Sudan University of Science and Technology

College of Graduate Studies

Prevelance and Some Risk Factors of Equine Piroplasmosis in Yassine Locality- Eastern Darfur State- Sudan

معدل انتشار بعض عوامل الخطر لمرض البابيروبلازما في الخيل في محلية ياسين- ولاية شرق دارفور- السودان

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DEDICATION

TO:

My parents

my sisters

my brothers

and

all my family

Acknowledgements

Thanks first and last to allah, who gives me the health and strength to make this work possible

I would like to express thanks to my supervisor, prof: Siham Elias for her advise and criticism in reviewing the dissertation.

Thanks to Sudan University of science and Technology, College of veterinary medicne , for giving me the chance to join the master degree of preventive veterinary medicine (M.P.V.M)

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Abstract

Cross-sectional study was conducted from march to April 2019 in Yassine locality,Eastern Darfure state , Sudan to investigate the prevelance of equine piroplasmosis infection in Horses and Donkeys and to assess the relationship between the occurrence of these equine piroplasmosis and Risk factors as age , sex, body condition, animals species, use of acaricides, tick infestation, animal activities and use of ivermectin in control of Ticks.

Atotal of 332 thin blood smears were prepared and stained with Giemsa stain and examined under microscopy. The samples were collected from five districts namely Yassine, Muhajiria, Sleaa, Kleakil and Om-alkhyrate for detection of *B. caballi* and *T. equi.* The results showed that *Babesia caballi* infection was more common than *Theilleria equi* infection in Horses and Donkeys and the prevelance of Babesia caballi in Horses and Donkeys were estimated 37.9% and 41.7% respectively. Prevelance of Theilleria equi in Horses and Donkeys were estimated 4.3% and 5.3% respectively. Univariate analysis by using Chi square test under significant level of P-value ≤0.5 showed positive association between prevelance of the disease and body condition score (X^2=4.419; P =0.036) and tick infestation (X^2=5.274; P=0.022). The tick infestation and body condition score are considering risk factors of equine piroplasmosis and confirmed that equine piroplasmosis was endemic in Yassine locality, Eastern Darfur state and the control of the disease should be focused of controlling of ticks and treatment of carrier animals

ملخص

اجريت دراسه مقطعيه من شهر مارس الي شهر ابريل من العام2019 وذلك لتقدير معدل انتشار مرض البايروبلازما في الخيل والحمير وتقييم العلاقه بين حدوث المرض0 وبعض عوامل الخطر كالعمر، الجنس ،حاله الجسم، نوع الحيوان، استخدام قاتلات الحشرات، وجود القراد علي الحيوان، نشاط الحيوان واستخدام الايفرمكتين للتحكم في القراد.

تم جمع 332 عينة شريحة دم رفيعه تم تجهيزها وصبغها بصبغة جرام وفحصت تحت المجهر الضوئى حيث تم جمع العينات من خمس مناطق وهي ياسين، صليعه، كليكل، ام الخيرات ومهاجريه وذلك للتقصي عن وجود طفيل البابيزيا العملاقه والثايليريا الخيليه والتنيجه اظهرت وجود البابيزيا العملاقه اكثر انتشارا من الثايليريا الخيليه حيث ان معدل انتشار البابيزيا العملاقه في الخيل والحمير تمثل 37% و41% على التوالي. ومعدل انتشار الثايليريا الخيليه في الخيل والحمير حوالي 4,3% و5,3% علي التوالي.

التحليل الفردي باستخدام مربع كاي تحت قيمه معنويه اقل من او يساوي 0.05 اظهرت علاقه موجبه بين معدل انتشار المرض وبين الحاله الجسمانيه(X^2=4.419; P = 0.036) ووجود القراد (X^2= 5.274; P= 0.022).

حالة الجسم ووجود القراد على الحيوان يعتبران عوامل خطر للاصابه بالبايروبلازما الخيليه

والدراسه اكدت ايضا ان داء البايروبلازما الخيليه مستوطن في محلية ياسين والتحكم في المرض يجب ان ترتكز على التحكم في القراد وعلاج الحيوانات الحامله للمرض.

INTRODUCTION

Equine piroplasmosis is a tick borne protozoal disease of horses, mules, donkeys, zebra (OIE,2014) caused by *Apicomplexan haemoprotozoan* parasites *Theileria equi* and *Babesia caballi* of the order Piroplasmida (Jesca *et al.*,2017). *Babesia equi* isassigned to the genus *Babesia* until Mehlhom and Scheinin 1998 transferred it to the genus *Theileria* on the basis of lifecycle and genetic differences. Certain ticks such as *Dermacenter, Hyalomma* and *Rhipicephalus* are biological vectors in which the parasites amplifies and then is transmitted to horses in certain conditions (Osman *et al.*,2016*).*Ticks are reservoir of infection with *B.caballi* because may persist in ticks throughout several generations, with transtadial and transovarial transmission, the disease can also be transmitted directly between animals by contaminated needles and syringes or by blood transfusions (CFSPH,2008, Friedhoff and Soule, 1996) The vectors of *Theileria equi* and *Babesia caballi* are the same although*T.equi* is more virulent than *B.caballi (*Jesca *et al.*,2017*).* The disease can be diagnosis by clinical signs but may confuse due to variety of other conditions, microscopic examination is known to be low sensitivity, serological test are more reliable and capable to detect antibodies against the parasites. Equine Piroplasmosis causes serious health effects to horse specially with respect to agricultural production including low working capacity, high cost of the control measures and impact on the transport of goods and international trade (Ming Wang *et al.*, 2013). Prevalence of equine piroplasmosis among horses and donkeys in the Sudan was 35.95% by using molecular technique (Salim *et al.*,2013). Osman *et al*. (2016) reported the prevalence of equine piroplasmosis in south Darfur by using microscopic examination (0.34%) *Babesia* and (2.04%) *Theileria*

Objectives:

1- To determine the prevalence of the equine piroplasmosis in Yassine locality

2-To investigate the risk factors associated with disease in Yassine locality.

