Sudan University of Science and Technology

College of Graduate Studies

Prevalence and Risk Factor of Bovine Babesiosis in South Darfur State, Sudan

نسبة الانتشار وعوامل الخطر لمرض بابيزيا الابقار فى ولاية جنوب دارفور؛ السودان

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

Prepared by:

Manasik Alhafez Edris Naser

B.V.Sc. (2014) College of Veterinary Science,

University of Nyala

Supervisor:

Dr:Sara Basher Taha Mohammed

August 2017

الآية:

بسم الله الرحمن الرحيم

اقْرَأْ بِاسْم ِرَبِّكَ الَّذِي خَلَقَ (1) خَلَق َالإِنْسَان َمِن ْعَلَقٍ (2)
اقْرَأ ْوَرَبُّك َالْأَكْرَمُ (3) الَّذِي عَلَّم َبِالْقَلَمِ (4) عَلَّمَ الْإِنْسَان َمَا
لَم ْيَعْلَمْ (5) كَلَّا إِنَّ الْإِنْسَان َلَيَطْغَى (6) أَن ْرَآهُ اسْتَغْنَى (7)

صدق الله العظيم

DEDICATION

To my parents, brothers and

sisters

To my friends who made this

work possible.

ACKNOWLEDGEMENTS

Thanks to Almighty Allah for blessing me the ability to complete this work.I would like to express my gratitude and appreciation to my Supervisor **Dr. Sara Basher Taha**for her advices, directions and continuous interest.I would like to thank**Suleman Ali Noga**, Central Laboratory (Nyala) for his kind support during all this work.My thanks extend also to**Prof. Mohammed Tajeldeen** for his help and guidance in data analysis.

I am very grateful to Ministry of Animal Resources And Fisheries of South Darfur, especially **Dr.Kaled Hamed Albeshare** (Department of Preventive Medicine) and **Dr Sharaf Yahia**. I also owe my great thank to **Dr.Ibrahim Mhamed Ahmed Sharaf Eldein** the Dean of Faculty of Veterinary Science University of Nyala.

My thanks are also extended to **Dr. Ahmed Elhag Bashar**, **Dr. Fathi Abdalla Mohammed**, **Dr. Yassein Mozamel Abdoel Mtalab** , **Dr. Abdalhakaim Aallaha hessian**, **Dr. Fadia Yagoub Ali** and **Dr. Ferdous Abdalla Koko** for their great help during my work.Special thanks to **Dr. Alia Said Amin**and **Dr.Mohammed Almurtada Abdalrhman Altegani** for financial and moral support.

Special thanks to all Doctors and Technical staff of Faculty of Veterinary Medicine, University of Nyala.My thanks also to all staff of slaughterhouse and cattle owners for their collaboration.

List of Contents

List of tables

List of figures

Abstract

Bovine babesiosis also known as red water, or tick fever is the worldwide most important arthropod-borne disease of cattle that causes significant morbidity and mortality. It is caused by intra-erythrocytic protozoan parasites of the genus Babesia, which is transmitted by ticks and affects a wide range of domestic and wild animals and occasionally humans. Two important Babesia species; *B.bigemina* and *B. bovis* infect cattle. They are widespread in tropical and subtropical areas including South Darfur State, Sudan and are transmitted by one host tick *Rhipicephalus* species.

A cross-sectional study was conducted from November to December 2016 to assess the prevalence of bovine babesiosis in Nyala town, South Darfur State, Sudan. In addition, the risk factors could be associated with the disease were also investigated.

A total of 300 blood samples were collected from the jugular vein of cattle. The overall prevalence of bovine babesiosis was 59.3% (178/300) using microscopic examination of Geimsa stained blood smear. A significant variation in the prevalence of bovine babesiosis ($P<0.05$) was observed between the areas (Slaughter house, Animal fauna and Farm).

The higher prevalence rate was recorded in male (65.3%) compared with female (58.1%). The increased in the prevalence rate was also recorded in young animals (62.5%) followed by adult (58.6%). According to the body condition, the highest prevalence rate was recorded in animals with poor body condition (64.3%). No significant ($P>0.05$) association was observed between the infection with Babesiosis and age, sex and body condition

Regarding mix-infection, 94.4% of infected animals showed infection with both Babesia spp. and other blood parasite, while 5.6% of infected animals were infected with Babesia spp. only. A significant increase in the prevalence rated was observed in the cross breed cattle (74%) compared with the local breed (52%). This significant was also reported in animal treated with a combination of drugs (74%) compared with animal treated with one drug only (52%).

The infection with Babesia was higher in the animal not infested with ticks (60.5%) compared with animal infested with ticks (55.8%). This fact could be refers to the miss using of acaricide in this area.

ملخص البحث

داء البابيزيا البقري المعروف أيضا باسم المياه الحمراء أو حمى القراد هو من أهم الأمراض المنقولة عن طريق المفصليات للماشية في العالم والتي تسبب في الاعتلال والوفيات بصورة كبيره ، هذا المرض ينجم عن طفيليات تعيش داخل كريات الدم الحمراء من جنس البابيزيا ، التي تنتقل عن طريق القراد ويؤثر على مجموعة واسعة من الحيوانات الاليف والبرية و في بعض الأحيان البشر.يوجد نوعان هامان من البابيزيا التي تصيب الماشية هما بابيزيا بوفيس وبابيزيا بايجمينا وهي منتشرة على نطاق واسع من المناطق المدارية وشبة المدارية بما في ذلك ولاية جنوب دار فور ، وتنتقل عن طريق نوع واحد من أنواع مضيفات الرايبوسيفلس . أجريت هذه الدراسة المقطعية من نوفمبر / تشرين الثاني إلى ديسمبر / كانون الأول 2016م لتقييم انتشار الإصابة بداء البابيزيا في الأبقار في مدينة نيالا بجنوب دار فور ، بالإضافة إلى ذلك تم التحقق من عوامل الخطر المرتبطة بالمرض. تم جمع 300 عينة دم من الوريد الوداجي للماشية وكان الانتشار الكلي لداء البابيزيا البقري 59.03 (178/300) باستخدام الفحص المجهري للشرائح الدموية المصبوغة بجمسا ، كما لوحظ وجود تباين معنوي (p<o.o5) بين المناطق التيتم جمع العينات (المسلخ – المواشي – المزارع) . سجل أعلى معدل انتشار للمرض لدى الذكور 65.3% مقارنة بالإناث 58.1%. كما سجلت الزيادة في معدل الانتشار في الحيوانات الصغيرة 62.5% يليها الاكبر عمرا 58.6% ، ووفقاً لحالة الجسم سجلت أعلى نسبة انتشار للمرض في الحيوانات ذات الحالة الجسمانية السيئة 64.3% . لم يلاحظ أي ارتباط معنوي (p>o.o5) بين العدوى والعمر والجنس وحالة الجسم . وفيما يتعلق بالعدوى المختلطة أظهرت 94.4% من الحيوانات المصابة ، الإصابة بعدوى البابيزيا وطفيليات الدم الأخرى في حين أن 5.6% من الحيوانات

المصابة كانت مصابة بالبابيزيا فقط . وقد لوحظ زيادة معنوية كبيرة في معدل انتشار المرض في الابقار الهجين 74% مقارنة بالسلالة المحلية 52%. كما لوحظت هذه الزيادة المعنوية في الحيوانات المعالجة بمجموعة من الأدوية 74% مقارنة بالحيوانات المعالجة بدواء واحد فقط 52%. من المثير للدهشة أن العدوى بالبابيزيا كانت أعلى في الحيوانات غير المصابة بالقراد 60.5 % مقارنة بالحيوانات المصابة بالقراد 55.8%، هذه الحقيقة يمكن أن تشير إلى سوء استخدام مبيدات القراد في تلك المناطق.

Introduction

Babesiosis is caused by genus Babesia and it is zoonotic tick-transmitted hemoparasitic disease, which is considered as the second most common tick borne parasites of mammals after Trypanosomes (Telford, 1993). Babesiosis spp are classified under apicomplexan, haemoprotozoan parasite, family *Babesiidae*, order Piroplasmida (Sharma *et al*., 2013). Babesiosis also knows as bovine babesiosis, piroplasmosis, Texas fever, Red water and Tick fever (Sahinduran, 2012). Although Bovine babesiosis can be found wherever the tick vectors exist, it is most common in tropical and subtropical areas (CFSPH, 2008). The disease is characterized by fever, anaemia, haemoglobinuria and weakness. Moreover, infection with babesia effects negatively on the health of the livestock, including production and productivity (Alekaw, 2000). Additionally, this disease threatens around half a billion cattle across the world (Saad *et al*., 2015).

Babesiosis commonly infect cattle, sheep, goats, horses, pigs, dogs and cats and occasionally man. More than 100 Babesia spp. have been identified, which infect many types of mammalian hosts. Out of these species, 18 cause disease in domestic animals (Hamsho *et al*., 2015). Bovine can infect by different species of Babesia, but three species are mainly found; *B. bovis*, *B. bigemina* and *B. divergens*. Additional species can also infect cattle include *B. major, B. ovate, B. occultans* and *B. jakimovi* (Spickler *et al*., 2010).

B. bigemina and *B. bovis have* a considerable impact on cattle health and their productivity, especially in tropical and subtropical countries (El-Ashker *et al*., 2015). They are present in most areas of the world, with the greatest incidence between the latitudes 32 °N and 30 °S, where their vector *Boophilus spp* are commonly occurring (Pohl, 2013). Moreover, they are particularly important in Asia, Africa, Central and South America, parts of southern Europe, and Australia. Generally, both parasites, *B.bovis* and *B. bigemina*, have the same distribution, but in Africa *B.bigemina* is more widespread than *B. bovis* because of the ability of *Boophilus decoloratus* and *Rhipicephalus evertsi* to act as vectors for *B. bigemina* (Pohl, 2013). *B. bovis* and *B. bigemina* are affect cattle, water buffalo and African buffalo. Furthermore, both parasites were recently discovered in white-tailed deer in Mexico.

