بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Epidemiological study of Babesiosis in Sheep in Gibash Locality, West Kordufan State

دراسة وبائية مرض البابيزيا في الضان في محلية غبيش – ولاية غرب كردفان

A thesis Submitted to the College of Graduate studies in partial Fulfillment of the Requirements for the Degree of Master of Veterinary Preventive Medicine

By

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October 2018

DEDICATION

To:

My parents and my sisters

Soul of my grand mother

Soul of my grand father

My brothers... and my friends...

Acknowledgements

Thanks first and last to Almighty 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 advice and constructive criticism in reviewing the dissertation.

Thanks to Sudan University of Science and Technology, College of Veterinary Medicine, for giving me the chance to join the master program of Preventive Veterinary Medicine (M.P.V.M) and to work in its laboratories.

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Abstract

A cross sectional study was conducted from January to May 2018 to investigate the prevalence *Babesia* infection in sheep and to assess the relationship between the occurrence of these parasites and factors of age, sex, district, body condition, rising system, ,presence of ticks uses of treatment, uses of acaricides in Gibash locality, west Kordufan state- Sudan.

A total of 255 bloods amples were collected from five districts namely: Aum-zagaia, Aum-sidare, Diglowa, Gibash Wad-gasim, respectively, and examined using microscopy examination.

The result showed that *Babesia* infection was common in sheep and the prevalence is estimated to be 12.2% (31/255).

The subsequent risk factors revealed association with sheep Babesiosis in the univariate analysis under significant level of P-value ≤ 0.5: BCS (p-value = 0.003), presences of ticks (P-value = 0.000), rising system (p-value = 0.000), district (p-value = 0.025). These factors may be contributing factors for the relatively high prevalence of *Babesia* infection among sheep.

ملخص

اجريت دراسه مقطعيه في الفتره من شهر يناير الى مايو2018 وذلك لتقديرمعدل الانتشار لمرض البابيزيا في الضان في محلية غبيش – ولاية غرب كردفان و تمت دراسة عوامل الخطر المرتبطة بالمرض من العوامل الاتية: العمر , الجنس, المنطقه, حالة الجسم ، العلاج المستخدم ، قاتل القراد ، نوع الرعي . تم جمع 255 عينة مسحة دموية من مناطق غبيش، ام صدير، ام زقاية، دقلوه و ود قاسم و جميعها مناطق تابعة لمحلية غبيش , و تم فحص العينات تحت المجهر.

اظهرت الدراسه ان نسبة انتشارالاصابه بمرض البابيزيا كانت 12.2% (255/31) في التحليل الفردي لمعرفة عوامل الخطر المرتبطه بالاصابه باستخدام مربع كاي وجدت علاقه معنويه تحت قيم معنويه اقل من او يساوي 0.05- بين حدوث المرض وكل من عوامل الخطر التاليه: حالة الجسم (القيمة المعنوية =0.003) و وجود القراد (القيمة المعنوية =0.000) و نمط التربية (القيمة المعنوية =0.000) و المنطقة (القيمة المعنوية =0.025).

INTRODUCTION

Background

World sheep population exceeded 1 billion head in 2010, which plays a vital socioeconomic role in the countries throughout the world (FAO, 2010). About 205 million of these sheep are found in Africa, mainly in arid and semi- arid areas of sub-Saharan and provide up to 30% of agricultural gross domestic product in developing countries (FAO, 2010).

Parasitic diseases in the tropics are responsible for great losses in the meat industry than any other infectious or metabolic disease (Gonfa *et al*., 2013)

Babesia infection is a tick-borne disease caused by heamotropicparasite of genus *Babesia* (Homer *et al.,* 2000). The disease affects mammals and birds and significantly impacts on the health of farm and pets animals. Therefore, *Babesia* infection is associated with huge economic losses worldwide. Several animal species are thought to be potential *Babesia* hosts. All vertebrates are potential carriers as they also serve as host for the parasite (Leonhard *et al*., 2012).

Babesia infection also occurs in domestic animals including cattle, sheep, goats, horses, dogs, cats, and pigs. The disease is very common in small ruminants (Theodoropouls *et al*., 2006).