CHAPTER ONE

LITERATURE REVIEW

1.1 Definition of equine piroplasmosis

Equine piroplasmosis is Tickborne disease of Equids (horses, donkeys, mules and zebra).The disease is caused by protozoan parasites belonging to the genera *Babesia* and *Theileria*, these two species responsible for causing EP are *Babesia cabali* and *Theilleria equi*. Theyare both transmitted by tick species belonging to the several genera such as *Hyalomma*, *Rhipicephalus* and *Dermacenter*. Infected animals may remain carrier for a long period and serve as source of infection to the vector (Thankgod *et al*., 2019).

1.2 History

Before 1901, equine piroplasmosis was not recognized as distinct disease and was often confused with other diseases. It was first reported in 1907 by Oliver in Sudan (Abdoon, 1984). Earlier it was also referred to as Anthrax fever. In the cape, a homogeneous condition was perceived and named biliary fever by Hutcheon. Nunn considered this condition as the bilious form of African horse sickness. However, in West Africa similar disease was described as equine malaria. In 1901-1902 it was revealed by Theiler that biliary fever is not similar to African horse sickness but may occur concurrently to it (Deepak, 2015).

1.3 Taxonomy of *Babesiacaballi* and *Theileriaequi*

Babesiacaballi and *Theileriaequi* belong to the genera *Babesia* and *Theileria*, and the families *Babisiidae* and *Theileridae*, respectively, Order *Piroplasmida*, Subclass *Piroplasmia*, Class *Sporozoa* and Phylum *Apicomplexa* (Soulsby 1982) (Table 1.1)

Table1.1 Taxonomy of the Protozoal Parasites that affecting equines:

Phylum	Apicomplexa
Class	Sporozoa
Subclass	Piroplasmia
Order	Piroplasmida
Family -1	Babesiidae
Genus	*Babesia*
Species	*B. caballi*
Family-2	Theileriidae
Genus	*Theileria*
Species	*T. equi*

1.4 Life Cycle and Transmission

*B.caballi*sporozoites invade red blood cells(RBCs) and transform into trophozoites which grow and divided into two round, oval, or pear shaped merozoites which in turn are capable of infecting new RBCs.*T. equi* inoculated via tick bite invade the lymphocytes and undergo development and eventually form theileria-like schizonts, merozoites released from these schizonts invade RBCs and transform into trophozoites which grow

and divide into pear-shaped tetrad merozoites(OIE., 2009) Fig.(1)

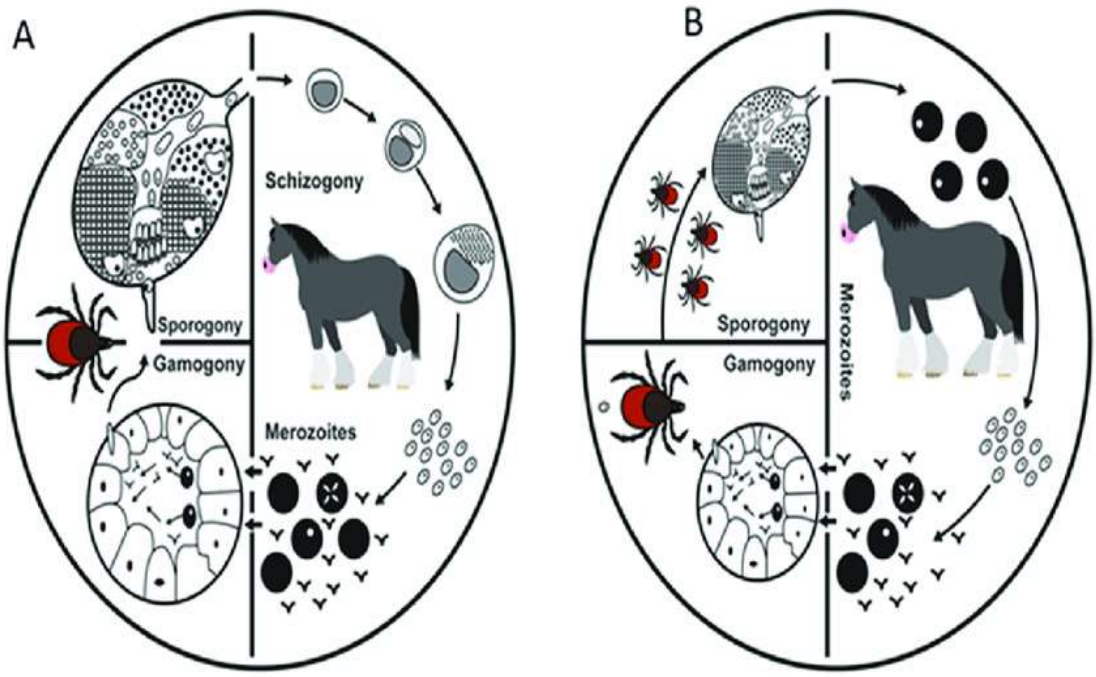

Figure 1 life cycle of (A) *Babesia caballi* and (B)*Theileria equi*i llustrated by MassaroUtei (Thankgod *et al*., 2019)