Host factors such as age, breed, and immune status can infect on the severity of the disease (Jabbar *et al.*, 2015). Bos indicus breeds are more resistant to Babesiosis compared with BosTaurus (Radostits *et al*., 2007). This reason for this phenomenon is due to the indigenous breed having lived for a long time in endemic areas. Thus, they have developed an innate resistance against the tick and tick borne disease. The severity of the clinical Babesiosis increases with age, so adult animal is more infected by Babesiosis as compared with calves (El Moghazy *et al*., 2014). The possible reason for that is the passive transfer of maternal antibody via colostrum from the mother to the newborn (Demessie and Derso, 2015). Comparing between Babesia species, *B. bovis* is usually more virulent than *B. bigemina* or *B. divergens*. Whereas the mortality rates over 50% are common in animals infected with *B. bovis*. In contrast, only a relatively small proportion of cases are fatal in animals infected with *B.bigemina* (CFSPH, 2008).

Babesiosis, especially in cattle has great economic importance, because unlike many other parasitic diseases, it affects adults more than young cattle, which leading to direct losses through death and the restriction of movement of animals by quarantine laws. Furthermore, the disease is a barrier to improve productivity of local cattle by crossbreeding due to the high mortality of the breed, which imported from Babesia free areas. Consequently, the quality of cattle in endemic areas remains low (Demessie and Derso, 2015).

Although, Sudan is the largest African country has livestock, the reduction in production and productivity due to tick borne disease are great. In South Darfur State (West Sudan), the major factors limiting livestock productivity are poor nutrition, lack of water, overgrazing, and diseases such as bacterial, viral and parasitic diseases. In order to control these diseases, especially tick borne disease, extensive studies on parasitic diseases are needed. Tick borne disease in general and Babesiosis in specific have not been studied extensively in South Darfur State before.

The objectives of this study are:

To estimate the prevalence of bovine babesiosis in South Darfur State.

To investigate the potential risk factors could be associated with disease.

Chapter one
Literature review

1.1 Etiology

Nowadays Piroplasmosis is a disease with a worldwide distribution affecting many species of mammals with a major impact on cattle and human. It has been recognized throughout the world as public health problems (Zanet *et al*., 2014). Babesia spp is a protozoan parasite transmitted mainly by ticks and able to infect erythrocytes of a wide variety of domestic and wild animals (Duh *et al*., 2008). Babesia is the second most common parasite found in the blood of mammals after trypanosomes (Yabsley and Shock, 2013). It causes direct economic losses, such as mortality, reduction in meat and milk yield, and the cost of control measures of ticks. The infection with babesia is characterized by anaemia, icterus, hemoglobinuria, which may finally lead to death (Wagner *et al*., 2002; Vial and Gorenflot, 2006).

Bovine babesiosis is caused by *Babesia bigemina* (*B.bigemina*), *Babesia divergens* (*B.divergens*), *Babesia bovis* (*B.bovis*), and *Babesia major* (*B. major*). Among these species, *B. bigemina* and *B. bovis*, have a considerable effect on cattle health and their productivity in tropical and subtropical countries (Iseki *et al*., 2010).

Examination of blood smears stained with Giemsa using microscopy has been considered as the "gold standard" for detecting *Babesia* organism in the blood of infected animals, particularly in acute cases. However, this method is not useful in a carrier animal, where the numbers of the parasites in the peripheral blood are very few (Bose *et al*., 1995; Nayel*et al.*, 2012). Polymerase chain reaction (PCR) is more sensitive and specific technique and offers an alternative approach to detect Babesia spp (AbouLaila *et al*., 2010; Zulfiqar *et al*., 2012 ; Shams *et al*., 2013).

Taxonomy of Babesia:

The genus Babesia is classified under Phylum *Apicomplexa* (also called *Sporozoa*), class *Aconoidasida* (*piroplasmea*) and Order *Piroplasmida* (Homer *et al*.,2000). *Piroplasms* are referring to the protozoan parasites that utilize mammalian erythrocytes in their life cycle and characterized by intra erythrocytic forms which can be pear-shaped (Levine,1971).

This order, *Piroplasmida*, includes two main families *Babesiidae* and *Theileriidae*. The primary distinction between Babesia and Theileriaon on one hand is the absence of apre-erythrocytic cycle in *Babesia* and on the other hand the absence of transovarial transmission in Theileria (Homer *et al*., 2000).

The Taxonomy of *Babesia* (Levine,1988) as following:

Domain: *Eukaryota*

Kingdom: *Chromalveolata*

Subkingdom: *Protozoan,*

Superphylum: *Alveolata*

Phylum: *Apicomplexa (Sporozoa*) (Homer *et al*.,2000)

Class*:* *Aconoidasida* (*Piroplasmea*) (Levine *et al*.,1980)

Order: *Piroplasmida* (Levine,1971)

Suborder: *Piroplasmorina* (Hunfeld *et al*.,2008)

Family: *Babesiidae*

Genus: *Babesia*

Species: *Babesia bigemina*, *Babesia bovis and Babesia divergens*. (M'Fadyean and Stockman,1911)

1.3 Mammals species affected by bovine Babesia spp:

B. bovis and *B. bigemina* are found in cattle, which are the main hosts. They also affect water buffalo, African buffalo and white-tailed deer in Mexico. Generally, animals other than cattle have been considered of epidemiological significance as reservoir for *B.bovis* and *B. bigemina*.

B. major, *B. ovata* and *B. occultans* also cause infection in cattle. *B. divergens* causes clinical signs in cattle, sheep and reindeer. Moreover, humans and non-human primates (including chimpanzees and monkeys) are also susceptible to infect with *B. divergens*. Experimental infections with *B.divergens* also established in red deer, roe deer, fallow deer and Mongolian gerbils but clinical signs are not usually seen. Mice, hamsters, rats and rabbits are highly resistant to get the infection with Babeisa spp. *B. jakimovi* can infect cattle, roe deer, Asian elk and reindeer (CFSPH, 2008).

1.4 Epidemiology of bovine Babesiosis:

Babesia species occur in Central and South America, parts of Europe and Asia, Australia and Africa. *Babesia bigemina* is much more widespread and present throughout southern Africa, except the arid and some high-lying parts (Bock *et al*., 2004). *B.bigemina* has been eradicated from the United States of America. In southern Africa, *B.bovis* is restricted to areas where *Rhipicephalus. microplus* is exist. *B. major* can be found in parts of Europe, Northwest Africa and Asia, as well as China. *B. ovata* has been described in Japan, China and other parts of eastern Asia. *B. occultans* was reported in Africa, and *B. jakimovi* occurs in Siberia (CFPH, 2008).

Endemic stability is defined as the state where the relationship between host, agent, vector and environment is such that clinical disease occurs rarely or not at all (Perry, 1996). In endemic area, passive acquired immunity against Babesia through colostrum remained until 2 months. Later is followed by innate immunity from 3 to 9 months of age (Mahoney and Ross, 1972; Mahoney, 1974). Therefore, calves exposed to babesiosis during the first 6 to 9 months rarely show clinical symptoms and develop a solid long-lasting immunity (Dalgliesh, 1993). Moreover, it is estimated that if at least 75% of calves were exposed to *B. bovis* infection by 6 to 9 months of age the disease incidence would be very low and a state of natural endemic stability would exist.

Under instability conditions, some animals become infected with Babesia after birth. Therefore, they may develop severe disease if they get the infection later in life (Callow, 1984; Mahoney, 1974).

1.5 Transmissions of Bovine Babesiosis:

Babesia species are transmitted by ticks, which become infected when they ingest the parasites during the blood meal. The major vectors of *B. bigemia* are *Rhipicephalus microplus* (formerly *Boophilu smicroplus*),*R. annulatus* (formerly *Boophilus annulatus*), *R.decoloratus*, *R. geigyi*, and *R. evertsi*. The major vectors for *B. bovis* are *R. microplus*, *R. annulatus* and *R. geigyi.*

B. divergens and *B.jakimovi* are mainly transmitted by *Ixodes* species. *Haemaphysalis punctate* transmits *B. major*, *Haemaphysalis longicornis* transmits *B. ovata*, and *Hyalomma marginatum* transmits *B. occultans*. (CFSPH,2008).

Inside the tick, Babesia zygotes multiply as ‘vermicules,’ which invade many of the tick’s organs including the ovaries. Babesia species are readily passed to the next generation of ticks through the egg. These parasites can sometimes pass several generations, though transovarial transmission. Although this method of transmission is mainly depending on the species of Babesia and ticks. For example, *B. divergens* can survive in tick populations for at least 4 years, even if cattle are not present. (CFSPH, 2008).

When an infected tick attaches to a new host, Babesia is stimulated to undergo their final maturation. The animal can become ineffective with *B. bovis* within 2-3 days after ticks attach. However, *B. bigemina* matures in approximately 9 days after attachment of ticks. Other biting flies might act as a mechanical transmission for Babesiosis.

Babesiosis is maintained in cattle populations through asymptomatic carriers, which have recovered from acute disease. *B. bovis* persists in cattle for years, while *B.bigemina* survives for a few months. Calves can be infected in utero; however, this way of infection appears to require pathological changes in the placenta (Barros, 2008).

1.6 Life cycle of *Babesia*:

The life cycle of *B.bovis* and *B.bigemina* was investigated extensively and more information is available regarding these species. The development of *B. bovis* and *B. bigemina* followa a similar patterns in adult *Boophilus spp*. (Friedhoff, 1988). Ticks introduce the sporozoites into the host during the blood meal. The sporozoite penetrates the membrane of erythrocyte with the aid of specialized apical complex (Potgieter and Els, 1977). Once inside, the sporozoites transform into a trophozoites and then merozoites, which they develop by a process of binary fission (Friedhoff, 1988). The merozoites later develop into gametocytes (Mackenstedt *et al.,* 1995). These gametes do not develop further until they ingested by ticks. Changes in the environment from host blood to the midgut of the tick vector stimulate the development of gametocytes (ray bodies from) (Gough *et al*., 1998). The ray bodies undergo further multiplication within the erythrocyte and then fuse together in pairs into form a spherical cell called zygote (Gough et al., 1998; Friedhoff, 1988). The zygote selectively infects the digestive cell of the tick gut where they multiply into motile kinetes (Agbede *et al*., 1986).

These motile knots, then escape into a variety types of cell and tissues, including the oocytes where successive cycles of secondary schizogony take place. Thus, transovarial transmission occurs with further development taking place in the larval stage. Motile kinetes enter also the salivary glands and transformed into multinucleated stages (sporogony) and then break up to form sporozoites (Mackenstedt *et al*., 1995). In all species, sporozoite development usually only begins when the infected tick attaches to the vertebrate host.

