Sudan University for Science and Technology

College of Graduate Studies

Epidemiological Investigation of Anapalsmosis in Sheep and Goats in Khartoum State, Sudan

دراسة وبائية لمرض الانابلازما في الضان والماعز في ولاية الخرطوم, السودان

A Thesis Submitted to the College of Graduate Studies in Partial Fulfillment of the Requirement for the Degree of Master in preventive veterinary medicine (MPVM)

By

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بسم الله الرحمن الرحيم

قال تعالى:

(ثمانية أزواج من الضأن أثنين ومن المعز أثنين قل ءالذكرين حرم أم الأنثيين أما اشتملت عليه أرحام الأنثيين نبئوني بعلم إن كنتم صادقين)

صدق الله العظيم

الانعام الاية(143)

Dedication

- **To my mother.**
- **To my father soul.**
- **To my brother & my sisters.**

Acknowledgement

All my thanks firstly and lastly to my god.

I would like to express my grateful thank to **professor**. **Siham Elias Suliman** for hers guidance, help, advising, directing, and carefully review of the research.

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ABSTRACT

Across sectional study was conducted in Khartoum State, Sudan, during the period extended from December 2016 to April 2017 to estimate the prevalence of anaplasmosis in sheep and goats and to investigate the risk factors associated with disease. Atotal of 150 whole blood samples were collected randomly from Sheep (80 samples) and goats (70 samples). Thin blood smear technique was applied. Questionnare was filled to collect a data of potential risk factors associated among with this disease. The results showed that the prevalence of the disease localities was 11% in Sharg Elneel and 14%inOmbeda. The overall prevalence of the disease was 12%. The risk factors were not associated with anaplasmosis. In conclusion, the presence of *A.ovis* in different localities was important factor.

ملخص البحث

أجريت الدراسة لمقياس انتشار مرض الانابلازما في الضأن والماعز وعوامل الخطر المرتبطة به المرض في الفترة من ديسمبر2016 الى أبريل 2017 في ولاية الخرطوم, السودان. تم جمع150 عينة دم عشوائيا من الضأن(80 عينة) والماعز(70 عينة). باستخدام المسحة الرقيقة للدم وجمع المعلومات في استبيان عن عوامل الخطر المرتبطة بحدوث المرض, أظهرت الدراسة اختلافا في معدل انتشار المرض بين المحليات التي اختيرت عشوائيا: شرق النيل 11% و14% في امبده وكانت النتيجة الكلية لتفشي المرض 12% ولم توجد علاقة معنوية لعوامل الخطر وحدوث المرض, ختاما وجود طفيل الانابلازما في الدم هو أهم عامل.

INTRODUCTION

Anaplasmosis is a disease caused by arickettsial of parasitic of ruminates, *Anaplasmaspp*. This bacteria (*Anaplasma*) belongs to the Genus *Anaplasma*, Family Anaplasmatoceae, order Rickettsiales and Kingdom Procaryota. *Anaplasmaspp* are mainly infected farm animals and to a lesser degree infected people. Members of *Anaplasma* are obligated intracellular microorganisms, gram negative bacteria, living in the blood cell of mammals, and caused severe disease in domestic animals (Schmidt*etal*., 2005).

The parasite (*A.ovis*) mainly exists in small ruminants such as sheep and goats, and it is presente has been confirmed in most regions of world both in farm and wild animals (Kuttler, 1984). Similarly to *A.ovis*, *A.marginale* and *A.centrale*, are liveing in the erythrocytes of the infected animal (Splitter *etal.*,1956).

The first report of anaplasmosis in sheep was presented in Zimbabwe (Bevan *etal.*, 1912). Anaplasmosis transmitted mainly cyclically by various Ixodid tick species, including; *Dermacentorspp*, *Rhipicephalsspp*, *Hyalommaspp*, *Boophilusspp*, *Argasspp* and *Amblyommaspp*. Anaplasmosis can also transmitted mechanically by Heamatophagus arthropods such as *Tabanusspp*, *Stomoxysspp* and *Mosqutoesspp*. It can also be transmitted through placenta during preganancy(Mohammad *etal* ., 1998; Rymaszewska and Grenda ,2008;Tembue*etal.*,2011).

