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Prevalence and Risk Factors of Tick Borne Blood Parasites in Calves in North Gezira Area, Gezira State, Sudan

معدل إنتشار وعوامل الخطر لطفيليات الدم المنقولة بواسطة القراد في العجول بمنطقة شمال الجزيرة – ولاية الجزيرة، السودان

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

By

Abdulmajeed Mujtaba Haydir Hassan

(B.V.M. (2014) College of Veterinary Medicine, Sudan University of Science and Technology)

Supervisor

Professor : Siham Elias Suliman Mohammed

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الإسْتِــهـلال

قال تعالى:

(وَإِنَّ لَكُمْ فِي الأَنْعَامِ لَعِبْرَةً نُسْقِيكُمْ مِمَّا فِي بُطُونِهِ مِنْ بَيْنِ فَرْثٍ وَدَمٍ لَبَناً خَالِصاً سَائِغاً لِلشَّارِبِينَ (66)

صدق اللهُ العظيم

سورة النحل الآية 66

DEDICATION

To my parents, my beloved wife,

brothers, sisters, and

all my family

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

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ABSTRACT

This study was conducted in North Gezira area – Gezira State to investigate the prevalence of tick borne blood parasites in calves and to assess the association between theileriosis, babesiosis and anaplasmosis and the risk factors (locality, breed, sex, age, body condition, tick infestation, use of acaricides, use of prophylactic treatment and presence of dogs). A total of 390 blood samples were collected from five localities namely Al-masudiyeh, Al-jadid althawra, Al-masid, Al-naapti and Al-nuba from March to April 2021. The samples were examined in laboratory microscopicly using Giemsa stain. The results showed that the prevalence of theileriosis was 41.2 %, babesiosis was 7.2% and anaplasmosis was 9%. Univariate analysis for the results was done using chi-square test showing no correlation between prevalence of theileriosis, babesiosis and anaplasmosis and risk factors except there was significant association between theileriosis and ticks infestation(Chi-square = 5.738 ; P-value 0.017) and presence of dogs (Chi-square = 18.100 ; P-value = 0.000). While ticks infestation (Chi-square =4.937 ; P-value = 0.026) was associated with babesiosis. Also usage of acarecide (Chi-square = 4.862 ; P-value =0.027) and prophylactic treatment (Chi-square = 8.690 ; P-value = 0.000) were as associated with disease. But presence of dogs (Chi-square = 8.121 ; P-value = 0.004) was associated with anaplasmosis. When multivariate logistic regression was done to risk factors after univariate analysis, the result revealed that Presence of ticks was more associated with theileriosis (Exp(B) = 1.776; P-value = 0.011) and babesiosis (Exp(B) 3.206 ; P-value 0.024). Whereas presence of dogs associated with *Theileria* infection (Exp(B) = 0.236 ; P-value = 0.000) and *Anaplasma* infection (Exp(B) = 0.328 ; P-value = 0.007). This study confirmed that tick-borne blood parasites were endemic in North Gezira area. The tick-borne blood parasites in calves of high economic importance by treatment of these diseases.

الخلاصه

أجريت هذه الدراسة في منطقة شمال الجزيرة – ولاية الجزيرة لتقدير مدي انتشار طفيليات الدم المنقولة بواسطة القراد في العجول ودراسة العلاقة بين الثاليرا، البابيزيا، الانابلازما وعوامل الخطر (المكان، السلالة، الجنس، العمر، حالة الجسم، وجود القراد، استخدام مبيدات الحشرات، استخدام العلاج الوقائي ووجود الكلاب). تم جمع 390 مسحة دموية من خمسة محليات وهي المسعودية، الجديد الثورة، المسيد، النابتي والنوبة من شهر مارس الي أبريل 2021 وتم فحص العينات في المختبر مجهرياً باستخدام صبغة جيمسا. أظهرت نتائج الدراسة أن معدل انتشار الثاليرا 41.2 % والبابيزيا 7.2% و الانابلازما 9 % . التحليل الفردي باستخدام مربع كاي ($p - value = \leq 0.05$) الذي أظهر بانه لا يوجد ارتباط بين معدل انتشار مرض الثاليرا والبابيزيا و الانابلازما وعوامل الخطر باستثناء وجود ارتباط معنوي بين تكرار مرض الثاليرا ووجود القراد (Chi-square = 5.738; P-value 0.017) ووجود الكلاب (Chi-square = 18.100 ;p -value = 0.000) بينما وجود القراد (Chi-square =4.937 ; P-value = 0.026) ارتبط بمرض البابيزيا. كما كان استخدام مبيد القراد(Chi-square=4.862;P-value =0.027)والعلاج الوقائي (Chi-square = 8.690 ; P-value = 0.000) مرطبتين بالمرض. لكن وجود الكلاب (Chi-square = 8.121 ; P-value = 0.004) كان ايضا مرتبطاً بمرض الانابلازما. عندما تم إجراء الانحدار اللوجستي متعدد المتغيرات لعوامل الخطر بعد التحليل أحادي المتغير. أظهرت نتائج الدراسة أن وجود القراد كان أكثر ارتباطاً بمرض الثاليريا (Exp (B) = 1.776 ; P-value = 0.011) و مرض البابيزيا (Exp (B) 3.206 ; P-value 0.024) . في حين وجود الكلاب المصاحبة للإصابة بمرض الثاليريا (Exp (B) = 0.236 ; P-value = 0.000) والإصابة بمرض الانابلازما (Exp (B) = 0.328 ; P-value = 0.007). أكدت هذه الدراسة أن امراض طفيليات الدم المنقولة بواسطة القراد متوطنة في منطقة شمال الجزيرة. طفيليات الدم المنقولة بواسطة القراد في العجول ذات الأهمية الاقتصادية العالية من خلال علاج هذه الأمراض.

INTRODUCTION

Tick borne diseases (TBDs) are one of the most important constrains to livestock production in developing countries (Makala *et al*., 2003). They are responsible for high morbidity and mortality resulting in decreased production of meat, milk and other livestock by- products together with loss of draught power (Osman *et al*., 2017). Despite the large animal population, their productivity is low due to poor nutrition, reproduction insufficiency, management constraints and prevailing livestock diseases (Bekele *et al*., 2010).

Tick-borne diseases (TBDs) are widespread in Sudan causing substantial economic losses and are a constant threat to the development of animal wealth. In the Sudan, tick fauna comprises over 70 species prevalent in diverse ecological zones (Abaker *et al*., 2017). Parasitic diseases have largely been neglected primarily because they do not often cause acute fatal diseases. Blood parasites feed on blood and constitute of nutrients. They are difficult to control due to their resistant to drugs, and lack of vaccine against most of them due to several factors such as antigenic variation and difficulties in propagation of these organism in artificial media (Mohamed, 2006).