In *B.bigemina,* some development takes place during the feeding of larvae, but infective sporozoites take about 9 days to appear. Therefore, the infection can occur only in the nymph and adult stages of the tick (Hoyte, 1961; Potgieter and Els, 1977). In the case of B. bovis, the formation of infective sporozoites usually occurs within 2 to 3 days of larva attachment, thus larva can also play a role in the transmission of *B. bovis* (Riek, 1966).

1.7 Pathogenesis and clinical signs of bovine babesiosis:

After penetration of the cell, Babesia multiplies via repeated binary fission within the erythrocyte, resulting in up to16 merozoites. Multiplication of the parasites leads to damage the membrane of erythrocyte cell, which increased the osmotic fragility, which in turn leads to intravascular and extravascular haemolysis and anaemia.(Máthé *et al* ., 2007). Babesia activates antibody-mediated cytotoxic destruction of erythrocytes leading to anaemia, haemoglobinaemia, haemoglobinuria, thrombocytopenia and in cases of massive infection leads to death caused by multiple organ dysfunction syndrome (Máthé *et al*., 2007). Immune-mediated haemolytic anaemia is assumed to occur with all *Babesia* species following the production of anti-erythrocyte membrane antibodies (Hunfeld *et al*., 2008). In severe *babesiosis*, tissue hypoxia is occuring specially in dogs and ruminants and particularly affecting the central nervous system, kidneys and muscles (Vercammen *et al.*, 1995; Matijatko *et al*., 2012; Schetters *et al* ., 1997). Furthermore, many of intra erythrocytic parasites can escape from the immune system through rapid antigenic variation, which has been demonstrated in *B. bovis* and *B. bigemina* (Radostits *et al*., 2007).

B. bovis is the highly pathogenic species compared with other bovine Babesia (Sahinduran, 2012). In acute cases of *B.bovis*, the maximum parasitaemia in circulating blood is less than 1%. This is in contrast to *B. bigemina* infections, where the parasitaemia often exceeds 10% and may be as high as 40%. Both *B. bigemina* and *B. bovis* have the same clinical signs, but show differences in pathogenesis and manifestation. Hence, *B.bigemina* can be characterized as a peripheral babesiosis with severe anaemia, whereas *B. bovis* often induces a visceral babesiosis because of thrombus formation (Pohl, 2013).

The incubation periods usually vary from 8 to 15 days. In acute manifestations, fever (>40°C) usually presents for several days before the onset of other clinical signs such as inappetence, depression, weakness, reluctance to move. Haemoglubinuria is often present, especially in *B.bigemina* infections (hence the common name "redwater"). Anaemia and icterus are especially obvious in more protracted cases. Diarrheal is common and pregnant cows may abort. Cerebral babesiosis, which occasionally develops in *B. bovis* infections, is manifested by hyperaesthesia, circling, head pressing, aggression, convulsions and paralysis these signs may or may not accompany other signs of acute babesiosis (Bock, 2004).

1.8 Lesion in bovine babesiosis:

Light red, watery blood, pale mucous membranes and pale carcass comparing with the normal character of the typical necropsy of babesia cases. The spleen is enlarged and congested .The liver is swollen, friable and yellowish-brown in colour, and the surface may have mottled appearance, with fatty degeneration to necrosis. The gallbladder is distended with viscous bile, which often contains dark brown granules up to 1 mm in diameter. The content of intestinal is usually lower and yellowish in color. The kidneys are mildly to moderately swollen and dark reddish-brown or yellowish-brown in color. The lungs are often oedematous, with foam present in the bronchi and trachea. The heart is usually flabby and pale (Bock,2004) .

If the animal survives longer, mild to moderate transudation into the body cavities such as hydrothorax, hydropericardium and ascites may be observed. The urine is discolored and may be deep yellow to yellow-brown (Bock, 2004 ; De Vos, 2004 ; De wall, 2006).

1.9 Babesiosis in other animals:

1.9.1 Sheep and goat Babesiosis:

Ovine and caprine babesiosis is an acute or chronic infectious disease of sheep and goats, caused by two species of Babesia; *B.motasi* and *B.ovis*. *B. motasi* is a larger and more virulent form, occurring singly or paired in erythrocytes; while *B. ovis*, is a small form.(Phillip and Peter, 2015).

Ovine and caprine babesiosis is widespread in tropical and subtropical regions in North Africa including Egypt, the Middle East, south-eastern Europe, and South America. This disease occurs in all breeds, sexes and age of sheep and goats, but animals 6-12 months old have a higher incidence than animals of other age groups. Ticks of the genera *Dermacentor*, *Rhipicephalus*, *Haemaphysalis*, and *Ixodes* have been reported as vectors. The organisms are transmitted transovarially or transstadially depending on the vector involved. The disease is characterized by fever, anaemia, hemoglobinuria and icterus. Economic losses result from the deaths among affected sheep and goats, and the cost of control programs..(Phillip and Peter, 2015).

1.9.2 Canine Babesiosis*:*

There are two species of Babesia ,*B. canis* and *B. gibsoni* ,caused Babesiosis in domestic dogs. Although canine Babesioisis is common throughout the world, the infection is more common in areas where tick infestation is high and acaricide is not used. Ticks are not able to transmit infection immediately upon first attachment to the host; they require a period of approximately 24 to 48 hours of initial feeding before organisms are able to pass across the salivary glands and into the vertebrate host. *Rhipicephalus sanguineus* and *D. variabilis* are the most common known arthropod vectors of canine babesiosis in the United States. Direct transmission of Babesia from an infected anima to a naïve animal can also occur following blood transfusion or with contaminated needles or surgical instruments. Canine babesiosis can also transmit directly from dog-to-dog via dog bites (Irwin, 2010; Sikorski *et al*., 2010 ; Di Cicco, 2012)

Canine Babesiosis is characterized by moderate to severe hemolytic anaemia, fever, anorexia, depression, pallor and splenomegaly. In general, the infection with *B. canis* is less severe comparing with *B. gibsoni* (Irwin, 2010;Sikorski *et al.*, 2010; Di Cicco , 2012)

1.9.3 Equine babesiosis :

Equine babesiosis is a febrile tick-borne disease of horses, mules and donkeys, caused by *B. equi* or *B. caballi*. Equine babesiosis occurs in North, Central, and South Africa, South and Central America, USA, Southern and Eastern Europe, Asia, the Middle East including Egypt, and India (El Sawalhy, 1999). Equine babesiosis is transmitted by ticks of the genera *Dermacentor*, *Hyalomma*, and *Rhipicephalus.* Contaminated needles, surgical instruments, and blood transfusion can also transmit the infection mechanically. *B. caballi* is transmitted transovarial from one tick generation to the next, while *B. equi* is transmitted only transstadial.

Generally, the disease is characterized by high fever, anorexia, oedema of the fetlocks joins, head and ventral abdomen, anaemia, hemoglobinuria, jaundice, abortions and around 17.5% mortality rate. *B. equi* is the more pathogenic as up to 20% of red blood cells are infected, which leading to severe clinical signs, while *B. caballi* causes less severe or asymptomatic disease as only about 1% of red blood cells are infected ((El Sawalhy, 1999).

Babesiosis in equine has a big economic effect result from the interference with racing and pleasure horse meetings and competitions. So equine babesiosis is an important risk, which concerns quarantine authorities in free countries particularly during Olympic and Paralympic games when so many horses will be imported into those countries. Thus, the Office International Des Epizooties (OIE) has placed this disease under the category of list (B) diseases (El Sawalhy, 1999).

1.9.4 Human Babesiosis:

The first case was identified on Nantucket Island, off Cape Cod, Massachusetts (Healy, 1970). The causative agent was *B. microti* and the vector was the *Ixodes dammini* (Spielman, 1976). Additional cases occurred on the island, and the disease became known as "Nantucket fever." During the past decade, the incidence and geographic distribution of babesiosis in human have been increased in the north-eastern and upper mid-western regions of the United States. Infection with *B. microti* is also common in southern New England, where the high prevalence of *B. microti* infected ticks in the region are observed (Krause *et al*., 2003; Tokarz et al., 2010).

The clinical manifestations of babesiosis range from subclinical infection to fulminating disease resulting in death. Most symptomatic patients become ill between 1 to 4 weeks after the bite of a *B. microti* infected tick and between 1 to 9 weeks after transfusion of contaminated blood (Vannier *et al*., 2008; Herwaldt *et al*., 2011). After a gradual onset of malaise and fatigue, fever usually develops, with a peak temperature that can be as high as 40.9°C (105.6°F). Chills and sweats are common and may be accompanied by headache, myalgia, anorexia, nonproductive cough, and nausea (Ruebush et al., 1977 ; Krause *et al*., 2003 and Joseph *et al*., 2011). Moreover, symptoms include vomiting, sore throat, abdominal pain, photophobia, weight loss, depression, and hyperesthesia may occur (Ruebush et al., 1977 ; Vannier *et al*., 2008). On physical examination, fever is the most common sign and it may be accompanied by splenomegaly, pharyngeal erythema, hepatomegaly, jaundice, or retinopathy with splinter hemorrhages, and retinal infarcts (Ruebush *et al*., 1977; Krause *et al*.,1996; Vannier et al., 2008).

1.10 Diagnosis of Bovine babesiosis:

1.10.1 Clinical findings:

The incubation period is often between 2–3 weeks after tick infestation. However, shorter incubation periods have been documented in the field and through experimental inoculation (4–5 days for *B. bigemina* and 10–12 days for *B. bovis*).

The severity of the disease varies according to the species of parasite and host factors (i.e. Age, immune status). Generally, infection with *B. bovis* is more pathogenic than *B. bigemina* or *B.divergens*. The infected animals develop a lifelong immunity against re-infection with the same species and cross-protection against other species (Homer *et al*., 2000and Kahn, 2005).

Generally, the disease is characterized by high fever, ataxia, anorexia, dark red to brown urine, signs of general circulatory shock, sometimes nervous signs associated with sequestration of infected erythrocytes in cerebral capillaries. Anaemia and haemoglobinuria may appear later in the course of the disease (Homer *et al*., 2000 and Kahn, 2005).