1.5 Clinical sign

The incubation period, ranges from 10 to 30 days for B. caballi and 12 to 19 days for *T. equi*. Clinical signs of equine piroplasmosis is characterized by fever, anaemia, icterus, hepatosplenomegly, intravascular hemolysis, ventral oedema, pale mucus membrane, dark urine, reduce feed intake and mild colic with reduced faecal output (Laus *et al.*,2013). The course of the disease may be peracute, acute or chronic. Per acute and acute may include fever, jaundice, anemia, hemoglobinuria, bilirubinuria, digestive or respiratory signs and occasionally death. Equids with subacutepiroplasmosis may display anorexia, lethargy, weight loss, anemia, limp edema, poor performance, increased heard and repiratory rate and splenomegaly. Chronic piroplasmosis is clinically indistinguishable from other chronic inflammatory disease and generally presents with nonspecific signs such as inappetence, poor body condition,

poor performance, anemia may be minimal or absent in equid with chronic or persistent infection (Rothschild and Knowles, 2007).Infected animals that recover from the acute infection may remain carriers of these blood parasites for long periods of time and act as sources ofinfection for tick vectors (OIE.,2014), It has been reported that *B. cabali* can be transmitted by intrauterine infection, leading to abortion or neonatal infection , but how often this occurs is not well documented. In some regions of the world where infection is common little or no clinical disease may be observed in native horses however , disease is frequently observed in adult horses suddenly introduced into areas with large numbers of infected ticks (Josie *et al* ., 2010).

1.6 Geographical distribution

The diseases are worldwide distributed and may endemic in tropical and subtropical area as well as in some temperate zone of the world.The equine piroplasmosisoccur in southern Europe, Asia countries of the common wealth of independent states, Africa, Cuba, south and centralAmerica, and certain parts of the southern united states of America. *Theileria equi* has been reported from Australia and is now believed to have a wider general distribution than *Babesia caballi* (OIE.,2014).Seroprevalences of equine piroplasmosis in China demonstrate that about 51.1%and 11.51% samples were positive for *B. caballi* and *T. equi* infection respectively. According to this result the equine piroplasmosis widespread in china (Ming *et al*.,2013). In Spain the overall seroprevalence were 52% consisting of 44% seropositive for *T. equi* and 2.1% for *B. Caballi* (Maria *et al*.,2017). In Africa Oduori *et al* . (2015) found that the overall seroprevalence of *T. equi* in donkeys from Nuudivision, Kenya was 81.2% using competitive ELIZA (cELIZA), while antibodies against *B.caballi* were not detected.in south Darfur state , Sudan parasitological and molecular detection of equine piroplasmosis

in horses and donkeys. Blood smears examination revealed that *B. caballi* was only seen in donkeys at Nyala locality but*T. equi* was seen in four (2.14%) and six (2.04%) horses and donkeys samples respectively (Osman *et al.*, 2016).

1.7 Diagnosis of disease

Equine piroplasmosis can be diagnosed by clinical signs and laboratory examination such as serological tests, molecular diagnosis and inoculation in the animals. So the clinical diagnosis is based onobservation of clinical signs and the microscopic examination but the clinical signs may confused with other diseases, so that the serological tests include complement fixation test, indirect fluorescent antibody test and enzyme link immunoassorbent assay are more reliable and capable of detecting antibodies against parasites but these tests do not distinguish between past and current infection *(*OIE,2014*;* Maria *et al.,* 2017 and Yahya,2018*).* It is impossible to differentiate between *T. equi* and *B.caballi* infections on the basis of clinical signs. So the definitive diagnosis depends on the identification of *B.caballi* and *T. equi*in blood smears stained by Giemsa or by Acridine orange (Salim *et al.*,2008).

1.7.1 Clinical Diagnosis

The clinical diagnosis of the equine piroplasmosis can be easily confused with other diseases because the signs of equine piroplasmosis are often nonspecific. The disease should be differentiated from surra , equine infectousanemia, daurine, African horse sickness, purpurahaemorrhagica and plant and chemical toxicities (OIE,2014)

1.7.2 Microscopic examination

Infected horses may be identified by demonstrating the parasite in stained blood collecting from superficial skin capilleries, Jugular vein or organ smears during the acute phase of the disease using Romanovsky-type staining methods, such as Giemsa. Stained blood smear with

Giemsausually give the best result. In acute clinical cases of *B. caballi* infection, the parasitaemia is very low and difficult to detect so that experienced workers sometimes use thick blood smear technique to detect very low Parasitaemia. Paired merozoites joined at the posterior ends are a characteristic feature of *B. caballi*, while the arrangement of the pear shaped merozoites forming a tetrad known as a (Maltes cross) is the characteristic one of *T.equi* (OIE,2014) **(**Fig2.2)