The infection with blood parasites can be suspected from general symptoms of the diseases such as decrease in production, emaciation, loss of appetite and jaundice (OIE, 2018). Cattle can be affected with blood parasites such as *Babesia*, *Theileria* and *Anaplasma*. The most important *Babesia spp*. that cause the disease in cattle are *Babesia bigemina*, *Babesia bovis* and *Babesia divergens* (Bouattour and Darghouth, 1996).

Babesiosis is one of the most not uncommon infections of free-ranging animals. It is a cosmopolitan disease and recently acquiring more attention and interest as an emerging zoonosis (Homer *et al*., 2000).

Babesia and *Theileria* species are apicomplexan–hemoprotozoan parasites transmitted by Ixodidae ticks (Preston, 2001; Silva *et al*., 2010). Theilerosis is caused by an intracellular blood parasite it invades lymphocyte and macrophage as well. The most important *Theileria spp*. are *Theileria parva* that is known to cause East Coast Fever (Brayton *et al*; 2007) and *Theileria annulata* which causes tropical theileriosis in cattle.

Anaplasmosis is an infectious blood parasitic disease cause by *Anaplasma marginale* and *Anaplasma centrale* also new species of *Anaplasma, A. phagocytophilum* and *A. bovis* reported by Dumler *et al.* (2001). Some of these diseases have an important zoonotic impact.

Objectives of this study:

The research aims were to achieve the following objectives:

- To investigate the prevalence of *Theileria*, *Babesia* and *Anaplasma* in calves in North Gezira.
- To determine the risk factors associated with the above mentioned tick borne protozoan infections.

CHAPTER ONE

1. LITERATURE REVIEW

1.1. Cattle Breeds in Sudan:

The Sudan has a hug wealth of animal resources. It has an estimated population of cattle of about 41.43 million heads (Anon, 2008). The size of this cattle population is ranking as the second biggest in Africa and the sixth worldwide, and breeds are either indigenous ecotypes of zebu cattle which include Foga, Baggara, Kenana, Gaash and Butana or cross-breeds of these ecotypes with exogenous breeds mainly Friesian, and others like Ayrshire and Holstein (Fadlelmoula *et al*., 2007). The cattle population has increased rapidly in Gezira State due to the increase in number of agricultural farms and availability of agricultural byproducts of sorghum, cotton plant, groundnut and wheat plant (Anon, 2013).

1.2. The Ticks :

Ticks (Acari: Ixodoidea) are important ectoparasites infesting livestock and human populations around the globe. Ticks carry and transmit a large number of pathogens, including bacteria, viruses, and protozoa. They are second to mosquitoes in importance as disease vectors, greatly impacting human and animal health. Ticks are voracious blood suckers, causing heavy blood losses leading to anemia, and the injuries (Mossaad *et al*., 2021). There are two families of ticks, the family Argasidae (soft ticks), which lacks scutum and the dorsum is covered by leathery integument and the family Ixodidae (hard ticks) whose scutum or dorsal shield covers the 4 entire upper surface of male and relatively a small area just behind the head in the female (Soulsbly, 1982).

1.2.1. Tick Biology:

Ticks are divided in two major families of which the Ixodidae (hard ticks) are of greater veterinary importance than the Argasidae (soft ticks). There are four stages in the life cycle of ticks which are egg, larva, nymph, and adult (Hoogstraal, 1956; Bowman, 1999).

1.2.2. Tick life cycle:

The life cycle of tick involves according to feeding habitat a characteristic number of host individuals. The Argasid species are characterized by multi-host feeding pattern, whereas the life cycle of most Ixodid species typically involves a 3-host cycle, though some species have a 1or 2 host cycle. According to the number of the hosts they require during their life cycle, ticks can be classified into three groups:

1.2.2.1. One-host tick:

One-host ticks parasitize large hosts mainly bovines and equines. Once the larva finds a suitable host, feeding and moulting proceed sequentially on the same host until the adult stage is reached. This type of life cycle is characteristic of *Boophilus spp*.

1.2.2.2. Two-host tick:

In these species, the larval and nymphal stages are spent on the same animal, but the nymph drops off to moult to the adult stage, which then seeks a final host. A few species in the genera *Hyalomma* and *Rhipicephalus*, (e.g. *H. marginatum rufipes and R. evertsi evertsi*). 6 Living typically in regions with long dry or cold seasons, and irregularly available hosts have a 2-host life-cycle.

1.2.2.3. Three-host tick:

In these species, the larvae, nymphs, and adult females feed on different host individuals. Larvae and nymphs detach and fall to the ground before moulting to the next stage and searching for a new host. In many instances larvae and nymphs of most species feed on small mammals (rodents) or birds, while adults prefer larger hosts (Suad, 2009).

1.2.3. Transmission of diseases:

Ticks can be carriers of pathogens, which they transmit from host to host during blood sucking and cause a large variety of diseases (FAO, 1998). The major diseases include babesiosis, theileriosis, anaplsasmosis, heartwater and some viral diseases. Generally, ticks become infected with the causative organisms of diseases while they are feeding on infected animals. Then, the organism may be transmitted from stage to stage in the tick (an example is *Theileria parva* transmitted by *Rhipicephalus appendicuatus*), or from the female tick through the egg to the larvae 10 an increase of several thousand times in vector potential (an example is *Babesia bovis* transmitted by *B. decoloralus*). When the next stage or generation subsequently feeds on another animal, the organism is transmitted to that animal if it is susceptible to the disease (Drummond, 1983). Tick- borne diseases generally affect the blood and / or lymphatic system (FAO, 1998).

1.3. Tick-borne diseases of importance:

The major tick-borne diseases of importance to the livestock industry can be classified according to Coetzer *et al*. (1994) and Kaufmann (1996) as follows:

1.3.1. Theileriosis:

1.3.1.1. Definition:

Theileriosis is a tick-borne disease of cattle, sheep, goats, buffalo and infrequently wild ruminants caused by species of protozoa belonging to the genus *Theileria*, The parasite is transmitted by a species of tick known as *Hyalomma anatolicum* (Soulsby, 1982; Losos, 1986). There are six identified *Theileria spp* that infect cattle ; the two most pathogenic and economically important are *T.parva*, and *T.annulata*. *T. lestoquardi* (*T.hirci*) is the only species of economic significance infecting small ruminants (OIE, 2009) .

1.3.1.2. Classification:

According to Levine *et al*. (1980) *Theileria* is classified as follows:

Sub-Kingdom	:	protozoa.
Phylum	:	Apicomplexa.
Class	:	Sporozoa.
Sub-class	:	Piroplasmina.
Order	:	Piroplasmida.
Family	:	Theileriidae.
Genus	:	*Theileria*.