Babesia ovis (*B. ovis*) is pathogenic especially in sheep and its case-fatality in susceptible hosts range from 30 to 50% in field infection. *Babesia* infection has also been reported in various wild carnivors and ungulate species (Hashemi-fesharki *et al*., 1977). *Babesia* infection in domesticated small ruminants is caused by threespecies, namely *Babesia motasi(B. motasi), Babesia carassa (B, carassa), and Babesia ovis (B.ovis),* (Friedhoff., 1988). The blood parasite is transmitted by ticks of the genus *Rhipicephalus* . *B.ovis* is endemic in southern Europe, the

Middle East and central Asia the geographical distribution of *Babesia* infection in south-east Asia and in Africa is however not well known (Friedhoff *et al*., 1997).

The economic loss associated with *Babesia* infection in sheep production in tropical and sub-tropical areas is enormous (Bai *et al*., 2002). *Babesia* infection of sheep remains an important impediment to meat and milk production because of the resulting parasitemia and death (Caracappa *et al*., 1999).

Objectives:

1- To determine the occurrence of *Babesia* infection in sheep reared in Gibash locality.

2- To identify the risk factors associated with *Babesia* infection of sheep in Gibash locality.

CHAPTER ONE

LITERATURE REVIEW

1.1 Aetiology and transmission

Babesia infection is as result of infection with the protozoa of the genus *Babesia* , which belongs to the family *Babesidae* , and the order *Piroplasmida* . *Babesia spp* in sheep caused by three species namely *B.motasi B.carassa B.ovis* (friedhoff *et al.,* 1988). The parasite in sheep is transmitted by ticks of the genus *Haemaphysalis (H. punctata, H. otophila), Dermacenter (D. silvarum), Hyalomma spp* and *Rhipicephalus (Rhipicephalus. Bursa)* (Rehman *et al.,* 2004). The ticks are sensitive to climatic condition and require a relative humidity of at least 80% in order to survive. Typical habitants of the ticks that transmit the infection include deciduous and coniferous woodland, heathland , moorland , rough pasture , forests and urban park (Gassner *et al.*, 2011).

1.2 Pathogenesis of Babesia infection

The infective form of *babesia* is known as sporozoites and is produced in the salivary gland of the tick vectors. The sporozoites are usually injected into the sheep by larvae or adult tick when feeding on the host. The parasite then attacks the host erythrocytes and then destroys them. This destruction leads to the release of hemoglobin in circulation (Alani *et al*., 1988). The erythrocytic cycle continues until the animal dies or its immune system is overwhelmed. *Babesia* parasites may be present in the blood system in small numbers sometimes even for many years without causing the disease (Alani *et al.,* 1988). There are a number of changes in hematological and biochemical profiles linked to the destruction of erythrocytes by *Babesia* following infection of sheep. As parasitemia advances, infected animals reveal a significant decrease in erythrocytes counts, packed cell volume (PCV) level, heamoglbin (HB)-concentration, mean corpuscular Volume (MCV), and mean corpuscular heamoglobin concentration (MCHC). The biochemical changes

seen in this case include alterations of total serum protein, as well as changes in levels of albumin, urea, creatinine, triglyceride, cholesterol, high-density lipoprotein (HDL) and a decrease in low-density lipoprotein (LDL) (Bijan *et al.*, 2012). In contrast, a further increase in parasitemia level has been reported to result in a significant increase in white blood cells (WBC) count, especially neutrophils. Changes in biochemical profiles such an increase in the concentration of blood urea nitrogen (BUN), creatinine, total protein, albumin, globulin, triglyceride, cholesterol, HDL, and LDL have also been documented (Peinado *et al.*, 1995).

1.3 Clinical signs of *Babesia* infection

After infection with the parasite, sheep develop fever and Parasitemia within 2 to 4 days followed by clinical signs of the disease, which include anorexia, listlessness, anemia, moderate jaundice, and haemoglobinuria. In immunocomptent animals, hyperthermia, usually returns to normal level on the fourth day after the peak pyrexia.

Thereafter, parasitemia may reduce to a low level or even zero during the course of the disease (Rahbari *et al*., 2008). Most cases of *Babesia* infection are seen in adults and animals younger than nine months, which usually remain asymptomatic. The level of the parasitemia and the degree of anemia are not usually correlated. The decrease in PCV has been reported to range from 30 to 40%. In some studies, parasitized erythrocytes have not been observed to block capillaries in the brain and it has been postulated that the failure of the cytoadherence to brain capillaries may contribute to the absence of nervous systems in acute babesiosis.(Yeruham *et al*., 1998).

