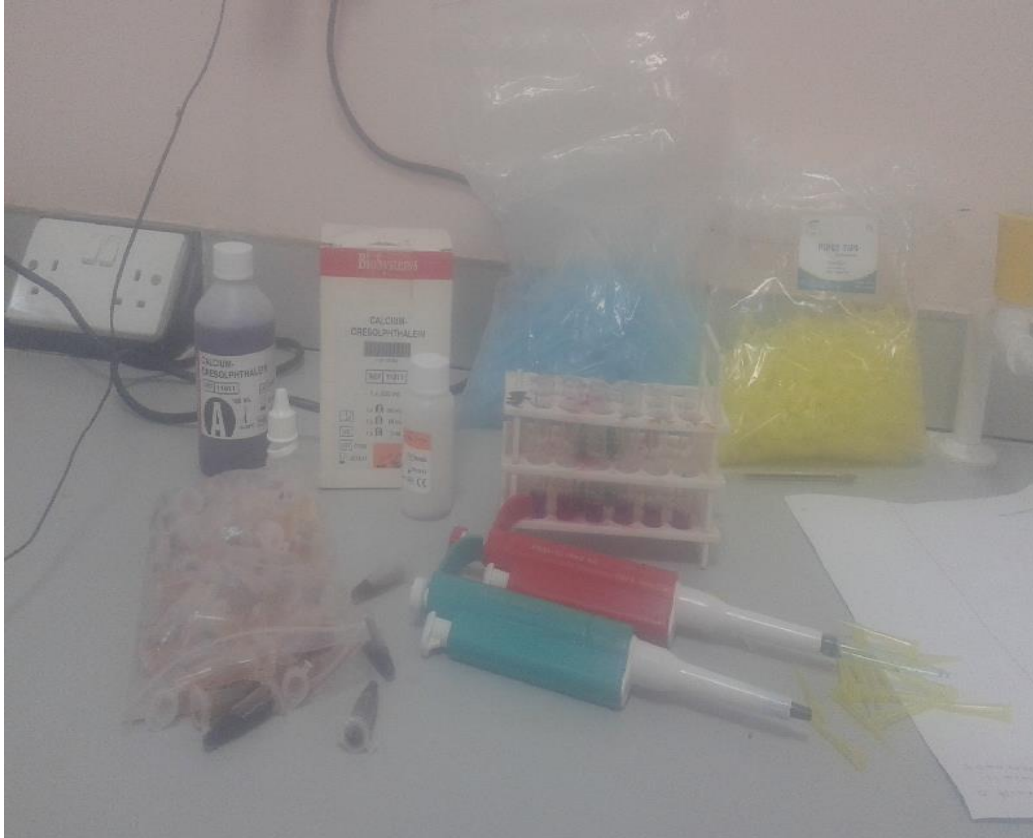


Fig. 5: Set of Biochemical Tests in Elrazi University Lab

Source: The researcher

2.5. Detection of tick species:

For identification of morphologic characteristic standard taxonomic keys were used [Estrada- Pena *et al.*, 2004]



Hyalomma anatolicum

Rhipicephalus eversi

Fig. 6: Tick identification in El-fasher (North Darfur State)

Source: The researcher

CHAPTER THREE

Result

The overall Prevalence of Bovine babesiosis

Examination of 227 smear samples and there were 133 samples positive to Babesia spp and 94 were negative . The overall prevalence of the disease was 58,5% [table 3.1]

Table3.1: The overall Prevalence of Babesiosis in cattle [n=227] in El-fasher locality - North Darfur State

	Number of animas	Frequencies
Positive	133	58.50%
Negative	94	41.50%
Total	227	100.00%

3.2. Biochemical analysis:

As shown in table 3.2 there was significant decreased in packed cell volume in the infected animals, compared with non- infected . While all biochemical parameters showed no statistical significant difference to disease . But serum calcium levels showed significant difference [p<0.01].

There were 2 genera of ticks were these were identified *Rhipicephalus Hyalomma*, included 7 species and these were *Rhipicephalus eversi eversi* [37%],*Hyalomma anatolicum* [21.9%],*H.impeltatum*[16.2%] , *H. dromedarii* [12.9%],*H .rufipes*[1.2%] and *H. truncatum* [9.0%],*H. predixtatus* [1.2%]

Table 3.2: The mean \pm Standard error for Some biochemical parameters associated with Babesiosis in cattle [n=227] in El-fasher locality – North Darfur State.