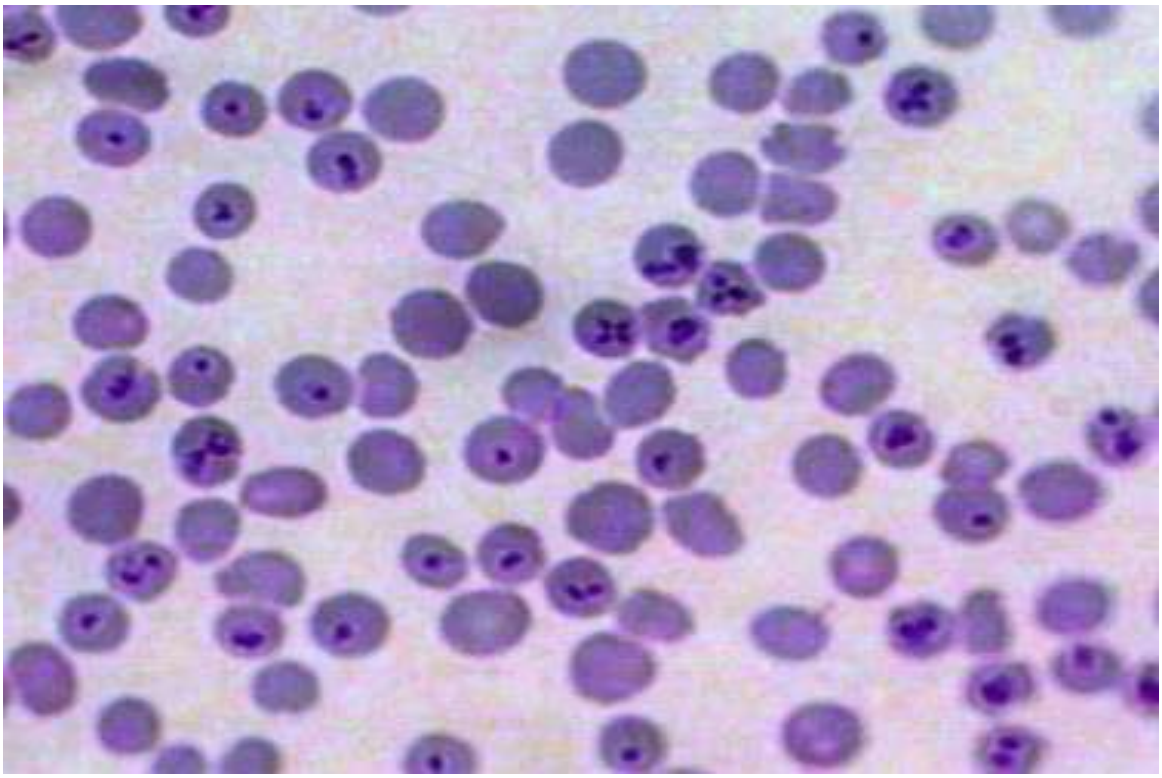

Figure2 Morphology of *Babesiacaballi* piroplasm under microscopic examination stained by Giemsa (Wise *et al*., (2013)

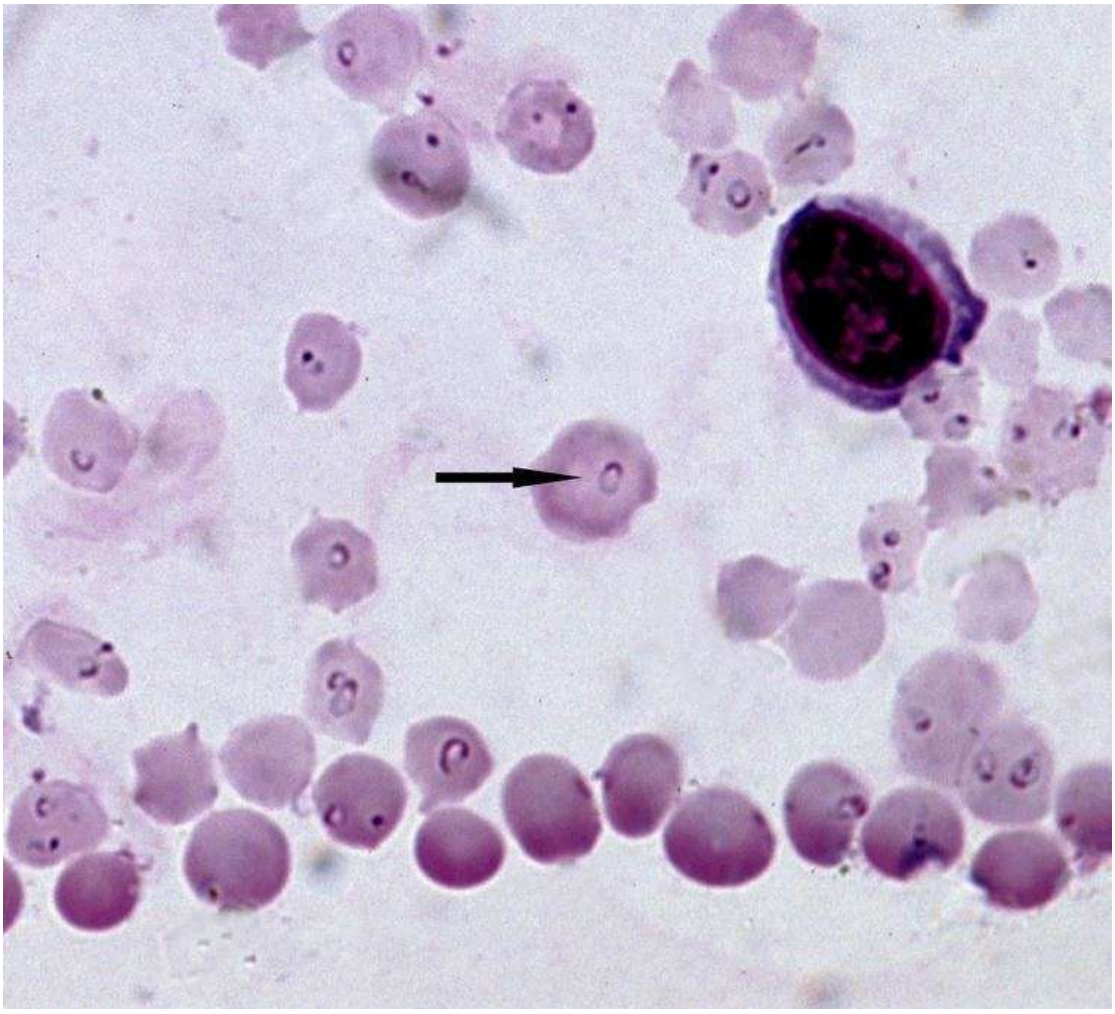

Figure 3 Morphology of *Theileria equi* piroplasm under microscopic examination by using giemsa (Wise *et al*., 2013)

1.7.3Serological tests

Enzyme-linked immunoassorbent assay, indirectflourecent antibody test and CFT are recommended and successful differential diagnosis of piroplasmosis. In addition, a simple and rapid immunochromatographic test for *T. equi* has also recently been described and might be a very useful test for the mass screening of serum samples (OIE, 2014)

1.7.3.1 Enzyme- linked immune assorbant assay

Number of recombinant antigens for the use in ELISA have been described recombinant *T. equi* (EMA-1; EMA-2) and *B. caballi* proteins (RAP-1; Bc48). Indirect ELISA using EMA-2 and BC48 have shown

high sensitivity and specificity in detecting antibodies in infected horses (OIE.,2014).