1.10.2 Direct microscopic examination:

Thin blood smears were prepared from the peripheral blood and then dried. After fixation with methanol for 5 minutes, slides were stained with 4% Giemsa for 30 minutes and then washed with distilled water. One hundred microscopic filed were examined under immersion lens oil (100×magnification). The presence of one piroplasm was considered as positive cases (Salih *et al*., 2007). Microscopic differentiation between Babesia species depends on the shape and size of these parasites. *B.bigemina* is a large pleomorphic Babesia and identified by the pear-shaped bodies joined at an acute angle within the erythrocyte. Comparison between

B. bigemina, and *B. bovis* the later is smaller, and identified by the pear shaped bodies joined at an obtuse angle. The differentiation between the round forms and the pear shaped of Babesia was mainly depends on the size. The size of the round forms of *B. bigemina* is around 2 µm, and of *B. bovis* is around 1–1.5 µm. The size of pear-shaped of *B. bigemina* and *B. bovis* are 4–5 µm and 1.5–2.4 µm, respectively (Taylor *et al*., 2007). These parasites and all divisional stages are found within RBCs (Zintl *et al*., 2003; OIE, 2008).

1.10.3 Polymerase chain reaction (PCR) assays and other techniques:

Polymerase chain reaction (PCR) assays can detect and differentiate *Babesia* species, and are more useful especially in a carrier animal. The infection with *Babesia* may also be diagnosed using vitro culture or blood transfusing into a test calf. Experimental animal techniques are expensive and seldom used as a routine diagnosis (Zintl *et al*., 2003; OIE, 2008).

1.11 Control of Babesiosis:

Babesiosis can be eradicated by eliminating of the vector; this can be performed by treating of all cattle every 2 to 3 weeks with acaricides. In countries where eradication is not feasible, tick control can reduce the incidence of disease. The development of resistance against acaricides must be concerned. Modification of the environment can also destroy the tick habitats, but in some cases, this may be difficult(CFSPH, 2008).

Live, attenuated strains of *B. bovis* or *B. bigemina* or *B. divergens* are used to vaccinate cattle in some countries. These vaccines have safety issues, including the potential virulence in adult animals, possible contamination with other pathogens, and hypersensitivity reactions to blood proteins. These vaccines are suitable to be used in animals less than one year of age. In some cases, vaccination of older cattle is necessary, especially when the cattle are moved into an endemic area. Older animals should be monitored closely after vaccination, and the treatment should be applied if any clinical signs are developed. Moreover, in some countries, animals may be vaccinated in the face of an outbreak. The use of genetically resistant cattle such as Bosindicus can also decrease the incidence (CFSPH, 2008).

1.12 Treatment of Babesiosis*:*

Treatment is most likely to be successful if the disease is diagnosed early; it may fail if the animal at the late stage. A number of drugs are reported to be effective against Babesia, but many of them have been stopped due to safety or residue concerns.

Currently, diminazenean and di midocarb dipropionate (imidocarb) are the most widely used. Diminazene works rapidly against *B. bovis* and *B. bigemina* at a dose of 3.5 mg/kg Intramuscular (De Vos, 1979). Imidocarb is used subcutaneously at a dose of 1.2 mg/kg for treatment, while 3 mg/kg provides protection against *B. bovis* for 4 weeks and against *B.bigemina* for 2 months (Taylor and McHardy, 1979). Moreover, imidocarb at high dose can eliminates *B. bovis* and *B. bigemina* from the carrier animals (de Vos Dalgliesh and McGregor, 1986). Treatment with long-acting Oxytetracycline following vaccination significantly reduces the parasitaemia without inhibiting the development of the immunity (Pipano *et al*., 1987; Jorgensen *et al*., 1993). However, Oxytetracyclines are not usually able to control the virulent field infections.

1.13 Economic impacts of Babesiosis:

The economic impact of babesiosis can be expressed in term of mortality, loss of production (including meat and milk production), the cost of control and the restriction of animal movement. In the areas where the management is best, there are few reliable estimates of economic losses resulting from babesiosis. The problem of babesiosis is becoming further complicated by the occurrence of more than one tick-borne disease in the region and control of ticks is recommended (Norval *et al*., 1991).

Chapter two
Material and Methods

2.1 Study area:

The present study was conducted at Nyala town (Figure 1), South Darfur State, Sudan. The State is located in the southwest of Sudan and covers around 139800 km^2, and located between latitude 13–9.30° North and longitude 27–24.30° east. The state has common borders with North Darfur State, West Kordofan State, Northern Bahr El Ghazal State, and West Darfur States. It also shared border with Chad and Central Africa Republic. Livestock species in south Darfur include camels, cattle, donkeys, goats, horses and sheep.The main important breeds of cattle in this area are: indogenous Baggara, butanna, kennana and some cross Frisian.

The climate in South Darfur State is savannah type, while is semi-desert in the north State. The meteorological annual data of 40 years were obtained from Nyala Airport Meteorological Station and it showed that the minimum and maximum temperatures are between 20.98°- 35.14°C. The annual relative humidity is 35.58% and the mean rainfall is 402 mm. There is one raining season, which occurs between June and October, but the bulk of the rainfall takes place during the period between Julys until September.

2.2 Study design:

The study was a cross-sectional study to estimate the prevalence of Bovine Babesiosis , and the risk factors associated with the disease.

Figure 1: The study area, Nyala town, South Darfur State, Sudan.

2.3 Sample size:

The sample size of animals was determined using the formula of Thrusfield (2007).

n= 〖(1.96)〗^2. Pexp (1-Pexp)/d^2

n=sample size

Pexp = expected prevalence

d = desired absolute precision (d=0.05)

The expected prevalence of bovine Babesiosis was obtained from a previous study (Shuaib *et al*., 2015), which was estimated as 5.20%. Consequently, the sample size was calculated as follows:

n= (〖1.96)〗^2*(0.0520)*(1-0.0520)]/(0.05)2=75 animals.

To increase the percussion of our result, the sample size was multiplied by 4. Thus, the sample size becomes 300 animals.

2.4 Sampling methods:

The samples were taken from three areas and these were blood samples (300 samples) collected by using Multi-stage sampling technique. Based on this technique the state was divided into three Areas. From each division, 100 cattle were selected Slaughterhouse, 100 cattle Almuashi area (animal fauna) and 100 cattle farms.

2.5 Blood samples:

The whole blood samples were collected from the jugular vein according to OIE method (OIE, 2010). Approximately, 3-5 ml of blood was taken from the jugular vein using labelled disposable syringe after disinfecting the site of injection. Immediately, the blood was transferred into tubes containing Ethylene Diamine Tetra-acetic Acid (EDTA) (Nayel *et al*., 2012).

The samples were transferred to Nyala University and kept at 4°C until use. Thin smears were prepared by applying one drop of blood onto a microscopic slide near to the edge. At an angle of 45°, another slide was placed and then the blood was spread by gently moving forward of the slide. The smears were first air dried and then fixed for 2 minutes using methyl alcohol (Absolute methanol). These Blood samples transferred to Nyala university lab for microscopic examination.

Figure 2: Collection of blood samples from slaughter house in Nyala town-South Darfur State.

2.6 Microscopic blood examination:

Thin blood smears were prepared from the peripheral blood and then dried. After fixation with methanol for 5 minutes, slides were stained with 4% Giemsa for 30 minutes, and then washed with distilled water. One hundred microscopic filed were examined under immersion lens oil (100×magnification). The presence of one piroplasm was considered as positive cases (Salih *et al*. 2007). Microscopic differentiation between Babesia species depends on the shape and size of these parasites. (Taylor *et al*., 2007).

2.7 Questionnaire:

Data regarding the characteristics of individual cattle, including age, gender, breed, body condition, presence of ticks, infected with other blood parasites, treatment and the Area, were obtained by asking the owner. These factors were divided into categories described by Thrusfield (Thrusfield, 2007).

2.8 Statistical analysis:

The data collected during the study period were stored in Microsoft Excel sheet and then analysed using statistical software program for Windows (SPSS version 16.0). The prevalence of Babesia was calculated by dividing the number of the positive cases by the total number of cattle examined. The associations of risk factors like age, sex, body condition and treatment with the positivity of Babesiosis were analysed using Chi-square test. The confidence interval (CI) was held at 95% and $P<0.05$ was set for statistical significance and then selected to analysed using Multivariate Analysis.

Chapter three

Results

3.1. Distribution of total results:

A total of 300 blood samples of cattle were selected randomly from three different areas Nyala Town - South Darfur State and microscopically examined for infection with bovine babesiosis. Among these animals, 178 (59.3%) were infected with Bovine babesiosis (**Table 3.1**).

Table 3.1: The number and distribution of animals examined for Bovine babesiosis from different areas in South Darfur State.

Areas	**No. of tested**	**No. of positive**
Slaughterhouse	100	54
Animal fauna	100	50
Farms	100	74
Total	300	178

3.2. Prevalence of Bovine babesiosis in relationship to the potential risk factors:

3.2.1 Cattle age group:

Cattle of different age group were examined for infection with babesiosis. The animals were divided into 2 groups; young cattle (age less than 3 years) and adult cattle (age ≥ 3 years). Fifty six and 244 cattle's were examined from each group, respectively (Table 3.2.1). Generally, the infection rate was highest in the young group (62.5%) compared with adult group (58.5%) (Table 3.2.2). However, there was no significant association between the disease prevalence and two age groups(p-value 0.593) (Table 3.2.3).

3.2.2 Cattle sex:

Cattle examined for Bovine babesiosis were classified according to sex. The total number of female examined was 251 animals and the total numbers of males examined was 49 animals (Table 3.2.1).The obtained result revealed that 146 out of 251(58.2%) of females and 32 out of 49 (65.3%) of males were infected with Bovine babesiosis (Table 3.2.2).The Chi- square test showed that, no significant association between the infection with Bovine babesiosis and gender of the animal (p-value =0.352) (Table 3.2.3).

3.2.3 Cattle breed:

The breed of examined cattle was classified into two breeds; cross breed and local breed. The total number of local breed was 200 cattle, 104 of them (52%) were positive for babesia infection. In cross breed cattle, 74 out of 100 (74%) were positive for Babesiosis (Table 3.2.2). By using Chi-square test, there was a significant association between the cattle breed and Bovine babesiosis (p-value= 0.000) (Table 3.2.3).

3.2.4 Body condition scores of Cattle:

The physical body condition of examined animals was divided into three groups;good (117 animals), medium (155 animals) and poor (28 animals). About 58.1% of cattle in a good condition were infected with Bovine babesiosis, while in the cattle of medium body condition 59.4% were infected with Bovine babesiosis. In the group of poor body condition, only 64.3% were positive for the infection with Babesiosis (Table 3.2.2). The Chi-square test showed no significant association between the infection and the body physical condition of cattle (p-value= 0.837) (Table 3.2.3).