The dynamics of infection by Anaplasmosis depend on several factors including ; transmission capacity of the vectors, susceptibility of sheep and goats, age and physiological and immune status. In addition, the agroecological and climatic condition of each geographical region also play role in the dynamics of infection (Tembue*etal*., 2011).

The severity of the disease depend on age, general condition of the animals and their breed. Generally, the disease is characterized by depression ,debility, weight loss, fever and aneamia and reduction in milk production . The infection is also lethal (Splitter *etal*.,1956;Shompole *etal*.,1989).

Clinical symptoms occurring in animals resulting from the presence of *A.ovis* in the blood cause huge losses in farming stock. This has significant effect specially in poor countries in tropical and subtropical regions where sheep and goats, are the main source of milk and meat, and also provide farms with natural manure which used in agriculture process (Jensen,1955).

Atentative diagnosis of anaplasmosis can based on clinical signs. However definitive diagnosis can be made by Giemsa stained of peripheral blood smears and polymerase chain reaction (PCR) techniques. The demonstration of anaplasma inclusion bodies in stained erythrocyte smears is commonly used technique that is sensitive during the acute phase of the disease, but parasite is seldom detected in chronic infection (Tembue*etal.*, 2011).The molecular diagnosis (PCR) allows reliable detection of the organism in chronic case. In addition in direct methods which detect specific antibodies against *A.ovis* surface antigens include the rapid agglutination test and competitive enzyme-linked immunosorbant assay (ELISA).

In the Sudan anaplasmosis is one of main constraint that retarded the intensive animal production system due to the considerable economic losses incurred. Yet there are scanty studies to handle the impact of bovine anaplasmosis. Occurrence of *A.marginale* parasites among dairy farm cattle was observed with prevalence rate ranging between 2.01 to 39.1 % using blood films and 50% using IFA test (Mohammed *etal*., 1998; Sulieman, 2004; Adam, 2005). Intra-uterine transmission of *A.marginale* has been reported for the first time in the Sudan in a calf 3-4 days old (Mohamed *etal.*,1998).

The objective:

1- To estimate the prevalence of Anaplasmosis in sheep and goats in Khartoum state, Sudan.

2- To investigate of the risk factors associated with the disease.

CHAPTER ONE

LITERATURE REVIEW

1.1. Etiology :

The first reports on *A.marginale* appeared as early in 18 century when Salmon and Smith (1896) detected the presence of inclusion in calf erythrocytes (Salmon and Smith *etal*.,1896). The first full description for the organism came from Theiler (1910), who observed bacteria in erythrocytes of South Africa cattle (Theiler *etal*.,1910).

A.ovis was first described in 1912 by Bevan (Bevan,1912). The subsequent years other species of Anaplasma which are pathogenic to animals, such as *A.bovis* and *A.platys* was also described. A number of research illustrated that Anaplasmosis might affect humans. However, the first case of Human Granulocytic Anaplasmosis(HGA) was reported in 1994 in USA caused by A.phagocytophilum (Rymaszewska*etal*.,2008).

1.2. Taxonomy:

In 2008, significant reorganizational changes were carried out within the order Rickettsiales . As a result of this reorganization, the family Ehrichiaceae was replaced by the family Anaplasmataceae, and some changes were made in the classification of the bacteria within the genera (Dumler*etal*., 2001).

Animal pathogens were attributed to the genus *Anaplasma* such as *A.marginale*, *A.centrale* ,*A.ovis*, *A.*bovis and also the etiological factor of human anaplasmosis*A.phagocytophilum.*

Therefore, the recent systematic classification (Rymaszewska*etal*., 2008) as follow :

Kingdom : Bacteria

Phylum :Proteobacteria

Order :Rickettsiales

Family :Anaplasmataceae

Genus :*Anaplasma*

Species :*A.marginale*

A.centrale

A.phagocytophilum

A. platys

1.3. Mammals species affected by *Anaplasmaspp* :

A.marginale and *A.centrale* infected cattle, *A.ovis* infected sheep,goats, *A.bovis* infected cattle, sheep and goats, *A.phagocytophilum* (HGA agent) infected sheep and goats and wild animals, horses , dogs and humans and *A.platys* infected dogs (Harvey *etal*., 1978; Kuttler, 1984).