1.3.1.3. Life cycle of the *Theileria spp*:

The life cycle of *Theileria spp* is complex, involving morphologically distinct phases in two hosts. Sporogony and merogony take place in the cattle host while zygote and kinete are formed in ticks (Gul *et al*., 2015). *Theileria* sporozoites enter their cattle host during tick feeding and they rapidly invade mononuclear leukocytes (lymphocytes and monocytes), where they mature into macroschizonts and induce proliferation in host cells (Shahnawaz *et al*., 2011). Microschizonts gradually develop into macroschizonts and ultimately into merozoites, which are released from leukocytes. These merozoites invade erythrocytes and develop into piroplasms (Khattak *et al*., 2012).

1.3.1.4. Transmission of *Theileria spp* :

Theileria species are spread by ticks and the most important vector for *T. parva* is *Rhipicephalus appendiculatus*, *Theileria annulata* is transmitted by ticks of the genus *Hyalomma*. Cattle that recover from *Theileria* infections usually become carriers. *Theileria orientalis* is transmitted by *Haemaphysalis spp*, or other genera of ixodid ticks and mechanical transmission through routine husbandry practices is another potential method for *T. orientalis* transmission (OIE, 2020).

Transplacental transmission of *T. orientalis* is of particular interest given the propensity of pregnant animals to abort and the susceptibility of young calves to disease. Prior studies indicate that transplacental *Theileria spp.* can occur in their respective hosts including *T. equi* in horses, *T. lestoquardi* in sheep and rarely, *T. annulata* in cattle (Swilks *et al*. 2017).

1.3.2.5. Pathogenesis and clinical signs of Theileriosis:

The pathological damage is induced in cattle by schizont stage of *T. annulata* and *T .parva* (Bishop *et al*., 2004). *Theileria spp* is classified into 2 groups. In first group (*T. parva* and *T. annulata*), proliferates in lymphocytes, but in the second group (*T. orientalis*) is seen in erythrocytes that causes hemolytic anemia (Magona *et al*., 2008). The cells infected by schizonts induce massive and uncontrolled proliferation of both specific and nonspecific T-lymphocyte resulting in enlarged lymph nodes (Schneider *et al*., 2007). Affected lymph nodes show reactive follicular hyperplasia, reticulo-endothelial hyperplasia, enlarged germinal centers and slight increase of inter-follicular lymphoid tissue within the paracortical and cortical regions (Hassan *et al*., 2012).

Due to schizogony in the lymphoid tissue hyperplasia occurs causing swelling of lymph nodes. Due to sudden release of toxins of macroschizonts high rise in body temperature and formation of the ulcers on the mucosal layers of abomasum. In acute cases there is disponea and death due to anemia. Pulmonary congestion, edema, hemorrhage and emphysema of variable extents are also observed in clinically infected cattle. These lesions are characterized by the occurrence of proteinacious fluid in alveolar spaces, enlargement of pulmonary blood vessels with erythrocytes, presence of emphysematous areas (interstitial and alveolar emphysema) and infiltration of inflammatory cells within the lungs (Hassan *et al*., 2012). Tropical theileriosis is characterized by hemolytic anemia and is caused by immune mediated hemolysis (Omer *et al*., 2002). The infected erythrocytes show morphological disorders which may be attributed to the presence of *Theileria* schizonts, immune-mediated processes and intravascular thrombi (Singh *et al*., 2001). Young calves also appear to be highly susceptible to theileriosis, with clinical disease and mortalities reported in herds where the disease is endemic(Eamens *et al*.,2013, Swilks *et al*., 2017).

1.3.2.6. Treatment of Theileriosis:

- ElGhali *et al.* (1994) reported that, Oxytetracycline at 5 mg/kg Bwt showed an efficacy of 70% when used for treatment of moderate cases and with overall efficacy of 52.9%. Oxytetracycline at 10 mg/kg Bwt showed an efficacy of 61.1% when used for treatment of moderate cases and overall efficacy of 55%. Diminazine aceturate efficacy when used for treatment of moderate cases was 47.1% and overall efficacy was 40%. They concluded both drugs are recommended to be used against this disease when other drugs are not available.

- Buparvaquone 2.5 mg / kg bwt.

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

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

1.3.3. Babesiosis:

1.3.3.1. Definition:

Babesiosis is a disease caused by the intraerythrocytic parasites of the genus *Babesia*. In cattle the protozoan parasites *B. bovis* and *B. bigemina* are the most important species (Homer *et al.*, 2000; Bock *et al.*, 2004; OIE, 2008). But infections with *B. divergens, B. major, B. ovate, and B. jakimovi* were also observed (Radostits *et al.*, 2007; OIE, 2009). The disease is transmitted by ticks; *Rhipicephalus microplus* is the principal vector of *B. bovis* and *B. bigemina*. Other important vectors include *Ixodes ricinus*, *Haemaphysalis* species, *Rhipicephalus* species and members of the subgenus *Boophilus* (Homer *et al*., 2000; Bock *et al*., 2004; Radostits *et al*., 2007; OIE, 2008; OIE, 2010). Bock *et al*. (2004) explained that infections of *B. bigemina* involve mostly direct destruction of erythrocytes, whereas that of *B. bovis* have more progressive haemolytic anaemia. Infected animals develop a life-long immunity against re-infection with the same species and some cross-protection is evident in *B. bigemina*-immune animals against subsequent *B. bovis* infections (WHO, 2012).

1.3.3.2. Classiffication:

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

Phylum : Apicomplexa.

Subclass: Sporozoea.

Family : Babesiidae.

Genus : *Babesia*.

1.3.3.3. Life cycle and Transmission of *Babesia spp:*

Ticks become infected by the ingestion of intraerythrocytic parasites. In the gut lumen of the tick, the parasites escape from the red cells and invade gut epithelial cells where they undergo massive multiplication. The end result is the production of large parasites called large merozoites, which are released into the hemolymph, which is blood of the ticks. Further development of the parasite outside the intestine occurs in a variety of tissues, the salivary glands and ovaries being especially important for transmission. Once the adult female tick is infected it can transmit the infection for 32 generations (Taylor *et al*., 2007). The merozoites are motile and are able to swim. Some enter the oviduct and invade the developing eggs in the female tick. Here, the parasites multiply again and then remain dormant until the eggs hatch and the larval progeny infest a suitable host. After attachment of infected seed ticks, sporozoites in tick salivary glands are injected into the mammalian host at the next blood meal and the *Babesia* is activated and development recommences. The infective forms of *B. bovis* are injected into cattle by larval ticks; those of *B. bigemina* are injected into cattle by nymphal and adult ticks. Sexual development occurs in the tick. *B. bovis* is transmitted transovarially but not transtadially (one tick stage to another stage) (Freeman *et al*., 2010).