1.4 Epidemiology and Risk factors of Babesia infection

The occurrence of the disease is dependent on the distribution of the ticks that transmit the disease. Many studies are available on associated risk factors of the protozoan infections in different countries. These countries include Iran (Bijan *et al.*, 2015), Kenya (Okuthe and Buyu, 2006), Greece (Theodoropoulos *et al.*, 2006),

Rwanda (Bazarusanga *et al.*, 2007), Bolivia (Gonzales *et al.*, 2007), and Uganda (Magona et al., 2008).

Babesiosis is a haemoparasitic disease belonging to a complex of several tick-borne diseases with different aetiological agents, such as protozoa, rickettsiae, and bacteria (Ranjbar-Bahadori *et al.* 2011) transmitted by ixodid ticks (Aktas, *et al.*, 2007). The high lethality and morbidity caused by Babesiosis explain its importance as a major constraint to livestock breeding development (Ahmed *et al*., 2002; Mehlhorn *et al.*, 1994). Several species of *Babesia* (*Babesiaovis, Babesia motasi, Babesia crassa,* and *Babesia* sp. Xinjiang) have been described in sheep; among them *B. ovis* and *B. motasi* are causative agents of sheep *Babesiosis* (Uilenberg *et al*., 2001; Schnittger *et al.*, 2003; Liu *et al.*, 2007; Ranjbar- Bahadori *et al.*, 2011).

Babesia motasi is moderately virulent, whereas *B. crassa* appears to have little or no pathogenicity (Hashemi-Fesharki, 1977). *Haemaphysalis punctata* is the vector of this species and is widespread in tropical Africa (Uilenberg *et al.*, 1980). The most important *Babesia* species infecting small ruminants is *B. ovis,* which has been reported in Europe, Africa, Asia, and the Far East (Ahmed *et al.*, 2006). *Babesia ovis* is highly pathogenic, especially in sheep; it causes severe infections characterised byfever, anaemia, icterus, and haemoglobinuria. Mortality rates in susceptible hosts range from 30% to 50% in natural infections (Aktas, *et al.*, 2005). *Babesia* infection is an emerging zoonotic disease that affects livestock and human with live-threatening implication, particularly in the immune compromised individuals. *Babesia* infection of sheep has been reported in a number of countries including; Iran, (Dekhordi *et al*., 2010), China (Guan *et al.*, 2012), Sudan (Osman *et al.*, 1997) and Somalia (Ahmed *et al.*, 2013). *Babesia* infection of sheep causes losses in production of meat, milk and other live-stock by-products with possibility of death in severe cases (Perez *et al.*, 2010). The infection causes severe economic losses to sheep farmers in tropical and sub-tropical regions

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1.5 Prevalence of ovine Babesia infection

The prevalence of *B. ovis* infection Lohi sheep in Pakistan has been found to be 50%(Iqbal *et al*., 2011), while a separate study reported a prevalence of 30% in infected sheep on detection by PCR (Shahzad *et al.*, 2013). In Nigeria, a prevalence of 7.5% has been reported for *B. ovis*-infection in sheep with prevalence of 40% for Yankasa breed, 26.7% for Ouda breed and 33.3% for the Koraji breed (Biu *et al.*, 2009).

1.6 Diagnosis of ovine babesiosis

The diagnosis of ovine Babesiosis infection has been done using a range of techniques such as microscopy, serological methods, and molecular techniques.

1.6.1 Microscopy

In the acute phase, diagnosis of ovine Babesiosis infection is mainly based on the microscopic examination of Giemsa-stained blood smear and clinical symptoms (Aktas *et al.*, 2005). The technique of microscopy may not detect the parasite during subclinical infections because of low parasitemia experienced during this stage.

Furthermore, examination of blood smear by microscopy may be influenced by the technical skills of the laboratory technician (Uilenberg *et al.*, 2001).

1.6.2 Serological detection of ovine Babesia infection

Serological tests used for the detection of ovine Babesiosis infection are usually antibody-based approaches, though some may be based on the detection of circulating antigens of parasite in the ovine blood. The samples used in these cases are mainly serum. The specific serological methods that have been used include ELISA, immune fluorescent antibody test (IFAT) and immune chromatographic test (ICT). The serological tests are useful for diagnosis of subclinical and chronic infections (Bose *et al.*, 1995).

1.6.3 Molecular detection of ovine Babesiosis

Molecular tools employing the detection of nucleic acids such as DNA have been used to detect the presence of *Babesia* species in sheep blood (Shayan *et al.*, 2008). For example techniques such as PCR targeting specific genus of the parasite have been used to detect *Babesia spp* such as *B. ovis*, *B. crassa*, *B. motasi* (Almeria *et al.*, 2001). The specific types of PCR that have been used to detect the parasite in sheep blood include conventional PCR, nested PCR, AND real-time PCR.