	Infected	Non-infected	Significant
PCV	24.02 \pm 10.26	34.08 \pm 10.57	**
Total protein	6.02 \pm 0.71	6.10 \pm 1.28	NS
Albumin	2.55 \pm 2.34	3.06 \pm 1.52	NS
Bilirubin	1.42 \pm 0.51	1.60 \pm 0.98	NS
Calcium	11.10 \pm 5.78	14.52 \pm 5.93	**
sodium	134.82 \pm 5.60	136.66 \pm 14.17	NS
potassium	3.80 \pm 0.41	3.97 \pm 0.85	NS

**=significant at $P < 0.01$, NS= No significant differences

CHAPTRE FOUR

Discussion

Tick-borne disease have affected the health of livestock leading to economical loss of productivity [El Ashker *et al.*, 2015; Omer *et al.*, 2015]. In the present study, the overall prevalence rate was 58.5% by microscopic examination of *Babesia* species [Table 3.1]. This prevalence is highest than prevalence in Danapur [35.5%] and Fatuna [26.21%] after retrospective data collected from Pantna using Giemsa stained thin blood smear method [Kumari and Kithin, 2018]. The result of Matuvu *et al.*, [2014] using microscopical prevalence for identification of *Babesia* species is lower . Also Shuaib *et al.*,[2015] found that after examination of 803 thin blood smears the prevalence rate is 5.20% and this is lower than this result. This result is higher than that reported by Salih *et al.*, (2008) , in equatorial region. (51.50%). Also Kivaria *et al.*,(2012) recorded the prevalence rate 52.80% in south region . But the overall prevalence in this study is lower[table3.1] than recorded by Radostits *et al.*,[1994] and Kumari and Jithin , (2018) during rainy season [58.55%] with increase humidity which favor the development of ticks as vector of the disease . immunofluorescent antibody test is more sensitive test and giving of high prevalence rate [68.7%] of bovine babesiosis [Shuaib *et al.*, 2015].

Packed cell volume was significantly decreased [Tabe3.2]. This finding in agreement with findings of Pandey and Misra, [1987] and Mohamed Said , [2017] who recorded that when the parasites are entered the blood circulation and invaded red blood cell membrane leading to intravascular hemolysis . Erythrocyte count, packed cell volume and hemoglobin level can be continued to decline steady after patency even the animals are recovered, but the hematopoiesis is increased [Lewis *et al.*, 1981; Soulsby, 1982].

The study showed there was decreased in serum calcium level, this result in accordance with data recorded in cattle babesiosis [O Neil, 1983; Zintil *et al.*, 2003 Mohamed Said, 2017]. Generrally, changes of calcium level due to mineral metabolism disturbances resulting from clinical or bone disease [Coles, 1986]. In this result, there were no changes in serum level of sodium, potassium, total protein an albumin These results in contrast with Mohamed Said ,(2017) who found that the increase in potassium level can be decreased, calcium , sodium and protein due to the harmful effect of toxic

metabolites of *Babesia* spp. Also in these results serum bilirubin was not altered, because no congestion of hepatic vessels due to the action of the parasites [Allen and Frerichs, 1975. Akinboade *et al.*, 1984]. From this study there were 2 genera of ticks were identified and these were *Rhipicephalus* and *Hyalomma*, for transmission of bovine babesiosis . Friedcoff, (1988) and Uilenberg,(2016) *Rhipicephalus* species have been implicated in the transmission of *Babesia* species to cattle and human. Also Altay *et al.* , (2008) recorded that *R. bursi* was the main vector of cattle babesiosis in eastern Turkey. There are several studies carried out in Mediterranean region confirming that *R. bursi* transmitted *Babesia bovis* and *B. bigemina* (Bouattour and Darghough, 1996; Ravindran *et al.*, 2006; Ghirbi *et al.*, (2010).

In conclusion, Bovine babesiosis is the most important tick-borne disease of cattle in E-fasher locality leading to death of the animal when the disease occurred in acute stage and may lowering the productive performance, specially the breed export for improving the productivity of the local cattle.

Conclusion and Recommendations

Conclusion

Similarly, the overall prevalence of Babesiosis in El-fasher locality – North Darfur State was 58.5% is the most higher than in a study conducted by Salih *et al.*, (2008); Kivaria *et al.*, (2012) the prevalence was 52.8%. Moreover , the prevalence in a study conducted by Awad *et al.*, (2011) was more lower than the prevalence in this study [0,4% for . *B .bovis* and 1.9% for *B. bigemina*].

Packed cell volume is the most lower in infected animals in compared with non - infected .In serum biochemical analysis calcium level is the most higher and showed a significant increased in negative animals than the positive once .The tick of the species *Rhipicephalus eversi eversi* in this study was 37.5% is higher than the other tick species ,we conclude that this species of tick has the major role in Babesiosis transmission in cattle in El-fasher locality- North Darfur State.

Recommendations

- 1- Cleaning of farms and grasses burning of for the insect reducing.
- 2- Using of acaricides is recommended to reduce tick infestation and it is negative impact.
- 3- Management developing by good husbandry practices specially the specific ways of drugs administrati ons.
- 4- Routine clinical and laboratory examinations for the newer animals before merging it with the old stock.
- 5- Further studies should be conducted to cover the other regions of North Darfur State.

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