1.3.3.4. Pathogenesis and clinical signs:

Babesia produces acute disease by two mechanism; hemolysis and circulatory disturbance. The rapidly dividing parasites in the red blood cells produce rapid destruction of the erythrocytes with accompanying haemoglobinaemia, haemoglobinuria and fever. This may be so acute as to cause death within a few days, during which the packed cell volume falls below 20% which will lead to anemia (Demissie and Derso, 2015). Despite, being closely related and transmitted by the same *Boophilus* ticks, then *B. bovis* and *B. bigemina* cause remarkably different diseases in cattle. In *B. bovis* infections, the disease pathology can be both due to over-production of pro-inflammatory cytokines and the direct effect of red blood cell destruction by the parasite. During an acute infection, macrophages activated by the parasite produce pro-inflammatory cytokines and parasitocidal molecule (Simuunza, 2009). The outcome of infection is related to the timing and quantity of production of these substances. Over-production of inflammatory cytokines results in severe pathology leading to vasodilatation, hypotension, increased capillary permeability, edema, vascular collapse, coagulation disorders, endothelial damage and circulatory stasis (Ahmed, 2002). Although stasis is induced in the microcirculation by aggregation of infected erythrocytes in capillary beds, probably, the most deleterious pathophysiological lesions occur from the sequestration of parasitized erythrocytes in microcapillaries of the lungs and brain. These results in cerebral babesiosis and a respiratory distress syndrome associated with infiltration of neutrophils, vascular permeability and edema. Coagulation disorders, cyto-adherence and the hypotensive state seen in *B. bovis* are not features of *B. bigemina* infections (Bock *et al*., 2004).

1.3.3.5. 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 *et al* ., 1986). In addition, supportive therapy such as blood transfusion , anti – inflammatory drugs, tick removal, iron preparations, dextrose, vitamin B complex, purgatives and fluid replacements, may be necessary in severe cases of babesiosis (Mosqueda *et al* ., 2012).Vitamin E also act as supportive therapy as vitamin E ameliorates the oxidative effect of *Babesia* by increase antioxidant effect (AbdelHamid *et al* ., 2014). 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.

1.3.4. Anaplasmosis:

Bovine anaplasmosis is a hemoparasitic disease caused by the tick-borne pathogen *Anaplasma marginale* (Dumler *et al*., 2001) and *Anaplasma centrale* (Inokuma *et al*., 2001).The microorganisms are gram negative, and infect red blood cells. which are usually transmitted by ticks, but may also be transmitted mechanically by biting diptera (e.g. Tabanidae and Stomoxys). Anaplasmosis affects domestic and wild ungulates and is widespread throughout the tropics. Anaplasmosis in cattle, caused by *Anaplasma* species is characterized by jaundice, anaemia, and debility. It is transmitted by the tick through transovarian and stage to stage transmission (Wanduragla and Ristic, 1993). Anaplasmosis can also be transmitted by use of surgical, dehorning, castration and tattoo instruments and hypodermic needles that are not disinfected between using (Hartelt *et al*., 2004).

1.3.4.1. Life Cycle of *Anaplasma*:

Life cycle starts with blood meal after tick bite to infected mammals. Infected erythrocytes are disrupted and release bodies which can then invade other erythrocytes. These bodies form vacuoles within the cytoplasmic membranes of the red blood cells and then undergo binary fission to form dense blue-purple round/cube shaped inclusion bodies. The inclustion bodies are most numerous during the acute phase of the infection, but some persist for years afterwards. (Urquhart *et al*., 1996). The number of infected erythrocytes doubles every 24 to 48 hours, and approximately 2 to 6 weeks later an acute infection can develop where 10 to 90% of erythrocytes are infected (>108 infected erythrocytes per ml). Some authors have claimed that at least 15% of infected erythrocytes are necessary for the animal to show clinical signs, while others suggest only 1% (Richey and Palmer, 1990).

1.3.4.2. Clinical signs of Anaplasmosis:

Dairy cattle upon its return from pasture usually becomes infected with variable severity of illness including dullness, anorexia, reduced milk production, respiratory distress, coughing, abortions, and stillbirth are common. The important finding that mild cases recover within 14 days and death is unusual outcome (Tuomi 1967; Taylor and Kenny 1980; Stuen *et al*. 1992; Grøva et al. 2011; CFSPH 2013). Cattle of all ages can become infected with *A. marginale*. However, mortality and disease severity is greater in adult cattle (Richey and Palmer, 1990). Young calves (less than 6 months of age) are equally susceptible to infection as adults, but they seldom develop clinical signs (Richey and Palmer, 1990; Kocan *et* *al*., 2003). Infection between 6 months and 2 years of age increases the risk of clinical illness but is rarely fatal. Cattle infected after 2 to 3 years of age are commonly affected by a peracute fatal form of the disease. Incidence of clinical disease (often fatal) increases with age (>3 yrs of age). Calves develop persistent infections and lifelong immunity (Kocan *et al*., 2003).

1.3.4.3. Treatment of Anaplasmosis:

Chemotherapies of choice for *A. marginale* are tetracycline compounds and imidocarb. Imidocarb is thought to block the entry and prevent the uptake of inositol by erythrocytes thus resulting in the starvation of any hemoparasite. Tetracyclines inhibit replication of *A. marginale* in cell culture by interfering with the ability of the organism to complete its replication cycle within the parasitophorous vacuole in the host cell cytoplasm (Blouin *et al*., 2002). Symptomatic treatment such as blood transfusion, drugs that stimulates erythropiosis, drugs which protects liver cells may help in recovery (lefevre *et al*., 2010).

1.3.5. Diagnosis of tick –borne blood parasites:

Tick – borne diseases are major economic constraint to livestock production. Identification of these haemoprotozan and rickettsial infection is essential in understanding the epidemiology and it is important to distinguish between species and subspecies involved. The microscopic techniques for diagnosis of tick-borne diseases are still considered as the “gold standard” technique. Microscopic examination shows *Theileria* schizonts in the lymph node smears and piroplasms alone or along with schizonts in blood smears (Salih *et al*., 2015). Conventional procedures such as microscopic and serological evaluations do not usually meet these prerequisites. Diagnostic contrivances, such as the complement fixation test (CFT), the indirect fluorescent antibody test (IFAT) and the enzyme linked immunosorbent assay (ELISA) have been efficaciously used for many years. Furthermore, DNA-based investigations for identification, differentiation and classification of different haemoparasites have also been established. Molecular diagnostic procedures, such as DNA hybridization, polymerase chain reaction (PCR), transcriptomics, proteomics, metagenomics and metabolomics, permit the uncovering of parasites in blood, tissues or ticks with optimal sensitivity, specificity and consistency(Adamu *et al*.,2016).