1.7 treatment and control

There are many drugs that have been used for the treatment of ovine Babesiosis including Quinuroium sulphate, Acaprin, Diminazene aceturate, (Berenil®) and Imidocarb dipropionate salt (Imizol®) (Hashemi-Fesharki, 1977). Treatment of *Babesia* infections is usually done to cure, control and even prevent the spread of the infection. The transmission and spread of infection of sheep with *Babesia* parasites is controlled mainly by using acaricides to control the tick-vectors and by practicing good pasture management (Ahlam *et al*., 2014).

CHAPTER TWO

MATERIAL AND METHODS

2.1 Study area

West Kordufan State is located in southwestern part of the region of Kordufan-Sudan, between latitude 11-20 north and 32.22-30.27 East, and lies between the south Kordufan state, north Kordufan state, and south Darfur (Gad Alkareem *et al.*, 2012).

The southern part of the state is characterized by heavy rainfall, vegetation, tree and heavy clay soils; the northern part is a medium-range rain and sandy soil.

The study was done in Gibash locality. The locality located in the west part in west Kordufan state. It has a population 143,619 and an approximate area of 15950Km². (Gad Alkareem *et al.*, 2012).

2.2 Sample size determination

Sample size was calculated as described by (Martin *et al*. (1987) using the formula

$N= (1.96^2 * P_{ex}(1- P_{ex}) / d^2$

Where n= required samples

P*ex*= expected prevalence

D= desired absolute precision

A prevalence estimate of 15% used based on a study done in Egypt by Hussein *et al*.(2017). A precision (d) of 0.05 was used. Thus

$1.96^2 * 0.15 * 0.85 / 0.05^2$

N= 196 heads of sheep

2.3 Data collection by questionnaire administration

Data for sheep characteristics include sex, age, body condition, presence of ticks, uses of treatment, uses of acaricides, rising system, flock location.

2.4 Blood sample collection and blood smear preparation for microscopic examination

A total of 255 thin smear of the blood samples were made on newly labeled glass slide. The blood was taken from ear vein using sterilized needles [Fig 1]. Care was taken to avoid any contamination of the sample. Then the dried blood smears were fixed in absolute methyl alcohol for one minute. To detect *Babesia*, Giemsa staining was done as described by (Almeria *et al.*, 2001). The stained smears observed by a microscope under oil immersion at 100X objective. The photos of smears that revealed *Babesia*-infected erythrocytes were taken directly by mobile camera and then saved for further documentation.

2.5 Data Management and Analysis

The collected data were coded and entered intoan Excel spread sheet (Microsoft Excel, 2007). Statistical analysis was performed using statistical package for the social sciences (SPSS), version 16 software. Percentage was used to calculate prevalence.Data were statistically analyzed using Chi-squared test to calculate degree of association between risk factors and prevalence of *Babesiosis* in sheep. 95% confidence interval (CI) and $p \leq 0.05$ was considered for statistically significant difference.

Figure 1: Taking of blood sample from ear vein of sheep in Gbash locality

CHAPTER THREE

THE RESULTS

3.1 The prevalence of *Babesia* infection in sheep in Gibash locality:

A total of 255 of blood sample of sheep were examined by microscopy examination for the presence of *Babesia* in Gibash locality. An overall prevalence of Babesiosis in sheep was 12.2%(31/255) in the current study. [table 1]

3.2 Risk factors analysis:

Considering sex of sheep examined, female animals had higher prevalence proportion (15.0%) than males (7.4%). There was no significant variation in *Babesia* infection between male and female. (X^2= 3.251; P-value= 0.071)

Regarding body condition score (BCS), 181 and 74 animals were found in good and poor body condition, respectively. There was statistical significant association observed between categories of body condition and infection of *Babesia* (X^2= 8.745E²; p-value = 0.003). However, sheep had poor body condition (21.6%) are more likely to be infected compared with good ones (8.3%). [table 2]

Considering presence of ticks in the body of sheep [Fig 2], the results show there was highly statistical significant association between sheep have a ticks on their body and *Babesia* infection (X^2= 2.457E2; P-value= 0.000). However, sheep had a ticks on their body (100.0%) are more likely to be infected compared with that sheep had no ticks on their body.[Fig 2].