3.2.5. Area:

As shown in the (Table 3.2.2), the prevalence of Bovine babesiosis in the different areas was 54 %, 50 % and 74 % in a slaughterhouse, Animal Fauna and Farms respectively. The Chi-square results showed a significant association between bovine babesiosis and the Areas of animal (p-value= 0.001) (Table 3.2.3).

3.2.6 Treatment of disease:

The prevalence of Babesia infection within animal treated by one drug was 52%, while the prevalence of Babesia infection within animal treated by compound drug was 74 % (Table 3.2.2). The Chi-square results showed a significant association between infection with bovine babesiosis and the number of drugs used for treatment (p-value= 0.000) (Table 3.2.3).

3.2.7 Ticks infestation:

All cattle in this study were investigated by visual examination for the presence of tick at the time of samples collection. The examination was shown that, 223 of the animals were not infested with ticks, while 77 of the animals were infested with ticks (Table 3.2.2).

Most of cattle 60.5% which were not infested with ticks where as 55.8% of cattle which were infested with ticks and showed positive results for babesia infection. The Chi-square analysis showed no significant association between bovine babesiosis and ticks infestation (p-value=0.470) (Table 3.2.3).

3.2.8 Other blood parasites:

A total of 300 cattle were also investigated for the infection with other blood (not Babesia). The results showed that, 289 animals were infected with one of other blood parasite, while only 11 animals were not infected with other blood parasite.

About 58.1 % of animals were shown mixed-infection (Babesia with other blood parasite), while 90.9% of animals were infected with Babesia only (Table 3.2.2). The Chi-square analysis showed a significant association between the infection with Bovine babesiosis and the presence of other blood parasites (p-value=0.030) (Table 3.2.3).

3.3 Multivariate analysis:

Multivariate analysis of potential risk factors was performed to determine which factors independently had associated with Bovine babesiosis. The animal breed and treatment were found as the major risk factors associated positively with the occurrence of the disease(p-value=0.000). Other risk factors such as areas (p-value= 0.001) and infection with another blood parasite (p-value=0.030) were also associated significantly with Bovine babesiosis (Table 3.2.4).

Table 3.2.1: The distribution and frequency of 300 cattle examined for Bovine babesiosis in Nyala Town-South Darfur State according to the potential risk factors.

Risk factors	Frequency	Relative frequency %	Cumulative percent %
Age group			
Adult	244	81.3	81.3
Young	56	18.7	100.0
Sex			
Female	251	83.7	83.7
Male	49	16.3	100
Breed			
Local breed	200	66.7	66.7
Cross breed	100	33.3	100
Body condition			
Good	117	39.0	39.0
Medium	155	51.7	90.7
Poor	28	9.3	100
Area			
Slaughterhouse	100	33.3	33.3
Animal fauna	100	33.3	66.7
Farms	100	33.3	100
Treatment			
One drug	200	66.7	66.7
Compound drug	100	33.3	100
Ticks infestation			
No	223	74.3	74.3
Yes	77	25.7	100
Other blood parasite			
Yes No	289 11	96.3 3.7	96.3 100

Table 3.2.2: The number of animal infected with bovine babesiosis according to the potential risk factors in 300 cattle examined by microscopic in Nyala Town- South Darfur State

Risk factors	Total of animal examined	No. of positive	No. of positive %
Age group			
Young	56	35	62.5%
Adult	244	143	58.6%
Sex			
Female	251	146	58.2%
Male	49	32	65.3%
Breed			
Local breed	200	104	52%
Cross breed	100	74	74%
Body condition			
Good	117	68	58.1%
Medium	155	92	59.4%
Poor	28	18	64.3%
Area			
Slaughterhouse	100	54	54%
Animal fauna(almuashi)	100	50	50%
Farm	100	74	74%
Treatment			
One drug	200	104	52%
Combination	100	74	74%
Ticks infestation			
No	223	135	60.5%
Yes	77	43	55.8%
Other blood parasite			
Yes	289	168	58.1%
No	11	10	90.9%

Table 3.2.3: Univariate analysis of the potential risk factors in association with bovine babesiosis infection in 300 cattle examined in Nyala Town- South Darfur State using the Chi-square test (p-value<0.05)

Risk factors	Total of animal examined	Number of positive %	d.f	X^2-value	p-value
Age group			1	.286	.593
Adult	244	143(58.6%)			
Young	56	35(62.5%)			
Sex			1	.866	.352
Female	251	146 (58.1%)			
Male	49	32 (65.3%)			
Breed			1	13.373	.000
Local breed	200	104 (52%)			
Cross	100	74 (74%)			
Body condition			2	.356	.837
Good	117	68 (58.1%)			
Medium	155	92 (59.4%)			
Poor	28	18 (64.3%)			
Area			2	13.704	.001
Slaughterhouse	100	54 (54%)			
Animal fauna	100	50 (50%)			
Farm	100	50 (74%)			
Treatment			1	13.373	.000
One drug	200	104 (52%)			
Combination	100	74(74%)			
Ticks infestation			1	.523	.470
No	223	135(60.5%)			
Yes	77	43(55.8%)			
Other blood parasite			1	4.718	.030
Yes	289	168(58.1%)			
No	11	10(90.9%)			

*df= degree of freedom
*X2-value= mean value
*p-value<0.05

Table 3.2.4: Multivariate analysis of potential risk factors in correlation with bovine babesiosis infection in 300 cattle examined in Nyala Town- South Darfur State:

Risk factors	No of positive (%)	Exp (B)	95% Confidence interval for Exp (B)		P-value*
			Lower	Upper	
Age group					.728
Adult	143(58.6%)	ref	-	-	
Young	35(62.5%)	.892	.467	1.703	
Sex					.189
Female	146(58.2%)	ref	-	-	
Male	32(65.3%)	.623	.308	1.262	
Body condition					.494
Good	68(58.1%)	ref	-	-	
Medium	92(59.4%)	.737	.430	1.263	
Poor	18(64.3%)	1.073	.429	2.686	
Area					.001
Slaughterhouse	54(54%)	ref	-	-	
Animal fauna	50(50%)	1.006	.562	1.802	
farm	74(74%)	.311	.153	.629	
Ticks infestation					.801
No	135(60.5%)	ref	-	-	
Yes	43(55.8%)	.929	.522	1.651	
Other blood parasite	168(58.1%)	ref	-	-	.111
Yes	10(90.9%)	.181	.022	1.484	
No					

*The level of significant was p<0.05

*EXP(B)=exponential(B)=Odds ratio

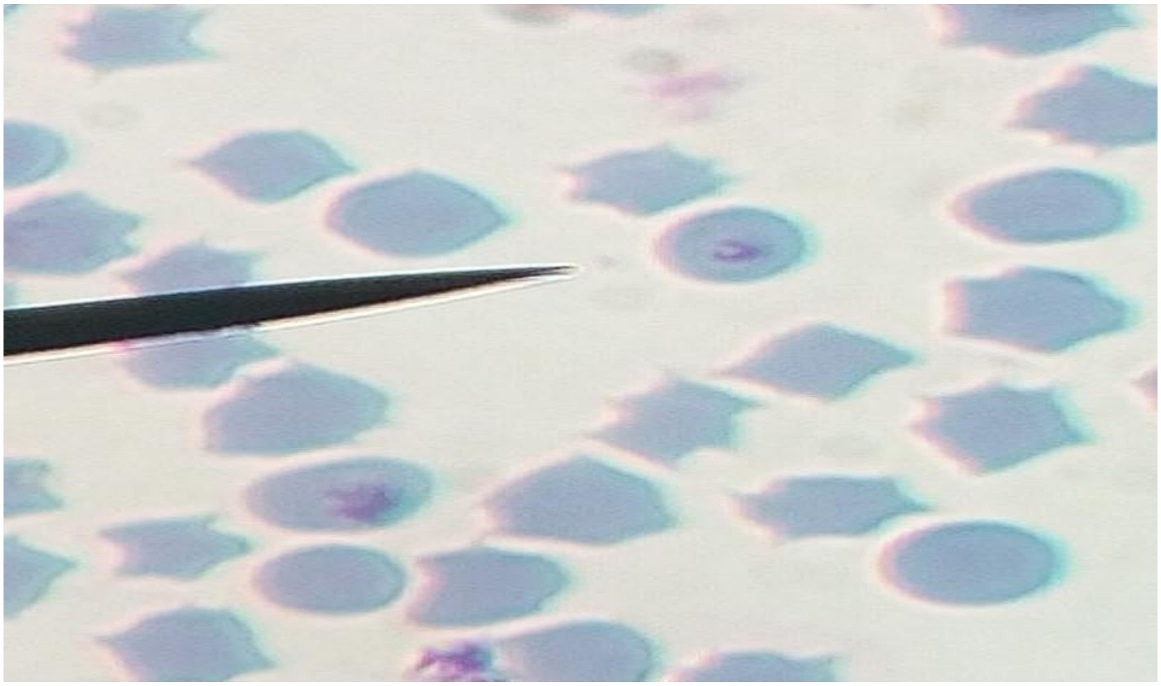

Figure 3: Babesia spp inside Red Blood Cells of cattle (n=300) in Nyala Town-South Darfur State