1.3.6.1. Control of tick borne blood parasites:

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

1.3.6.2. Tick control:

1-3.6.2.1. Acaricids control:

Control of ticks and tick-borne diseases in Sudan is based mainly on the use of chemical acaricides (Aziz, 2003). However, there is no official policy on tick control in the Sudan. Cypermethrin was registered for veterinary use in the Sudan in 1990. It has since become the predominantly acaricide used for ticks control in Khartoum State and other parts of the Sudan. The development of resistance is a potential problem resulting from the over-use of any pesticides. Unfortunately, information concerning tick-resistance to this acaricide is limited although attempts to monitor tick resistance at the larval and other stages have been made (Elhaj *et al*,. 2016). Acaricides are often inappropriately used, have residual effects in milk and meat subproducts, and are not environmentally friendly, being responsible for the increase of acaricide – resistant ticks. Resistance is associated with mutations in genes related to drug susceptibility (Mona, 2019).

1.3.6.2.2. Biological control:

For many years, acquired resistance to ixodid tick, has been recognized as possible biological control method. Such resistance acquired after repeated infestation by ticks, is immunologically mediated. Acquired immunity is expressed by reduction in the number of ticks which attach to the host, reduced ingorgement weight, and reduced egg and larval production resulting in significantly reduced tick population. The occarence of this type of resistance varies with the tick species and the type of breed and between individuals. Probably depending on natural selection of animals exposed to the tick in question over many generations (Jongegon and Uilenberg, 1994). Ticks have numerous natural enemies, but only a few species have been evaluated as tick biocontrol agents (BCAs). Some laboratory results suggest that several bacteria are pathogenic to ticks, but their mode of action and their potential value as biocontrol agents remain to be determined. The most promising entomopathogenic fungi appear to be Metarhizium anisopliae and Beauveria bassiana, strains of which are already commercially available for the control of some pests. Development of effective formulations is critical for tick management. Entomopathogenic nematodes that are pathogenic to ticks can potentially control ticks, but improved formulations and selection of novel nematode strains are needed. Parasitoid wasps of the genus *Ixodiphagus* do not typically control ticks under natural conditions, but inundative releases show potential value. Most predators of ticks are generalists, with a limited potential for tick management (Samish *et al,*. 2004).

1.3.6.2.3. Vaccines:

Immunization using anti tick vaccine, exploitation of natural resistance phenomenon of tick infestation, an observed phenomenon during the tick-host relationship is the development of resistance against ticks. This resistance appears to be maintained on subsequent infestation with the same tick species and sometimes show the ability to cross react with other tick species. This consists either of innate or acquired components and featured by reduced attachments engorgement and development of the ticks (Latif, 1984).

CHAPTER TWO

2. MATERIALS AND METHODS

2.1. Study area:

This study was carried out in Gezira State which is located in the eastern region of the central Sudan and lies between latitudes 13° 32′ - 15° 30′ N and longitudes 32° 22′ - 34° 20′ E. It is bordered by Khartoum State to the north, Sinnar State to the south, Gedarif State to the east and White Nile State to the west. It has a total area of about 27,549 km2 and a human population of 3,529,992 that subsists mainly on agriculture and livestock rearing (Sudan census, April 2008). Livestock population is about 9,824,922 heads including cattle (3,618,418), sheep (3,780,015), goats (2,317,881) and camels (108,608) (Ministry of Agriculture and Animal Resources, Gezira State, 2013). The mean daily maximum temperature is 42 °C in May and the mean daily minimum temperature is 14 °C in January. The rainy season is from June and October, the peak is in July and August. The mean relative humidity ranges from 24% in May and 80% in August (Anon, 2013). Five areas along the north region of the state were selected to conduct this investigation. These were Al-masudiyeh, Al-jadid althawra, Al-masid, Al-naapti and Al-nuba. This study was conducted during March and April 2021.

2.2. Sample size:

Atotal of 390 blood samples were collected according to formula of Thrusfield (2007) This formula is: $n = (1.96)^2 . pexp(1-pexp)/d^2$

Where:
n = sample size
1.96 = constant
Pexp = expected prevalence
d = desired accuracy level at 95% confidence interval

2.3. Questionnaire:

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

2.4. Diagnosis of tick borne blood parasites:

2.4.1. Preparation of blood smears:

Blood smears were prepared from the blood collected from the jugular vein of each animal by using EDTA tubes through asepetic method and microscopic examination of the slides used according to OIE (2012) and Burgdorfer (1970) by using Giemsa's staining procedure, spreader kept at an acute angle in order to obtain one thin layer smear. The slides were air dried and immediately fixed in absolute methyle alcohol for 2-3 minutes.

2.4.2. Giemsa's Staining procedure:

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

2.5. Data Analysis:

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

CHAPTER THREE

3. RESULTS

3.1. Overall prevalence rate of tick borne blood parasites in calves in North Gezria area:

A total of 390 blood samples of calves were collected and examined microscopically for the presence of tick borne blood parasites. The overall prevalence of theileriosis was 41.3% (161/390), babesiosis was 7.2% (28/390) and anaplasmosis was 9% (35/390) (Table 1).

Table 1: The Overall prevalence of tick-borne blood parasites in calves in North Gezira area - Gezira state

Blood parasites	No. of samples	No. of positive samles	Prevalence%
Theileria	390	161	41.2%
Babesia	390	28	7.2 %
Anaplasma	390	35	9 %

3.2. The prevalence rate of tick borne blood parasites in calves based on localities of North Gezria area:

In this study 79 blood samples were collected from Al-masudiyeh, 86 from Al-jadid althawra, 63 from Al-masid, 79 from Al-naapti and 82 blood samples from Al-nuba. The chi- square test showed no significant association between localities and infection with *Theileria*, *Babesia* and *Anaplasma* (Table 2).

Table 2: The prevalence of tick-borne blood parasites in calves in different localities in North Gezira area - Gezira state

Locality	No. of animal Examined	+ve *Theileria(%)*	+ve *Babesia(%)*	+ve *Anaplasama(%)*
Al-masudiyeh	79	30 (38)	2 (2.5)	10 (12.7)
Al-jadid althawra	86	32 (37.2)	8 (9.3)	5 (5.8)
Al-masid	63	27 (42.9)	5 (7.9)	5 (7.9)
Al-naapti	80	40 (50)	5 (6.25)	10 (12.5)
Al-nuba	82	32 (39)	8 (9.8)	5(6.1)

3.3. Analysis of other risk factors in calves :

3.3.1. Risk factors analysis with *Theileria spp* :

3.3.1.1. Sex of animals:

According to the sex of calves examined, the result showed that 66 out of 163 males (40.5%) and 95 out of 227 females (41.9%) were infected with *Theileria*. There was no significant variation between *Theileria* infection and sex (x^2 =0.072; p-value =0.788) (Table 3).