Regarding uses of treatment there was no statistical significant association between uses of treatment and infection of *Babesia* (X^2 = .048; P-value = 0.827). However, sheep were get drugs against *Babesia* infection are more likely to be healthy (3.1%) than that sheep had not get drugs (9.0%).

Regarding uses of acaricides there was no statistical significant association between uses of acaricide and *Babesia* infection (X^2 = .048; P-value = 0.827). However, sheep were sprayed by acaricide are more likely to be healthy (3.1%) than that sheep had not sprayed (9.0%).

In the Chi-squared test, the result showed that there was no association between *Babesia* infection and the age of animal (X^2 = 3.896; p-value 0.273).[table 2]

Concerning rising system, open and close there was highly statistical significant association between *Babesia* infection and rising system (X^2 = 2.457E^2; P-value = 0.000). However the closed system was highly infected (100%) than open system (4%).

Concerning district of examined animals, the rate of infection was in Aum-zgaia, Wad-gasim, Aum-sidare, Diglowa and Gibash (14.7%), (3.3%) (19.3%), (5%), and (20%), respectively. there was association found between the infection with *Babesia* and district of the examined sheep (X^2 = 11.157E^2; p-value = 0.025).

Table 1 : An overall prevalence of *Babesia* infection in sheep in Gibash locality

Sheep examined	Positive	Negative	Prevalence %
255	31	224	12.2

Table 2: Summary of univariate analysis for risk factors associated with *Babesia* infection(n=255) using the Chi-squared test in Gibash locality west kordufan State, Sudan.

Risk factors	No.tested	No.positive	Df	X^2	p-value
Sex					
Female	160	24(15%)	1	3.251	0.071
Male	95	7(7.4%)			
BCS					
Good	181	15(8.3%)	1	8.745	0.003*
Poor	74	16(21.6%			
Presence of tick					
No	225	1(0.4%)	1	$2.457E^2$	0.000*
Yes	30	30(100%)			
Treatment					
No	185	23(12.4%)	1	0.048	0.827
Yes	70	8(11.4%)			
Acaricide					
No	185	23(12.4%)	1	0.048	0.827
Yes	70	8(11.4%)			
Age					
>1 year	14	0(0%)	3	3.896	0.273
1-3 year	118	13(11%)			
3-5year	99	16(16.2%)			
<5year	24	2(8.3%)			
Rising system					
Open	225	1(4%)	1	$2.457E^2$	0.000*
Closed	30	30(100%)			
District					
Aum zagaia	68	10(14.7%)	4	11.157	0.025*
Wad gasim	60	2(3.3%)			
Aum sidare	57	11(19.3%)			
Diglowa	40	2(5%)			
Gibash	30	6(20%)			