1.7.3.2 Complement fixation test

The test is accurate for detection of early infections only for which purpose it showed good sensitivity and specificity but it may not identity all infected animals especially those that have been drug-treated or that produce anti-complementary reaction , or because of the inability of IGg to fix guinea- pig complement (OIE.,2014).

1.7.3.3 Indirect fluorescent antibody test

The IFAT has been successfully applied to differentiate of *T.equi* and *B. caballi* infections. One challenge with the IFAT is the need to dilute sera to reduce non-specific binding and subsequent background, which may preclude identification of the intra-erythrocytic parasites (OIE.,2014).

1.7.4 Molecular Technique

Molecular technique considered a highly sensitivity and specificity to detect the pathogens so polymerase chain reaction (PCR) test targeting the 18s r RNA gene as well as BC48(*B. caballi*) and EMA-1(*T. equi*) genes are available for molecular detection (OIE.,2014)

1.8 Treatment

Treatment of equine piroplasmosis is not well established but many drug have been used, but combination of imidocarb and buparvaquone appears to be the only efficient treatment capable of eliminating *T. equi*infection. *B. capalli* is more susceptible but it is expected to develop resistance quickly (Radostits *et al.*, 2006). Diminazene aceturate and diaminazene diaceturate have been used with success against *T. Equi* and *B. Caballi* at a dose of 3.5mg/kg IM every 48 hours for 2 treatments. Diminazene aceturate is more effective than diaminazene diaceturate, but both drugs have been reported to cause significant injection site muscle damage.

Efficacy of both drugs increases with the 2nd dosage. Signs of toxicity include respiratory distress and lethargy (Wise *et al*., 2013).

1.9Prevention and control

The control measures of equine piroplasmosis require effective diagnosis that can detect carrier or chronically infected animals *(*Ming *et al.*,2013*).* Imported horses must be quarantined and tested for evidence of piroplasmosis and the causative organisms(OIE, 2009*).* Application of acaricides before removal from the endemic nation is used to ensure that ticks are not introduced with the horses. In the United states, a horse that is identified as positive on cELIZA must be immediately quarantined and the state and federal authorities must be notified. Until a reliable means of sterilization is identified, these horses must remain quarantined, be exported, donated to research facilities, or euthanized. Non endemic nations that border endemic nations cannot completely prevent introduction of ticks, so diligent measures must be taken to reduce horses contact with ticks. This includes routines application of acaricides, surveillance for the presence of ticks, and reduction in vegetation (Wise *et al*., 2013).

CHAPTER TWO

Material and Methods

2-1 Study area:

This study was conducted in period from March to April 2019 in eastern Darfur state that is located in south western Sudan. The area is located between latitude 10-13 degree North and longitude25-27 degree East.The mean annual rainfull about 250-300 mm. in the North and 800-1000 in South. The area is bordered from the east with western Kordofan state, from the north with Northern Darfur state, from the west with South Darfur state and from the south republic of South Sudan (Annual report 2018).

2-2 Study design and sample size

Cross-sectional design was conducted and samples calculated to achieve the study objectives. Calculation of sample size by using 27.4% expected prevalence (Yahya, 2018) and 95% level of confidence interval with 5%desired absolute precision according to the formula of Thrusfield (2007).

Formula: $n = 1.96^2 * P_{exp}(1-P_{exp})/d^2$

Where n = required sample size

1.96= constant

P_{exp} = expected prevalence

D = desired absolute precision

2.3 Animals

Multistage random sample used to collect blood samples from equines. The study animals play multifunctional roles in their production system such as riding and draught. In the current survey, the questions of the owners were about animal species, age of the animal, breed, body condition, use of acaricide for eradication of tick infestation and use of ivermectin for controlling of external and internal parasites (Henneke *et al*.,1983). The location of study wereYassine, Muhajiria, Sileaa, Kileakil and Om-alkhyrate.

2-4 Blood sample collection:

Three hundred and thirty two blood samples was collected from horses(163 samples) and donkeys(169 samples) from both sexes in all breeds with different ages from jugular vein by dispossiplesyrings and immediatly smeared and fixed by methanolalcohol and then transferred to theNyala Central veterinary and research laboratory. The smeared slides (332 samples) were stained by 10% Giemsa for 30 minutes. Allslides were examined under 100x oil immersion lens of light microscope for identification of parasites (OIE.,2014)

2.5 Statistical analysis

Statistical package for social sciences (SPSS) version 16.0 software was used. Prevalence's and frequencies were calculated as percentages. Chi square test was used to identify associated risk factor with piroplasmosis and the level of significance was set as p-value <0.05

CHAPTER THREE

RESULT

3.1 Prevalence of equine Piroplasmosis in horses and donkeys in Yassine locality:

Atotal of 332 of blood sample of horse and donkey were examined microscopically for the presence of *Babesia caballi* and *Theileria equi* in Yassine locality. An overall prevalence of *Babesia caballi* infection and *Theileria equi* infection in horses and donkeys were 39.8% (132/332) and 4.8%(16/332) respectively.According to the geographical distribution out of 332 examined horses and donkeys the infection rate was showed the highest prevalence of *Babesia caballi* in both species was found in Muhajiria 47.1% (33/70) followed by Kileakil 45.8%(38/83), Sileaa 44.4%(24/54),Yassine 30.8%(28/91) and the lowest one Om-alkhyrate 26.5%(9/34)(table2). The prevalence of *Theileria equi* was found highest in Sileaa(14.8%) flowed by Muhajiria(5.7%), Yassine(3.3%) and Kleakil (1.2%) (Table 3.1).