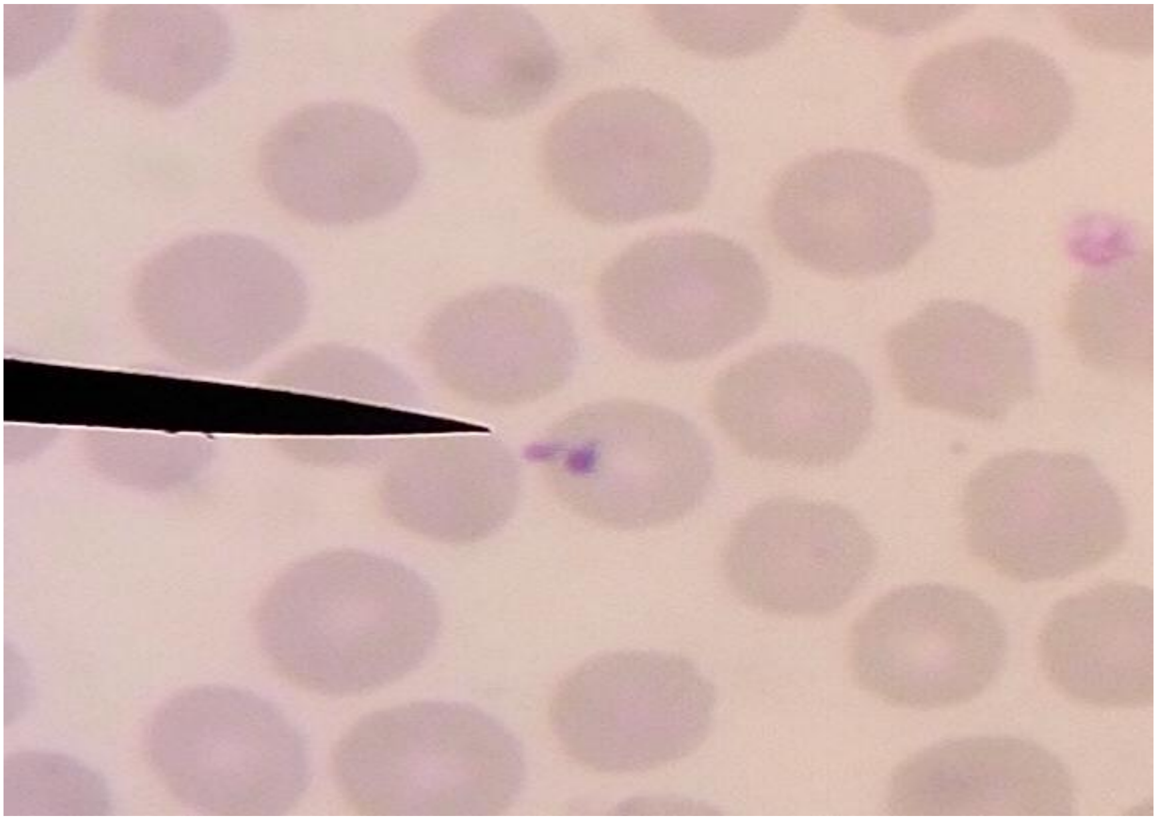

Figure 4: Babesia spp inside Red Blood Cells of cattle (n=300) in Nyala Town-South Darfur State

Chapter four

Discussion

In this study, the overall prevalence rate of bovine babesiosis was 59.3% using Giemsa stain for microscopic examination. The prevalence of bovine babesiosis in this study is higher than that in other studies in different countries. In Malak and Agency the prevalence was 6.6% (Ahmad and Hashim, 2007), in Pakistan the prevalence was 9.9% (Ayaz *et al*., 2013), and in Kanchanaburi province the prevalence was 26.6% (Patcharathorn*et al* ., 2013). The discrepancy in the prevalence of bovine babesiosis might be due to different factors such as; management condition in the farm , farm system, using acaricides or anti-parasitic drugs during tick infestation, fluctuations of the parasite during the course of the disease and the sensitivity of the test used for diagnosis (Cadder *et al*., 1996 ;Gubbels *et al.*,1999; Homer, 2000). Moreover, the variation in the prevalence rate may be due to the variation of the geographical conditions, the variation of cattle breeds, the distribution of the vectors and the accessibility of animals to have a contact with these vectors (Cadder *et al* ., 1996 ;Gubbels *et al*., 1999; Nasir *et al*.,2000 and Homer, 2000) .

In the present study, the highest prevalence rate of Bovine babesiosis was noted in young cattle (62.5%) comparing with adult cattle (58.6%). This result in agreement with previous work performed by Amorim *et al* (2014), who demonstrated that the calves were more susceptible to infect with Babesia spp. compared with adult cows (Amorim *et al*., 2014). In contrast, this result was not in the line with the finding of Ayaz and co-worker, who reported a high prevalence rate in old animals 13.4% (61/452) compared with young animals 11.7% (48/409)

(Ayaz *et al* ., 2013). This variation can be because young animals have less rate of infestation with tick as compared to old animals. On the other hand low prevalence rate in young animals attributed to restricted grazing of young animals which in turn reduce their chance of contact with the vectors of this disease (Kamani *et al.*,2010).

Although, in the present study a slightly higher infection rate was recorded in male(65.3% 32/49) compared with female (58.2% 146/251), this variation was statistically not significant. This observation was in contrast with the report of Kocan *et al*(2010) who found higher prevalence rate of babesiosis in female cattle (11.2%) compared to male cattle (6.96%) (Kocan *et al*., 2010). Moreover, the higher prevalence of tick borne diseases in female animals may be due to the fact that female animals kept longer for breeding and milk production purposes (Kamani,2010) . On the other hand, the hormonal affect, milk production and breeding, which reduced the immune defence of the female (Kamani, 2010).

In present study, the cross breed were more infected by bovine Babesiosis (74%) as compared with local breed (52%). The statistical analysis was revealed a significant association between the infection with bovine Babesiosis and animal breed (P-value = 0.000). This result is in agreement with previous study conducted by Shuaib (Shuaib *et al* ., 2015) .They are found the highest prevalence in cross breed but the lowest prevalence estimated in local breed .

The prevalence of the disease based on the body condition of the animals was 58.1%, 59.4%, 64.3% for good, medium and poor scoring respectively with no significant association between disease and body condition (P-value= 0.837). This result is agreement with previous study conducted by (Hamsho *et al* ., 2015) . This could be due to the fact that animals with poor body condition have lower immunity defence which in turn increased the susceptibility of animal to get infection with different organisms including Babesia spp.

In the present study, prevalence of bovine babesiosis was recorded in different Area in the south Darfur state: slaughterhouse (54%), animal fauna (50%) and farms (74%). The high infection rate of bovine babesiosis in the farm, because in the farms the breeding is semi open system breeding and a mixed animal in one farm.

The result of Chi -square showed a significant association between the prevalence of bovine babesiosis and the Area (P-value=.001) . This result is in agreement with previous work, in which they reported a significant different in the prevalence of the disease according to the geographical region (Terkawi *et al* ., 2011). Several studies reported that humidity plays an important role in tick population density (L'Hostis and Seegers, 2002) . An increase in temperature may allow vectors to migrate into new areas or to allow a significant development of parasites (Coles, 2001 and El-Fayomy, 2013) .

In this study, the prevalence of Babesia infection within animal treated by one drug was 52% (104/200), while the prevalence of infection within animal treated by compound drug was 74% (74/100).Clearly, the infection rate was higher in the animals treated with more than one drug. There was a significant association between the infection rate and treatment (P-value= 0.000). This finding could be observed in area where the treatment of animals using anti-parasitic drugs (*babesiocide* drugs) was irregular and also the administration of drug was not clear (Heinz *et al* ., 2000).

There is no strategic treatment and application of preventive measures in the majority of livestock farm in Nyala town. Moreover, the animal owners use the drugs randomly without a clear plan. There are many reasons for that such as; the infrastructure defect of the veterinary services including the absent of proper slaughterhouses, the lack of the laboratory and facilities, wars, conflicts and financial problems. Furthermore, the absence of public awareness and the limitation of extension programmers in this area lead to increase the effect of tick-born disease. Recently after Darfur civil strife, changes on the livestock movements were noticed (Henry, 1978).

The prevalence of the disease according to the ticks infestation on cattle in the south Darfur state was 55.8% (43/77). This result is in agreement with Maiju (2005). It is well known that the number of tick increases in a relation to the humidity of claimed and the rain fall. Thus, ticks are widely distributed in the moderate to high rain fall season (Maiju, 2005).

As shown in the results 94.4% (168/178) of infected animals were infected with both Babesia and one of other blood parasite, while 5.6% (10/178) of infected animals were infected with Babesia only.There was a significant association between the infection with Bovine babesiosis and the presence of other blood parasites (p-value=0.030). This result is agreement with results reported by El Moghazy *et al* (2014). They found that mixed infection, using Giemsa-stained for blood smears of Babesia and Theileria spp .

It is well known that the microscopic examination considered as the "Gold standard test" for detecting a number of organisms in the blood of infected animals.

Conclusion:

This survey was thought to provide answers to the question about the prevalence and risk factor of Ambola (the local name for bovine babesiosis in South Darfur State).This study indicates that the overall prevalence of bovine babesiosis was 59.3%.

Strong significant association was observe between bovine babesiosis and breed and also between the diseases and the number drugs are used for treatment (p=0.000).

Our study showed a significant association between infection rate and area where the samples were collected (p-value=0.001) and also between the infection with babesia and infection with other blood parasite (p-value=0.030).

RECOMMENDATIONS

1-Structured studies must be conducted within national and regional programs, monitor the vector distribution and dynamics, the interplay of the different parasites and their virulence must be determined, and investigation of the carrier states.

2-Reproducible and simple-to-use tests should be developed and to establish collaboration between scientist to address the development, production and distribution of these techniques.

3-Studies on the productivity of cattle should be incorporated with the planning stages of future epidemiological and control programs for tick-borne diseases at both national and donor levels.

4-Using of strategic acaricide, exposure of young cattle to natural challenge, immunization with available vaccines and using of chemotherapy.

5-Live vaccines for tick-borne diseases.

6-The poor body condition scores that were observed during the dry season req uire the use of feed supplements, to maintain ahigh nutritional status in cattle throughout the year. Improved nutrition results in improved immunity to babesiosis and many cause adecrease in the clinical cases of disease druing the dry seasons.

Reference

Abou Laila, M ; Yokoyama, N and Igarashi, I(2010). Development and evaluation of two nested PCR assays for the Cattle through PCR. Pakistan J. Zool., 42(2): 201-204.

Agbede, R .I .S ; Kemp, D . Hand Hoyte, H . M .D (1986). Secretory and *Babesiabovis* ; light and electron microscope studies. In Morphology, Physiology and Behavioural Biology of Ticks (ed. Sauer, J. R. & Hair, A. J.), pp. 457–471. New York, Wiley and Sons.

Ahmad, N and Hashim, H (2007).A comparative study on the incidence of ticks and ticks borne diseases on local and crossbred cattle in Malakand Agency. J Anim Pl Sci 17: 56-58.

Alekaw, S . (2000) .Distribution of ticks and tick born diseases at Metekel ranch, Ethiopia. J Ethiop Vet Assoc 4: 40-60.