3.3.1.2. Breed of animals:

Considering breed of cavles examined, the result showed 37 out of 90 (41.1 %) local breed and 124 out of 300 (41.3 %) Gross breed were infected with *Theileria* (Table 3). There was no significant difference between *Theileria* infection and breeds (x^2 = 0.001; p-value =0.970).

3.3.1.3. Age of animals:

According to the age of animals examined, the animals were classified based on age into three groups, < 3 month, 3-6 month and > 6 month. The result showed that 9 out of 28 (32.1 %), 25 out of 70 (35.7 %) and 127 out of 292 (43.5) were infected with *Theileria* respectively. No association between *Theileria* infection and the age of animals (x^2 = 2.449 ; p-value =0.294) (Table 3).

3.3.1.4. Body condition of animals:

According to the body condition, the animals examined were classified as good, moderate, and poor body condition, the animals in good body condition were 42.6 % , 35.5 % in moderate and 59 % in poor body condition (Table 3). The chi – square test showed there was no significant association between body condition and *Theileria* infection (x^2 =4.779; pvalue = 0.092).

3.3.1.5. Presence of ticks:

The animals in this study were examined for the presence of tick on their bodies (Table 3). About 112 animals out of 244 (45.9%) were showed ticks on their bodies and positive to *Theileria* and 49 animals out of 99 (47.5%) were showed no ticks on their bodies but positive to *Theileria*. There was no significant association between tick on animal bodies and *Theileria* infection (x^2 =5.738 ; p-value = 0.017).

3.3.1.6. Usage of acaricides:

Regarding use of acaricides, there was no significant association between using of acaricides and *Theileria* infection (x^2=2.099 ; p-value =0.147) . However, calves were sprayed by acaricide were more likely to be healthy (39.2%) than that calves had not sprayed (47.5 %) (Table 3).

3.3.1.7. Usage of prophylactic treatment:

Regarding use of prophylactic treatment, there was no significant association between using of prophylactic treatment and *Theileria* infection (x^2=1.285 ; p-value =0.257). However, calves were treated by prophylactic are more likely to be healthy (40%) than that calves had not treated (26.8 %) (Table 3).

3.3.1.8. Presence of dogs:

The animals in this study were examined for the presence of dogs in their houses. About 126 animals out of 339 (37.2%) were showed dogs on their houses were positive to *Theileria* and 35 animals out of 51 (68.6%) showed no dogs in their housses were positive to *Theileria*. There was a significant association between dog on animal houses and *Theileria* infection (x^2 =18.100 ; p-value = 0.000) (Table 3).

Table 3: Univariate analysis of risk factors associated with Theileriosis infection in calves (n=390) using chi-square test in North Gezira area - Gezira state

Risk factor	No. tested	No. positive	Df	X^2	P-Value
Locality					
Al-masudiyeh	79	30 (38%)	4	3.690	0.450
Al-jadid althawra	86	32 (37.2%)			
Al-masid	63	27 (42.9%)			
Al-naapti	80	40 (50 %)			
Al-nuba	82	32 (39 %)			
Sex:					
Male	163	66 (40.5%)	1	0.072	0.788
Female	227	95 (41.9%)			
Breed:					
Local breed	90	37 (41.1%)	1	0.001	0.970
Gross breed	300	124 (41.3%)			
Age :					
<3 months	28	9 (32.1%)	2	2.449	0.294
3-6 months	70	25 (35.7%)			
>6 months	292	127 (43.5%)			
Body condition:					
Good	244	104 (42.6%)	2	4.779	0.092
Moderate	124	44 (35.5%)			
Poor	22	13 (59.1%)			

Presence of ticks Yes No	244 146	112 (45.9%) 49 (33.6%)	1	5.738	0.017
Use of acaricides Use Not use	291 99	114 (39.2%) 47 (47.5%)	1	2.099	0.147
Use of prophylactic treatment Use Not use	318 127	127 (40%) 34 (26.8%)	1	1.285	0.257
presence of dog Yes No	339 51	126 (37.2%) 35 (68.6%)	1	18.100	0.000

3.3.2. Risk factors analysis with *Babesia spp* in calves :

3.3.2.1. Sex of animals:

Considering sex of calves examined, the result showed 8 out of 163 males (4.9%) and 20 out of 227 females (8.8%) were infected with *Babesia* (Table 4).There was no significant association between sex and *Babesia* infection (x^2 = 2.168 ; p-value = 0.141).

3.3.2.2. Breed of animals:

According to the breed, the result showed 6 out of 90 local breed (6.7%) and 22 out of 300 Gross breed (7.3%) were infected with *Babesia* (Table 4). The statistical analysis showed no significant association between breed and *Babesia* infection (x^2 =0.046; p-value =0.830).

3.3.2.3. Age of animals:

According to the age, 2 animals out of 28 (7.1 %) < 3 months, 8 animals out of 70 (11.4 %) 3-6 years and 18 animals out of 292 (6.2 %) 6 months were positive to *Babesia* (Table 4) . In the chi-square test, the result showed no significant association between age and *Babesia* infection (x^2 = 2.348; p-value =0.309).

3.3.2.4. Body condition of animals:

According to the body condition, the infection rate was higher in animals that had poor body condition (18.2%) compared with those had good and moderate body condition (7.4 % and 4.8 % respectively) (Table 4). There was no significant association between body condition and *Babesia* infection (x^2=5.030; p – value = 0.081).

3.3.2.5. Presence of ticks:

According to the presence of tick in the body of calves (Table 4), the result showed calves had a tick on their bodies were highly infected with *Babesia* (9.4%) than calves had no ticks on their bodies (3.4%). The chi-squared test showed significant association between tick infested in animals and *Babesia* infection (x^2= 4.937; p value =0.026).

3.3.2.6. Usage of acaricides:

In this study, the used of acaricides (Table 4) was highly statistical significant association with *Babesia* infection (x^2=4.862; p-value =0.027).

3.3.2.7. Usage of prophylactic treatment:

In this study, the used of prophylactic treatment (Table 4) was highly significant with *Babesia* infection (x^2=8.690; p-value =0.003).

3.3.2.8. Presence of dogs:

The animals in this study were examined for the presence of dogs in their houses (Table 4). 26 animals out of 339 (7.7%) were showed dogs on their houses were positive to *Babesia* and 2 animals out of 51 (4%) were showed no dogs on their housses were positive to *Babesia*. There was no significant association between dog in animal houses and *Babesia* infection (x^2 =0.934 ; p-value = 0.334).