3.2 Association between some risk factors and prevalence of *Babesi acaballi* infection and *Theileria equi* infection in Yassine locality.

In this study a total of 332 blood samples were obtained (169 donkeys and 163 horses) in 5 districtsin study area and tested with Giemsa stain. Overall prevalence of *Babesia caballi* in horses and donkeys in the study area was 37.9% (64/169)and 41.7% (68/163) respectively (Table3.2).

The prevalence of *Theileria equi* in horses and donkeys were 4.3% (7/163) and 5.3% (9/169) (Table3.3).

Out of 332 examined equines(donkeys and horses) 88% (292/332) males and 12% (40/332) females. The prevalence of *Babesia caballi* was38.7(113/292) in males and 47.5(19/40) in females. The prevalence of

Babesia caballi showed no association between male and female (X^2=.1.138,p=.286) (Table3.2).

The prevalence of *Theileria equi* infection in males and female swere found 14(4.8%) and 2(5%) respectively. Chi square test indicated that no association in the prevalence of *Theileri aequi* infection between male and female (x^2= .003; P = .955)(Table3.3)

Based on the result out of 332 examined horses and donkeys 22.3%(74/332) animals were less than 5years old, 47.9%(159/332) from 6 to 10 years old and 29.8%(99/332) more than 10 years old. The prevalence of *Babesia caballi* infection were 29(39.2%) in less than 5 years old, 62(39%) from 6 to 10 years old and 41(41.4%) more than 10 years old (Table3.2). Chi square test showed no association between the prevalence of *Babesia caballi* infection and different age groups (X^2=.162; p-= .922).

The prevalence of *Theileria equi* infection in three age groups were 5.4%,4.4% and 5.1% respectively (Table3.3). The result demonstrated no association between the prevalence of *theileria equi* infection and different age groups (X^2 = .127; P = .938).

The breeds of these animals were local breed 242(242/332) animals, foreign breed 46(46/332) animals and crossbreed 44(44/332) animals. The infection rate of these animals by *Babesia caballi* were 42.1%, 34.8% and 31.8% (Table3.2). Therefore was no association between the prevalence of *Babesia cabali* infection and breed of tested equines (x^2= 2.211; P = .331).

The prevalence of *Theileria equi* infection in local, foreign and crossbred were found 12(5%), 2(4.3%) and 2 (4.5%) respectively (Table3.3). Chi square test showed no association between the prevalence of *Theileria equi* infection and different breed (X^2= .040;P= .980).

Surveyed horses and donkeys about 62% were good body condition while 38% were poor body condition. Prevalence of *Babesia caballi* infection in good body condition and poor body condition were 91(44.2%) and 41(32.5%) animals respectively (Table3.2). Therefore was found association between the prevalence of *Babesia caballi* infection and body condition score (X^2 = 4.419; p = .033).

The prevalence of *Theileria equi* infection in poor and good body condition were 12(5.8%) and 4(3.2%) respectively (Table3.3). The result of Chi square test showed no association between the prevalence of *Theileria equi* infection and body condition score (X^2 = 1.197; P = .274).

Prevalence of *Babesia caballi* infection was 31(36%) in study animals that were no use of acaricides for controlling of ticks infestation and 101(41.1%) was use of acaricides (Table3.2). Chi squared test was showed no association between the prevalence of *Babesia caballi* infection and the use of acaricides (X^2 = .668; p = .414).

the prevalence of *Theileria equi* infection in that animals exposed acaricides that used as tick chemical control, but the animals not exposed were13 (5.3%) and 3(3.5%) respectively (Table3.3). There was no association between the prevalence of *Theileria equi* infection and usingacaricides(X^2 = .448; P = .503).

Prevalence of *Babesia caballi* infection was 41(50.6%) in animals that infested by ticks and 91(36.3%)that were not infested by ticks (Table3.2). Chi squared test demonstrated association between the prevalence of *Babesia caballi* infection and tick infestation (X^2 = 5.274, P= .018).

The prevalence of *Theileria equi* infection was 3(3.7%) in animals that infested by ticks and 13(5.2%) that not infested by ticks(Table3.3). Chi square test showed no association between the prevalence of *Theileria equi* infection and Tick infestation(X^2 = .291; P = .590).

Examined animals used for riding were 23.8%,whereas animals used for draught were 76.2%. The prevalence of *Babesia caballi* in draught and riding was 97(38.3%) and 35(44.3%) respectively (table3.2). Chi squared tested showed no association between prevalence of the disease infection and purpose of using of the animals (X^2= 0.894; P= 0.344).

The prevalence of *Theileria equi* infection in riding and draught animals were 3(3.8%) and 13(5.1%) respectively(Table3.3). The result of Chi square test demonstrated no association between the prevalence of *Theileria equi* infection and purpose of using of the animals (X^2= 1.271; P= .260).

Prevalence of *Babesia caballi* infection was 36.8% in animals that were not subjected to ivermectin, while the prevalence of *Babesia caballi* infection was44.9% in animals that were subjected to ivermectin that used against internal and external parasites (Table3.2). Chi square test showed no association between the prevalence of *Babesia caballi* infection and use ofivermectin in the previous treatment (X^2 = 2.151; P = 0.142).