Amorim, L.S ; Wenceslau, A. A ; Carvalho, F. S; Carneiro, P.L. S and Albuquerque, G.R(2014). Bovine babesiosis and anaplasmosis complex: diagnosis and evaluationof the risk factors from Bahia, Brazil. Braz J Vet Parasitol Jaboticabal 23: 328-336.

Ayaz, S ; Shams, S ;Abdel-Reheem MAT, khan, S and Ullah, R (2013) . Epidemiology and molecular detection of *babesiosis* in household Dairies in Districts Kohat and Karak, Khyber Pakhtunkhwa Pakistan.Life Science Journal, 10(10s).

Barros, C.S .L and Fighera, R (2008).*Babesiosis*. In: Foreign animal diseases. 7th edition. Boca Raton, FL: United States Animal Health Association. p.147-158.

Bock, R .L ; Dackson, A.D.E vos and Jorgensen,W(2004). Babesiosis of cattle parasitology, 5247-5269.

Bose, R ; Jorgensen, W. K; Dalgliesh, R . J ; Friedhoff, K .T and de Vos , A . J (1995). Current state and future trends in the diagnosis of *babesiosis*. Vet Parasitol, 57:61–74.

Callow, L .L (1984). Piroplasms. In Animal Health in Australia, Protozoal and RickettsialDiseases , vol. 5. Animal Health in Australia , pp. 121–160. Canberra, Australian Bureau of Animal Health, AGPS.

Cadder, J ; Reddy, G;Chieves, L;Courtney, C and Little, R(1996) . Monitoring *Babesia bovis* infection in cattle by using PC based tests. J Clin Microbiol 34: 2748-2755.

Coles, G .C (2001) . The future of veterinary.Parasitol.,32: 601-610 .

Heinz Lüllmann, M . D ;Albrecht Ziegler, Ph. D and Klaus Mohr, M . D. Detlef Bieger , M . D (2000) . Color Atlas of Pharmacology 2nd edition, revised and expanded

CFSPH : Center for Food Security and Public Health(2008).*Bovine babesiosis*, Iowa state university, Ames, Iowa.

Dalgliesh , R . J(1993).*Babesiosis*. In Immunology and Molecular Biology of Parasite Infections (ed. Warren, S. K.), pp. 352–383. Oxford, Blackwell.

Demessie, Y and Derso, S(2015). Tick Borne Hemoparasitic Diseases of Ruminants: A Review. Advance in Biological Research, 9(4), 210-224.

DE VOS, A . J (1979) .Epidemiology and control of bovine babesiosis in South Africa. Journal of the South African Veterinary Association 50, 357–362.

DE VOS, A .J ; Dalgliesh, R.J. and Mcgregor, W (1986). Effect of imidocarbdipropionate prophylaxis on the infectivity and immunogenicity of a Babesiabovis vaccine in cattle. Australian Veterinary Journal 63, 174–178.

DE VOS, A . J (2004). Bovine babesiosis. In: COETZER, J.A.W. and TUSTN R.C. (eds). Infectious diseases of livestock (2nd edition). Cape Town: Oxford University Press: 406-424.

Dewaal, D.T(2006). Live vaccines against bovine babesiosis. Veterinary Parasitology 138: 88-96.

Di Cicco (2012) . Re-emergence of Babesiaconradae and effective treatment of infected dogs with atovaquone and azithromycin. Vet Parasitol. 8;187(1-2):23-7. PubMed PMID: 22305297.

Duh, D ; Punda-Polić , V ; Trilar, T and AvšičŽupanc, T (2008). Molecular detection of Theileria sp. in ticks and naturally infected sheep. Vet Parasitol, 151(2–4):327–331.

El-Ashker, M ; Hotzel, H ; Gwida, M ; El-Beskawy, M ; Silaghi, C and Tomaso, H (2015). Molecular biological identification of *Babesia, Theileria,* and Anaplasma species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray. Veterinary parasitology, 207(3), 329-334.

El-Fayomy, A .O ; Ghoneim, M .A ; Abu-Samak, A .O and Khidr, A .A (2013) . Contribution of *Babesia* to the Illness of Cows in Port Said Governorate, Egypt. Glob. Vet., 11: 118-122.

El Moghazy, H .M ; Ebied, M . M ; Abdelwahab, G . M and El Sayed, A . A (2014) . Epidemiological studies on bovine Babesiosis and Theileriosis in Qalubia governorate. BenhaVeterinary Medical Journal, 27: 36-48.

El Sawalhy, A .A (1999): "Veterinary Infectious Diseases" 2nd Edit. Ahram Distribution Agency, Egypt.Babes V. Sur l'hemoglobinuriebacterienne du boeuf. C R AcadSci 1888;107: 692-4.

Friedhoff, K .T (1988). Transmission of Babesia . In Babesiosis of Domestic Animals and Man (ed. Ristic, M.), pp. 23–52. Boca Raton, Florida, CRC Press, Inc.

Gough, J. M ., Jorgensen, W . K and Kemp, D . H (1998). Development of tick gut forms of *Babesia bigemina* in vitro . Journal of Eukaryotic Microbiology 45 , 298–306.

Gubbels, J .M ; de vos, A . P, Van der Weide, M;Viseras, J and Schouls, L. M (1999) . Simultaneous detection of *bovine Theileria and Babesia* species by reverse line blot hybridization. J Clin Microbiol 37: 1782-1789.

Hamsho, A ; Tesfamarym, G ; Megersa, G and Megersa, M (2015).A Cross-Sectional Study of *Bovine Babesiosis* in Teltele District, Borena Zone, Southern Ethiopia.Journal of Veterinary Science & Technology, 2015.

Henry, J (1978) . A Handbook of Veterinary Parasitology Domestic Animals Of North America Henry J. Griffiths Professor, Veterinary Pathobiology University of Minnesota

Herwaldt, B . L ; Linden, J .V ; Bosserman, E;Young, C ;Olkowska, D and Wilson, M(2011). Transfusion- associated babesiosis in the United States: a description of cases. Ann Intern Med 2011;155:509-19.

Homer, M , J ;Aguilar-Delfin, I;Telford, S . R ; Krause, P. J and Persing , D. H . M (2000). *Babesiosis*.ClinMicrobiol Rev 13: 451-469.

Hunfeld, K . P ; Hildebrandt, A and Gray, J . S (2008). – *Babesiosis*: recent insights into an ancient disease. Int. J. Parasitol., 38 (11), 1219–1237.

Hoyte, H. M (1961). Initial development of infectious *Babesia bigemina* . Australian Veterinary Journal 8 , 462–466.

Irwin (2010).*Canine babesiosis.* Vet Clin North Am Small AnimPract. 40(6):1141-56. PubMed PMID: 20933141.

Iseki, H ; Zhou, Z; Kim, C;Inpankaew, T ; Sununta, C ; Yokoyama, N ; Xuan, X ; Jittapalapong, S and Igarashi, I (2010). Seroprevalence of *Babesia* infections of dairy cows in northern Thailand. Vet Parasitol, 170:193-196.

Jabbar, A ;Abbas, T ; Sandhu, Z . U. D ;Saddiqi, H . A ; Qamar, M . F and Gasser, R .B (2015). Tick-borne diseases of bovines in Pakistan: major scope for future research and improved control. Parasit Vector, 8, 283.

Jorgensen, W. K;Bock, R . E ; Kingston, T. G ; DE VOS, A . J andWaldron , S. J (1993). Assessment of tetracycline and Babesia culture supernatant as prophylactics for moderating reactions in cattle to live Babesia and Anaplasma vaccines. Australian Veterinary Journal 70, 35–36.

Kahn, C .M .Ed (2005). Merck Veterinary Manual.Merck and Co. Inc. and Merial Ltd.

Kamani, J; Sannusi, A;Egwu, O . K ; Dogo, G. IandTanko, T . J (2010) . Prevalence and significance of haemoparasitic infections of cattle in North-Central, Nigeria. Vet World 3: 445-448.

Kocan, K .M; dela-Fuente, J ; Bouin, E . F ; Coetzee, J . F and Ewing, S .A (2010) . The natural history of Anaplasma marginale. Vet Parasitol 167: 95-107.

Krause, P. J ; McKay, K and Gadbaw, J (2003). Increasing health burden of human babesiosis in endemic sites.Am J Trop Med Hyg ;68:431-6.

Krause, P .J ; Telford, S , R III and Spielman, A (1996). Concurrent Lyme disease and babesiosis: evidence for increased severity and duration of illness. JAMA ;275:1657- 60.

Levine, N. D (1971) . Taxonomy of the piroplasms .Trans . Am. Microsc. Soc.90:2-33.

Levine, N. D ; Corliss, J . O and Cox, F. E . G (1980) . Anewly revised classification of the protozoa .J.Protozool.,1980,27:3758.

L'Hostis, M and Seegers, H (2002) . Tick-borne parasitic diseases in cattle: current knowledge and prospective risk analysis related to the ongoing evolution in French cattle farming systems. Vet. Res., 33: 599-611.

Mackenstedt,U ; Gauer, M ; Fuchs, P ; Zapf, F ; Schein, E and Mehlhorn, H (1995). DNA measurments reveal differences in the life cycles of *Babesiabigemina* and *Babesia canis* , tow tropical members of the genus *Babesia* parasitology Resesrsh 81,595.604.

M'Fadyean, J and Stockman, S (1911). A new species of piroplasm found in the blood of British cattle . J .comp .pathol. 24:340-354.

Mahoney, D . F (1974). The application of epizootiological principals in the control of Babesiosis in cattle. Bulletin of Office International Des Epizooties 81 , 123–138.

Mahoney, D . F and Ross, D . R (1972). Epizootiological factors in the control of bovine babesiosis. Australian Veterinary Journal 48 , 292–298.

Martin,S.W;Meek,A.HandWillebergv,P (1987).Veterinary epidemiology .Principles and methods. Iowa State University Press,Ames.

Matijatko, V ; Torti, M and Schetters , T. P (2012).*Canine babesiosis* in Europe: how many diseases? Trends Parasitol., 28 (3), 99–105.

Máthé, A ; Dobos-Kovács, M and Vörös, K (2007). Histological and ultrastructural studies of renal lesions in Babesiacanis infected dogs treated with imidocarb. Acta Vet. Hung., 55 (4), 511–523.