Table 4: Univariate analysis of risk factors associated with Babesiosis infection (n=390) in calves using chi-square test in North Gezira area - Gezira state

Risk factor	No. tested	No. positive	Df	X^2	P-Value
Locality					
Al-masudiyeh	79	2 (2.5%)	4	4.117	0.390
Al-jadid althawra	86	8 (9.3%)			
Al-masid	63	5 (7.9%)			
Al-naapti	80	5 (6.25%)			
Al-nuba	82	8 (9.8%)			
Sex:					
Male				2.168	0.141
Female	163	8 (4.9%)	1		
	227	20 (8.8%)			
Breed:					
Local breed	90	6 (6.7%)	1	0.046	0.830
Gross breed	300	22 (7.3%)			
Age :					
<3 months	28	2 (7.1%)	2	2.348	0.309
3-6 months	70	8 (11.4%)			
>6 months	292	18 (6.2%)			
Body condition:					
Good	244	18 (7.4%)	2	5.030	0.081
Moderate	124	6 (4.8%)			
Poor	22	4 (18.2%)			
Presence of ticks	244	23 (9.4%)	1	4.937	0.026
Yes	146	5 (3.4 %)			
No					

Use of acaricides Use Not use	291 99	16 (5.5%) 12 (12.1%)	1	4.862	0.027
Use of prophylactic treatment Use Not use	318 72	17 (5.3%) 11 (15.3%)	1	8.690	0.003
presence of dog Yes No	339 51	26 (7.7%) 2 (3.9%)	1	0.934	0.334

3.3.3. Risk factors analysis with *Anaplasma spp* in calves :

3.3.3.1. Sex of animals :

Considering sex of calves examined 13 out of 163 males (8%) were infected with *Anaplasma* compared with 22 out of 227 females (9.7%) (Table 5). There was no association between sex and *Anaplasma* infection (x^2 = 0.342; p-value =0.559).

3.3.3.2. Breed of animals :

According to the breed , the result showed 4 out of 90 local breed (4.4%) and 31 out 300 Gross breed (10.3%) were infected with *Anaplasma* (Table 5). There was no statistical association between breed and infected with *Anaplasma* (x^2=2.393; p-value =0.086).

3.3.3.3. Age of animals :

Regarding age groups, there was no significant association (Table 5) between age and infection with *Anaplasma* (x^2= 4.520; p-value =0.104).

3.3.3.4. Body condition of the animals:

According to the body condition. The infection rate (Table 5) was higher in poor body condition (13.6%) compared with those had moderate and good body condition (8.9% and 8.6% respectively) There was no significant association between body condition and infected with *Anaplasma* (x^2 =0.627; p value =0.731).

3.3.3.5. Presence of ticks:

In the chi-squared test (Table 5), the result showed that there was no association between *Anaplasma* infection and presence of tick in the body of calves (x^2 = 3.489 ; p-value = 0.062).

3.3.3.6. Usage of acaricides:

According of using of acaricides (Table 5), the result showed that there was no association between *Anaplasma* infection and used of acaricide (x^2 = 1.608; p value =0.206).

3.3.3.7. Usage of prophylactic treatment:

According of using of prophylactic treatment (Table 5), the result showed that there was no association between *Anaplasma* infection and used of prophylactic treatment (x^2 = 1.344; p value =0.246).

3.3.3.8. Presence of dogs:

In the chi-squared test (Table 5), the result showed that there was high association between *Anaplasma* infection and presence of dog in the house of calves (x^2 = 8.121 ; p-value = 0.004).

Table 5: Univariate analysis of risk factors associated with anaplasmosis infection (n=390) in calves using chi-square test in North Gezira area - Gezira state

Risk factor	No. tested	No. positive	Df	x^2	P-Value
Locality					
Al-masudiyeh	79	10 (12.7%)	4	4.495	0.343
Al-jadid althawra	86	5 (5.8%)			
Al-masid	63	5 (7.9%)			
Al-naapti	80	10 (12.5%)			
Al-nuba	82	5 (6.1%)			
Sex:	163	13 (8%)	1	0.342	0.559
Male	227	22 (9.7%)			
Female					
Breed:	90	4 (4.4%)	1	2.939	0.086
Local breed	300	31(10.3%)			
Gross breed					
Age :					
<3 months	28	4 (14.3%)	2	4.520	0.104
3-6 months	70	11 (15.7%)			
>6 months	292	21 (7.2%)			
Body condition:					
Good	244	21 (8.6%)	2	0.627	0.731
Moderate	124	11 (8.9%)			
Poor	22	3 (13.6%)			

Presence of ticks Yes No	244 146	27 (11.1%) 8 (5.5 %)	1	3.489	0.062
Useof acaricides Use Not use	291 99	23 (7.9%) 12 (12.1%)	1	1.608	0.206
Use of prophylactic treatment Use Not use	318 72	26 (8.2%) 9 (12.5%)	1	1.344	0.246
presence of dog Yes No	339 51	25 (7.4%) 10 (19.6%)	1	8.121	0.004

Table 6: Summary of Multi variate analysis of risk factors associated with *Theileria*, *Babesia* and *Anaplasma* infection in calves (n=390) using Logistic Regression test in North Gezira area - Gezira state

Risk factors	**Exp(p)**			**P –value**		
	Th	Ba	An	Th	Ba	An
Body condition						
Good	Ref	Ref	-	-	-	-
Moderate	1.395	1.547	-	0.161	0.375	-
Poor	0.510	0.419	-	0.152	0.174	-
Presence of tick						
Use	Ref	Ref	Ref	-	-	-
Not use	1.776	3.206	2.138	0.011	0.024	0.071
Use of acaricide						
Use	Ref	Ref	-	-	-	-
Not use	0.632	1.455	-	0.64	0.724	-
Presence of dog						
Use	Ref	-	Ref	-	-	-
Not use	0.236	-	0.328	0.000	-	0.007
Use of prophylactic treatment						
Use	-	Ref	-	-	-	-
Not use	-	0.244	-	-	0.192	-

*X^2-value= mean value * p-value<0.05 *EXP(P)=exponential(P)=Odds ratio
*Th= *Theileria* *Ba= *Babesia* *An= *Anaplasma*

Multivariate analysis showed no significant association between tick borne blood parasites and risk factors except significant association between Presence of tick with *Theileria*, *Babesia* infection (P-value = 0.011, 0.024 respectively) and presence of dog with *Theileria*, *Anaplasma* infection (P-value = 0.000, 0.007 respectively).

CHAPTER FOUR

DISCUSSION

The target of this study was to investigate the prevalence of tick borne blood parasites in calves in North Gezira area and studying the association between prevalence of tick borne blood parasites and risk factors. The results of the present study showed that the prevalence of theileriosis was 41.2 %, babesiosis was 7.2% and anaplasmosis was 9%. The study of blood parasites in calves during March and April in North Gezira area revealed a higher prevalence of *Theileria* species infection compared to *Babesia* infection. Similarly, different workers recorded the presence of blood parasites in both intensive and pastoral production systems of Sudan (Abdalla, 1984 and Hassan, 2003). Farms carried out as a secondary business and calves that are more susceptible to the occurrence of blood parasitic diseases, because breeders have limited time to clean farms and not observed the health status of animals due to other major activities.