Prevalence of *Theileria equi* infection was 5.9%in animal that were not subjected to ivemectin, while the prevalence of *Theileria equi* infection was 3.1%(4/57) in animal that were subjected to ivermectin (Table3.3). According to Chi square test showed no association between the prevalence of *Theileriaequi* infection and previous treatment by ivermectin (X^2 = 1.271; P = 0.260).

3.3 Multivariate analysis

Multivariate analysis showed significant association between Babesiacaballi infection and body condition and tick infestations (Table3.4)

(Table3.1): Prevalence of*Babesiacaballi* and *Theileriaequi* infection in Yassine locality, Eastern Darfur State

Area	No. examined	No. infected		Prevalences %	
		T.equi	*B. caballi*	*T.equi*	*B.equi*
Yassine	91	3	28	3.3	30.8
Muhajiria	70	4	33	5.7	47.1
Sileaa	54	8	24	14.8	44.4
Kleakl	83	1	39	1.2	45.8
Om-alkhyrate	34	0	9	0	26.5
Total	332	16	133	4.8	39.8

Table3.2 Association between some risk factors and prevalence of *Babesia caballi* infection in horses and donkeys in Yassine locality, Eastern Darfur State

Factor	No.examined	No.infected	DF	X^2	p-value
Species					
Horsess	169	64(37.9%)	1	.513	.474
Donkeys	163	68(41.7%)			
Age					
>5year	74	29(39.2%)			
5-10year	159	62(39%)	2	.162	.922
<10	99	41(41.4%)			
Sex					
Male	292	113(38.7%)	1	1.138	.286
Female	40	19(47.5)			
Breed					
Local	242	102(42.1%)			
Foreign	46	16(34.8%)	1	4.419	.036*
Crossbred	44	14(31.8%)			

Table 3.2 (continued)

Body condition					
Good	206	91(44.2%)	1	4.419	.036*
Poor	126	41(32.5%)			
Acaricides					
Yes	246	101(41.1%)	1	.668	.414
No	86	31(36%)			
Tick infestation					
Yes	81	41(50.6%)	1	5.274	.022*
No	251	91(36.3%)			
Animal purpose					
Riding	79	35(44.3%)	1	.894	.344
Draught	253	97(38.3%)			
Use of ivermectin					
Yes	127	57(44.9%)	1	2.151	.142
No	204	75(36.8%)			

Table 3.3 Association between some risk factors and prevalence of*Theileriaequi* infection in Yassine locality, Eastern Darfur

Factor	**No.examined**	**No.infected**	**Df**	**X^2**	**p-value**
Species					
Horse	163	7(4.3%)	1	.192	.661
Donkey	169	9(5.3%)			
Age					
>5years	74	4(5.4%)			
5-10years	159	7(4.4%)	2	.127	.938
<10years	99	5(5.1%)			
Sex					
Male	292	14(4.8%)	1	.003	.955
Female	40	2(5%)			
Breed					
Local	242	12(5%)			
Foregn	46	2(4.3%)	2	.04	.980
Crossbred	44	2(4.5%)			

Table 3.2 (continued)

Body condition					
Good	206	12(5.8%)	1	1.197	.274
poor	126	4(3.2%)			
Acaricides					
Yes	246	13(5.3%)	1	.448	.503
no	86	3(3.5%)			
Tick infestation					
Yes	81	3(3.7%)	1	.291	.590
no	251	13(5.2%)			
Animal purposes					
Riding	79	3(3.8%)	1	.236	.627
draught	253	13(5.1%)			
Use of ivermectin					
Yes	127	4(3.1%)	1	1.271	.260
no	204	12(5.9%)			

Table 3.4 Summery of multivariate analysis for association between some risk factor and prevalence of *Babesiacaballi* in horses and donkeys in Yassine locality

Risk Factor	**Exp(B)**	**p-value**
Body condition	1.670	0.033
Tick infestation	1.855	0.018
Use of ivermectin	0.421	0.186

Chapter Four

Discussion

The current study was conducted in the period from March to April 2019 to investigate the prevalence and some risk factor for equine piroplasmosis in horse and donkeys inYassine locality, Eastern Darfur using Giemsa stain. According to our investigation equine piroplasmosis was prevalent in all places within Yassine locality with an overall prevalence of *Babesia caballi* and *Theileria equi* infection were 39.8%(133/332) and 4.8%(16/332) respectively. This prevalence was higher than that reported by Osman *et al*. (2016) who found only one sample was positive (0.34%) in sick donkeys at Nyala locality and *Theileria equi* piroplasms were seen in 2.14%and 2.04% in horse and donkey samples respectively by Giemsa staining Technique. The result was lower than reported by Salim *et al*. (2013) who detected the prevalence of equine piroplasmosis in Ed-alfirsan (41.7%) and Tulus(43.5%) by PCR amplification of the 18s rRNA gene of both *Theileria equi* and *Babesia caballi*. Prevalence of equine piroplasmosis in other countries has been reported in Sao Paulo ahigher over all seroprevalence of *B. Caballi* was 54.1 than *T. equi* 21.6 (Claudia *et al*., 2009).The infection rate was 19.5% in working donkeys of central Ethiopia (Tesfie *et al*., 2018), 100% examined donkeys was positive for *T. equi* and 100% negative for *B.caballi* by serology in Caramoja sub-region Uganda(Jesca *et al*., 2017) these differences could be due to difference in geographical distribution and diagnostic test.

According to our surveys,no significant association between equine piroplasmosis and species of the animals(X^2=.513; P= .474) (X^2= .192; P^2=.661) for *Babesia* and *Theileria* respectively. Prevalence of *B.caballi*

and *T. Equi* piroplasms were lowerin horses37.9% and 4.3% respectively than donkeys 41.7% and 5.3% respectively.