Maiju, J .K (2005). Ecological and epidemiological studies on ticks (Acari: Ixodidae) in Pibor area, Jonglei state. Sudan. M. Vet. Sci. Thesis, University of Khartoum, pp. 59-60.

Nasir, A. A; Hashmi, H. A and Afzal, M (2000). Prevalence of haemoparasites in exoticcattle. Int J Agri Biol 2(4): 402-403.

Nayel, M; El-Dakhly, K. M ; Aboulaila, M ;Elsify, A ; Hassan, H ;Ibrahim, E ;Salama, A and Yanai, T (2012). The use of different diagnostic tools for Babesia and Theileria parasites in cattle in Menofia, Egypt.Parasitol. Res., 111: 1019-1024.

Norval, R . A .I ; Barreit, J . C;Perry, B . D and Mukhebi, A .W (1991). EcGnomics, epidemiology and ecology: a multi-disciplinary approach to the planning and appraisal of tick and tick-borne diseases control in Southern Africa. In: Firaz, B. ed. Ticks and Tick Borne Diseases of Southern Africa. Grahams Town: Rhodes University, in preparation.

OIE :WorldOrganisation for Animal Health (2004). Bovine babesiosis. In: Manual of diagnostic tests and vaccine for terrestrial animals, 5th ed. (Web-page: http:// www.oie.int/fr/normes/mmanual/.../2.04.02_BOVINE_BABESIOSIS.pdf).

OIE :World Organisation for Animal Health (2008). Manual of diagnostic tests and vaccines [online]. Paris: OIE; 2008. Bovine babesiosis. Available at: http://www.oie.int/eng/normes/mmanual/2008/pdf/2.04.02_Bovinebabesiosis* Accessed 4 Dec 2008.

OIE : World Organisation for Animal Health (2010) Bovine Babesiosis, Terrestrial manual.

Patcharathorn, S ; Nantawan,Y;Nantiya, S ; Sinsamut, S and Nongnuch, P (2013) . Prevalence and factors associated with Babesia spp. infections of cattle raised nearby forest in Salakpra Wildlife Sanctuary, Kanchanaburi province.

Perry, B .D .(1996). Epidemiological indicators and their application to the control of tick-borne diseases. In Manual on Tick and Tick-borne Disease Control . Rome, FAO.

Phillip, D .Carter and Peter Rolls (2015).BVSc , MVS,Queensland Department of agriculture, fisheries and forestry's Tick fever center., BVSc, MVS,veterinary officer, tick fever center, Department of Agriculture, fisheries and forestry, Biosecurity Queesland.(2015).

Pipano, E ; Makovics, A ; Kriegel, Y ; Frank, M and Fish, L (1987) . Use of long-acting oxytetracycline inntheimmunisation of cattle against *Babesia bovis a*nd *B. bigemina*. Research in Veterinary Science 43, 64–66.

Pohl, A.E (2013). Epidemiology study of tick-borne diseases in cattle in Minas Gerais, Brazil (Doctoral dissertation, lmu).

Potgieter, F. T (1977). The life-cycle of *Babesia bovis and Babesia bigemina* in ticks and in cattle in South Africa. Ph. D. Thesis, Rand Afrikaans University, 1977.

Radostits, O. M; Gay,G . C ; Hinchiff,K . W ;and Constable,P. O (2007). Veterinary Medicine: A text book of the disease of cattle, sheep, goat, pigs and horses. 10 th Ed London: Saunders Elsevier, pp: 1110-1489,1527-1530.

Riek, R . F (1966). The life cycle of *Babesia*argentina . (Lignie ` res, 1903) (Sporozoa: Piroplasmidea) in the vector Boophilus microplus (Canestrini). Australian Journal of Agricultural Research 17 , 247–254.

Ruebush, T. K II ; Juranek, D . D; Chisholm, E . S ; Snow, P.C ; Healy, G . R and Sulzer, A .J (1977).*Human babesiosis* on Nantucket Island: evidence for self-limited and subclinical infections. N EnglJ ;297:825-7.

Saad, F ; Khan, K ; Ali, S and ul Akbar, N (2015). Zoonotic significance and Prophylactic Measure against babesiosis. Int. J. Curr. Microbiol. App. Sci, 4(7), 938-953.

Salih, D.A;El Hussein, A .M ; Seitzer, U and Ahmed, J.S (2007). Epidemiological studies on tick-borne diseases of cattle in Central Equatoria State, Southern Sudan. Parasitol. Res., 101,1035–1044.

Sahinduran, S. (2012) . Protozoan diseases in farm ruminants.In A Bird's Eye View of Veterinary Medicine.Perez Marin, C. C. (Ed). In. Tech. pp. 473-477. http://dx.doi. org/10.5772/30251.

Schetters, T. M . P ; Moubri, K ;Precigout, E ;Kleuskens, J ; Scholtes, N . C and Gorenflot, A (1997) . Different *Babesiacanis* isolates, different diseases. Parasitology, 115 (5), 485–493.

Sharma, A; Singla, L . D ; Tuli, A;Kaur, P ;Batth, B . K ;Javed, M and Juyal, P. D (2013). Molecular prevalence of Babesiabigemina and Trypanosomaevansi in dairy animals from Punjab, India, by duplex PCR: a step forward to the detection and management of concurrent latent infections. BioMed research international, 2013.

Shuaib, A .Y ;Osman, M . H ;Hussien, O . M ; Bakhiet, A . M ; Omer, A. R ;AL-Nahas, N ; Suliman, E . S ;Abdalla, A . M and Ismail, A . A (2015). Seroprevalence, antibodies, babesia, cattle, iELISA, Sudan.

Shams, S ;Ayaz, S ;Ali, I ; Khan, S ; Gul, I ; Gul, N and Khan, S . N (2013). Sensitivity and Specificity of PCR and Microscopy in detection of *Babesiosis* in domesticated cattle of Khyber Pakhtunkhwa, Pakistan. International Journal of Advancements in Research & Technology, 2, Issue 5, 37 – 41.

Sikorski (2010). Babesiosis caused by a large *Babesia* species in 7 immunocompromised dogs. J Vet Intern Med. 24(1):127-31. PubMed PMID: 20002547.

Spickler, A .R ; Roth, J . A and Dvorak, G (2010). Emerging and exotic diseases of animals, 4th ed CFSPH Iowa State University, lowa USA. Pp: 102-105

Spielman, A (1976) . *Human babesiosison* Nantucket Island: transmission by nymph al Ixodes ticks. Am J Trop Med Hyg ; 25:784-7.

Taylor, R . J. and Mchardy, N (1979). Preliminary observations on the combined use of imidocarb and Babesia blood vaccine in cattle. Journal of the South African Veterinary Association 50, 326–329.

Telford 3rd, S .R ;Gorenflot, A ; Brasseur, P and Spielman, A (1993). Babesial infection in human and wildlife. In: Kreier, J.P. (Ed.), Parasitic Protozoa, vol. 5. Academic Press, San Diego, CA, pp. 1–47.

Terkawi, M .A ; Thekisoe, M . O ; Katsande, C ;Latif, A .A ; Mans, J .B ; Matthee, O ;Mkize, N;Mabogoane, N ; Marais, F ; Yokoyama, N ; Xuan, X and Igarashi, I (2011) . Serological survey of Babesia bovis and Babesia bigemina in cattle in South Africa. Vet. Parasitol., 182: 337-342

Tokarz, R ; Jain, K;Bennett, A ; Briese, T and Lipkin, W . I (2010) . Assessment of polymicrobial infections in ticks in New York State. Vector Borne Zoonotic Dis 2010;10:217-21.

Thrusfield , M (2007). Veterinary Epidemiology.(3rd Edition). UK, Blackwell Science Ltd. pp 274 - 282.

Vannier, E ; Gewurz, B . E and Krause, P. J (2008) . Human babesiosis. Infect Dis Clin North Am 2008;22:469-88.

Vercammen, F; De Deken, R and Maes, L (1995) . Clinical and serological observations on experimental infections with Babesia canis and its diagnosis using the IFAT. Parasite, 2 (4), 407–410.

Vial, H . J and Gorenflot, A (2006). Chemotherapy against babesiosis. Vet. Parasitol., 138: 147-160.

Wagner, G .G ; Holman , P ;Waghela, S (2002). Babesiosis and heartwater: threats without boundaries. Vet Clin Food Anim 18:417–430. Yabsley, M.J., Shock, B.C. 2013. Natural history of Zoonotic Babesia: role of wildlife reservoirs. Int.J. Parasitol, 2:18–31.

Yabsley, M . J and Shock, B .C(2013). Natural history of Zoonotic Babesia: role of wildlife reservoirs. Int.J. Parasitol, 2:18–31.

Zanet, S; Trisciuoglio, A ; Bottero, E ; Fernández de Mera, I . G ; Gortazar, C; Carpignano, M .G and Ferroglio, E (2014). Piroplasmosis in wildlife: Babesia and Theileria affecting freeranging ungulates and carnivores in the Italian Alps. Parasites & Vectors, 7:70

Zintl, A., Mulcahy, G., Skerrett, H.E., Taylor, S.M., Gray, J.S., (2003). Babesia divergens, a bovine blood parasite of veterinary and zoonotic importance. Clin. Microbiol. Rev. 16, 622–636

Zulfiqar, S ; Shahnawaz, S ; Ali, M ; Bhutta, A . M ;Iqbal, S ;Hayat, S ; Qadir, S ; Latif, M ;Kiran, N ; Saeed, A ;Ali, M and Iqbal, F (2012). Detection of *Babesiabovis* in blood samples and its effect on the hematological and serum biochemical profile in large ruminants from Southern Punjab. Asian Pacific Journal of Tropical Biomedicine, 104-108.

Appendix

Questionnaire: Investigation of Bovine Babesiosis in South Darfur State.

Locality…………………………………………….date………………………

Herd Owner…………………………….…………Herd Code…………………………

Address………………………………………….……………………………………

The individual risk factors:

1-Cattle age group:(years):

Less 3( ) more 3( )

2-Cattle gender:

Male ( ) Female ( )

3-Cattle breed:

Local breed ( ) Cross breed ( )

4-Body condition:

Good ( ) Medium ( ) Poor ( )

5-Area:

Slaughterhouse ( ) Animal fauna ( ) Farm ( )

6-Treatment:

One drug ( ) Combination ( )

7-Tice infestation:

No ( ) Yes ( )

8-Other blood parasite:

No ( ) Yes ( )