The sensitivity of blood smear examination in the detection of *Theileria* parasites in blood smears was low, and 161 (41.3%) of 390 blood smears were positive for *Theileria spp.* while Salih (2003) reported 3.9%, Abdallah (2005) reported 2.8%, and Jaafer (2015) reported 3.6%. Ali (2005) reported 16.6% as the prevalence of *Theileria spp*. piroplasm in Khartoum State. However the prevalence of babesiosis studied by Shuaib *et al*.(2015) in cattle in North Kordofan state was 5.2% by using blood smear technique. Different prevalences of babesia-infection, ranging from 1.75% to 42%, were reported using blood smear technique in Ethiopia, Malaysia, Pakistan, and Brazil (Ahmad and Hashmi, 2007; Rahman *et al*., 2010; Atif *et al*.,2012; Amorim *et al*., 2014; Hamsho *et al*., 2015). Teferi and Mukarim (2018) reported that the prevalence of anaplasmosis in bovine as 11.46% this result agree with the finding of Angwech *et al*. (2011), Zein *et al*. (2013), Kasozi *et al*. (2014). While Mushtaqa *et al*. (2015) who found 10.4% (Gulu district, Notheren Uganda), 8% (South Kordofan, Sudan), 14.4% (Central and Western Uganda), and 11.25% (Lahore district, Pakistan) respectively found that by using Giemsa stain method.

These variation between there studies and the present study is possible due to variation in areas, grazing system, immunological status of animals, different ages, different breeds and sexes.

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

In the present study *Anaplasma spp* slightly high in female (9.7%) than male animals (8%) and there was no statistical significant difference. But this finding is low compare with other studies recorded by Kispotta *et al*. (2016) in Dinajpur district, Bangladesh (male 13.48% , female 22.52%). Whereas, the result of Atif *et al*. (2012) is near to our results (male 7% , female 10.8%).

Cattle of all ages are susceptible to anaplasmosis, whereas severity and mortality rate increases with increase of animal age (Aubry and Geale, 2011). In the present study the slightly high prevalence was recorded among <3 months (14.3%) then 3-6 months (15.7%) although the difference not statistically significant (P>0.005). This result was in line with the finding of Khan *et al*. (2017) from Pakhtunkhwa-Pakistan and Zein *et al*. (2013) from south Kordofan state, Sudan. The highest prevalence rate of Bovine babesiosis was noted in 3-6 months (11.4%) comparing with <6 months (6.2%). In the present study this result in agreement with previous work performed by Amorim *et al*. (2014), who demonstrated that the calves were more susceptible to infection *Babesia* spp. compared with adult cows.

The prevalence of tick borne blood parasites was higher in poor body condition than good and moderate. This result in 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, Theileria* and *Anaplasma spp.*

The presence of tick were examined in this study. The results showed that prevalence of tick borne blood parasites was higher in animals infested with ticks than those have no ticks. This explains the importance of tick in transmission of infection. However, in the present study the infection was lower in animals when using acaricides than in animals where not using acricides, this may be attributed to wrong usage of acaricides by owners.

In present study, the cross breed were more infected by tick borne blood parasites as compared with local breed. The statistical analysis revealed no significant association between the infection with tick borne blood parasites and animal breed. This result is in agreement with previous study conducted in bovine babesiosis by Shuaib *et al* . (2015). They are found the highest prevalence in cross breed but the lowest prevalence estimated in local breed. Considering the presence of dogs in the animals fram, the result showed that the higher prevalence of tick borne blood parasites occur in those farms which explains that dogs plane an important role in the transmission of the disease by carrying ticks to other animals.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

North Gezira area is considered as an important center of raising calves in Sudan, the findings of this study reported that tick borne blood parasites are prevalent in North Gezira area and there was no relation between blood parasites and the risk factors (locality, breed, sex, age, body condition, tick infestation, usage of acaricides, usage of prophylactic treatment and presence of dogs, except the positive but there was significant association between babesiosis and tick infestation, usage of acarecide and usage of prophylactic treatment. Also there was significant association between theileriosis and tick infestation and presence of dogs. A significant association was observed between anaplasmosis and presence of dogs.

Recommendations:

1. Conduction of more survey on tick borne blood parasites in North Gezira area by using high sensitive tests such as serological and molecular techniques.

2. Additional studies focusing on both dry and wet seasons should be done to determine the effect of seasonal change on the disease prevalence.

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

4. Enhace of extension focusing acaricides usage and the hazard of dogs rearing.

5. Treatment and vaccination if possible.

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APPENDIXS

Appendix 1

Questionnaire:

Date : ………………………………..

Number of blood samples : { }.

Locality : …………………………… Breed : ……………………………….

Sex : ………………………………

Age : (1) < 3 month { }.

(2) 3-6 month { }.

(3) > 6 month { }.

Body condition :

(1) Good { }.

(2) Moderate { }.

(3) Poor { }.

Presence of tick :
(1) Yes { }. (2) No { }.

Use of acaricides :

(1) Use { }. (2) Not use { }.

Use of prophylactic treatment

(1) Use { }. (2) Not use { }.

presence of dogs

(1) Yes { }. (2) No { }.

Appendix 2

1-Multivariate analysis of *Theileria spp*

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	BC			4.764	2	.092	
	BC(1)	.333	.238	1.968	1	.161	1.395
	BC(2)	-.674	.470	2.056	1	.152	.510
	TI(1)	.574	.227	6.423	1	.011	1.776
	ACARICIDE(1)	-.459	.248	3.425	1	.064	.632
	PD(1)	-1.444	.332	18.876	1	.000	.236
	Constant	.391	.174	5.060	1	.024	1.479

a. Variable(s) entered on step 1: BC, TI, ACARICIDE, PD.

2. Multivariate analysis of *Babesia spp*

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	BC			3.203	2	.202	
	BC(1)	.436	.492	.785	1	.375	1.547
	BC(2)	-.870	.640	1.844	1	.174	.419
	TI(1)	1.165	.516	5.100	1	.024	3.206
	ACARICIDE(1)	.375	1.061	.125	1	.724	1.455
	PT(1)	-1.410	1.082	1.699	1	.192	.244
	Constant	2.460	.306	64.645	1	.000	11.709

a. Variable(s) entered on step 1: BC, TI, ACARICIDE, PT.

3. Multivariate analysis of *Anaplasma spp*

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	TI(1)	.760	.420	3.267	1	.071	2.138
	PD(1)	-1.116	.412	7.321	1	.007	.328
	Constant	2.298	.231	98.593	1	.000	9.957

a. Variable(s) entered on step 1: TI, PD.