In the present survey, no significant association between the prevalence of *B. Caballi* piroplasms and ages groups (X^2 = .162; P = .922). However , the prevalence of *B. caballi* infection was higher in age group more than 10years old followed by age group less than 5years old (39.2%) and from 5-10years old (39%). Benfenacti *et al*. (2016) in Algiera urban area using cELISA and microscopic examination, reported that ages groups had significant effect on the prevalence of *Theileria equi* infection.

In the current study, no significant association between the prevalence of *T. equi* and age groups (X^2 = .127; P = .938). The result showed that a higher prevalence in age group less than 5years old(5.4%) followed by age group more than 10 years old (5.1%) and 5-10years old (4.4%). These results was disagree with Yahya (2018) who showed that prevalence of equine piroplasmosisin different age groups was statistically different.

The results demonstrated that no significant association between the prevalence of *B. caballi* infection and sexes of animals (X^2 = 1.138; P = .286). The prevalence of infection was ahigher in females (47.5%) than males (38.7%). These result are similar to Tesfie *et al*. (2018) in Ethiopia who showed that both sexes are equally susceptible and exposed to this disease and disagreement with Benfanakti *et al*. (2016) who, reported that sexes had significant effect on the prevalence of *Theileria equi* infection.

In this observational study, no significant association between the prevalence of *T.equi* infection and sexes animals (X^2 = .003; P = .955).The prevalence of *T.equi* in females(5%) was higher than males(4.8%).

Regarding breed of animal, no significant association between *B. caballi*infection and breed (X^2= 2.211; P= .331), however the prevalence of *B.caballi* infection was higher in local breed (42.1%) compared with

foreign breed(34.8%) and crossbred (31.8%).Also no significant association between prevalence of *T. equi* and breed of animals. Both results were similar to reportin Khartoum stateof local breed of horses (Yahya, 2018).

Our result showed significant difference in the prevalence of *B.caballi* infection among body condition score (X^2 = 4.419; P = .036). Prevalence of *B. caballi* infection was higher in poor body condition (44.2%) than good body condition(32.5%). But no significant association between *T. equi* infection and body condition score (X^2 = 1.179; P = .274).Previous investigations of Liv *et al.* (2010) in horse in Switzerland and Hawkins *et al.*(2015) in Grevy's zebras and donkeys in Kenya confirmed that there was no association between equine piroplasmosis and body condition.

Our results showed no significant association between the prevalence of *B. caballi* infection and animal activities (X^2 = .894; P = .344). However the prevalence of *B. caballi* infection was high in riding animals (44.3%) than draught animals(38.3%), these result due to owners interested for draught animals.

No significant association between *T.equi* infection and animal activities (X^2 = 1.197; P = .627). The prevalence was showed higher in draught animals(5.1%) than riding animals(3.8%). These result could be due to draught animals are more exposed to stress leading to decrease immunity system.

In our observation, on significant association between the prevalence of *Babesia caballi* infection and using of acaricides for controlling of tick vector (X^2= .668; P= .414). The infection rate was a high in that not usedacaricides (41.1%) than that used acaricides (36%). In our survey, no association significant in the prevalence of *T. equi* and using of acaricides(X^2= .261;P= .590).

According to tick infestation, prevalence of *B. caballi* infection was 50.6%of the animals infested by ticks at the time of sampling and 31.1% in animals that were not exposed to ticks. Chi square test showed significant difference of *B. caballi* infection between the two categories (X^2 = 5.274; P = .022).These result indicated that owners were not apply tick control programme that related to increase *B. caballi* infection.

Babesia caballi infection was more prevalent in animals that subjected to ivermectin treatment(44.9%). No significant association result between the both prevalence of *B. Caballi* and *T. Equi* infection and using of Ivermectin as the during for treatment of internal and external parasite (x^2 = 2.121; p = .142)and (X^2 = 1.271; P = .260) respectively.This result indicated that using of ivermectinhave no effect in prevalence of *Babesia caballi* and *Theileria equi* infection in horses and donkeys. This might be due to use of ivermectin after animals infested by ticks that are vector of equine piroplasmosis, or may be due to ticks resistance of ivermectin.

Coclusion and recommendation

Conclution

The overall prevelances of *B. Caballi* and *Theileria equi*were 39.8% and 4.8% respectively. Muhajiria was considered the highest infected area by *B. caballi*(47.1%) andSileaa was found highest infected areaa by *T. equi*(14.8%). The equine piroplasmosisinfection were endemic in Yassine locality and this could be attributed to tick infestation and body condition score.

Recommendation

1-Additional studies should be applicated by using tests of high sensitivity and specificity to assess the exact prevalence of both infections.

2-effective control program should be based on tick control and elimination of disease from carrier horses and donkeys.

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APPENDECS

Appendix 1

QUESTIONARE:

Fase to fase questionnaire will be conducted and includedin the data analysis

-Date: ……

1- Location: ……..

-Owner,s name: …….

-Address and telephone number: ……..

2- Age of animal: ……

3- Sex: ☐ male ☐

female ☐

4 -Breed: local foreign: crossbred: ☐ ☐

5-Body condition:

good ☐

poor ☐

6-Use of acaricide :

yes: ☐ specify …….

No ☐

7-Tick infestation:

yes ☐ no ☐

8-use of animals : riding ☐

Draught ☐

Racehorse ☐

9- use of ivermectin

Yes ☐

No ☐

Apendix2

Multivariate analysis

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	bodycon	.513	.240	4.556	1	.033	1.670
	tickinf	.618	.262	5.549	1	.018	1.855
	ivermec	-.363-	.234	2.405	1	.121	.695
	Constant	-.865-	.653	1.751	1	.186	.421

a. Variable(s) entered on step 1: bodycon, tickinf, ivermec.