figure 2: ticks infestation in the body of the sheep in Gibash locality

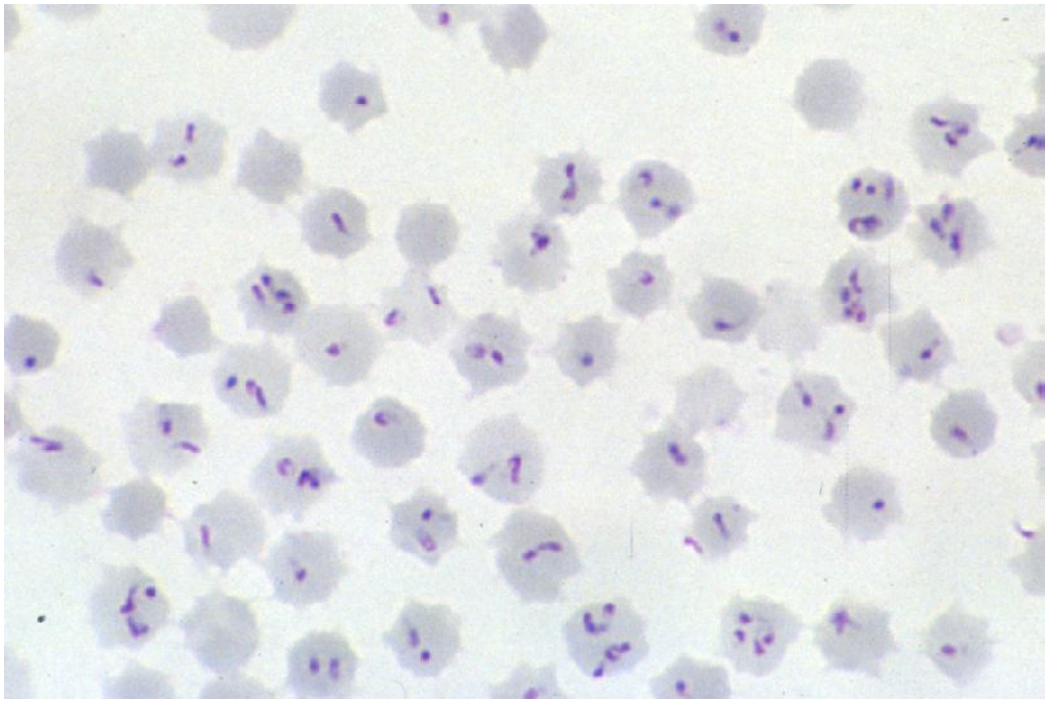

figure 3: Babesia parasite into erythrocyte of sheep in Gibash locality

CHAPTER FOUR

DISCUSION

The prevalence of ovine Babesiosis in certain district is influenced by a multi-factorial system. This system consists of hosts, parasite and environmental factors.

Ovine babesiosi are blood haematoprotoan parasite that cause losses like anemia, reduced productivity and death in heavily infected animals (Slousby , 1982).

In our study, the prevalence was 12.2%.The observed prevalence in sheep in the current study is higher than Abdalbasit (2006) in Khartoum state and Khansa (2017) in Ethiopia who reported 0.6 and 3.3% of ovine *Babesiosis*, respectively. This variation in prevalence between these studies is possibly due to variation in climatic condition.

According to the current study, detection of prevalence of ovine babesiosi,12.2 % (31\255) in Gibash locality was similar with the findings of Ziapour *et al*, (2010) 16.0 .This is may be due to the wide distribution of the ticks in the world.

In our study, sex was investigated. The higher prevalence of infection was in females (15.0%) as compared to males (7.4%). There was no significant association between ovine babesiosis and gender (P= 0.071).This finding was similar observed by Razmi *et al.,* (2003).

The prevalence of ovine babesiosis in different body condition score of sheep was 8.3% in good body condition and 21.6 % in poor body condition according to our results. Highly association between the body condition score and ovine babesiosis infection was observed (P =0.003). This is in line with prior studies suggesting that animals with poor body condition have a little tolerance and lack of immunity and therefore more susceptible to infection (Gonfa *et al.,* 2013).

According to our results, highly significant association between the present of ticks and ovine babesiosis infections (P =0.000). the presence of ticks on sheep suggest that there is risk of animals being infected with *Babesia* as well as other tick-borne disease.

In our survey, use of treatment has been investigated for the prevalence of ovine babesiosis. The highest prevalence of infection was in those not using treatment (12.4%) while the prevalence was lower in those used treatment (11.4%). No association was reported (P =0.827).

Use of acariside was considered an important risk factor in ovine babesiosis infections. Our data showed that the sheep subjected to acaridae are less infection than those not exposed with prevalence rate (11.4%) and (12.4) respectively.

In the presence study, different ages of sheep have been investigated for the prevalence of ovine babesiosis infection. The highest prevalence of infection was recorded in old (16.2%) followed by adult(11.0%), young (8.3%) and lastly suckling (.0%). No association between ovine babesiosis infection and different investigated ages of sheep was reported (P =0.273).

According to our observations, there was a significant association between raising system and prevalence of ovine babesiosis (P=0.000). Closed system was more likely to have ovine babesiosis (100.0%) than open (.4%).

Different districts of Gibash locality have been investigated for the prevalence of ovine babesiosis infection. The highest prevalence of infection was recorded in Gibash (20.0%) followed by Um-sidare (19.3%), Um-zgaia (14.7%), Diglowa (5.0%) and the lowest one recorded in wad gasim (3.3%). positive association between ovine babesiosis infection and different investigated areas was reported (P =.025) and this could be due to similarity in management system adopted in these areas.

Conclusion and Recommendation

Conclusion:

sheep reared in Gibash locality are infected with *Babesia* parasites and the prevalence of the infection appears to be high. The microscopic examination used in this study could detect *Babesia*parasites, but about species seemed not to be appropriate, that means the test have low sensitive, so more studies is required used test with high sensitive.

4.1.Recommendations:

1- Additional surveys should be conducted to cover west kordofan state and further investigation for the epidemiology and control of the disease.

2- Effective control measures must be applied to reduce ticks burden and consequently sheep health and production.

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

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