1.4. Epidemiology:

Anaplasmosis is common and wide spread in all continent with coinciding distribution of the divers group of biological and mechanical vectors (Nabil, 2003). The present of enormous population of these vectors contributed to endemic infection in tropical and subtropical areas. infection occurs more sporadically in temperate climate (Nabil, 2003) .Endemic regions are usually characterized by a high tick infection rate and the disease maintained by the prevalence of both vectors and reservoir hosts. The efficient reservoirs for vector transmission are carrier animal, which are asymptomatic (Whittier *etal*., 2009).

The disease epidemiology in USA is complex and not well understood. The prevalence rate of *A.marginale* in cattle vary widely, and contributed of geographically stable and enzootic region (Braz*etal*., 2000).

Anaplasmosis is enzootic throughout the Southern Atlantic, Gulf coast several of the

mid western and states and it is endemic in the southeastern and much in the west caste (Braz*etal*.,2000). A variety of species of wild ruminants in both North America and Africa have been regarded as equally important reservoirs with cattle. Anaplasma

survives in nature through deer-to-deer (Nabil, 2003).

Differences in enzootic and epizootic areas in Africa are related to tick distribution and climate .The dynamics infection depends on vector competence, host susceptibility which varies among breed, age and agrocoiogical and climatic condition (Tembue*etal*., 2011).

In Europe countries including France ,Switzarland , Netherlands Australia , the disease distribution area advanced northward with the infection cases (Magnarelli*etal*.,2006) . In Australia infection is related to the Northern areas closely related to distribution of *Boophilus* the potential vector. In Europe, the existence of cattle infected with *A.marginale* has been noted in a local area the determinants of tick-and fly-borne transmission are not well understood (Bakirci*etal*., 2011).

Contemporary research conducted by various scientific centres shows that *A.ovis* is problem not only in poorer countries ,these bacteria have been detected in USA , Italy and Hungary,.(de al Fuente*etal* ., 2002 ,2005;Hornok *etal* .,2007).

In the Sudan, the presence of *A.marginale* in cattle very significantly between testes in different age groups, while gender seems not to have a significant effect (Mohammed *etal*., 1998;Sulieman, 2004; Adam, 2005; Awad*etal*., 2011).

In the Sudan, the blood samples from four states of the Sudan were collected from apparently healthy cattle (n=692). They were tested by PCR. The results confirmed the presence of *Anaplasmamarginale* in cattle with an overall prevalence rates of 6.1% Statistical analysis revealed that the prevalence of *A.marginale* varies significantly between Sudanese states as well as in different age groups. Sex has no significant effect on the prevalence of these pathogens among Sudanese cattle. The highest positive sample of *A.marginale* was reported in River Nile (Awad*et*al., 2011)

1.5. Transmission:

The organism transmites to animal by multiple routes, depending on geographic location. Carrier animal is considered as the principal source of infection. Spread from host to host occurs by arthropods either biological or mechanical vectors. A variety types of arthropods act as vectors but ticks, family of Ixodiae and biting flies, family of Tabanidae, are the main vectors (Ewing *etal*.,1981) .

Ticks are destructive blood sucking parasite, found in all countries of the world, but the great economic significance exists in tropical and subtropical zones (Anon *etal*., 1979). The family Ixodidae included the genera (*Dermacentorspp*, *Rhipicephalusspp* ,*Argasspp*, *Ixidesspp* and *Hyalommaspp*) are transmit Anaplasmsis in sheep and goats(Kahn *etal*., 2005).

1.6. Life cycle:

Anaplasmas are transmitted by a wide range of tick species and other insects.

Maturation can occur only in tick hosts, infectious bacteria reside and replicate within the salivary gland of vector and transmitted to mammalian hosts during blood feeding.

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.

This amplifies infection within the host and increases the likelihood of transmission when insects blood feed (https://en.wikipedia.org>wiki).

1.7. Pathogenesis and clinical signs of Anaplasmosis in sheep and goats:

Anaplasmamarginale invades and proliferates in red blood cells (RBCs) of domestic and wild ruminant. As the disease progresses red blood cells are destroyed in liver and spleen, resulting in, fever, weakness, loss of appetite, increase respiratory rate, anaemia and jaundice even death in severe cases .*A.ovis* similarly to

A.marginale and *A.centrale* ,these bacteria live in erythrocytes, but mainly parasitizes

small ruminant(sheep , goats) , and its presence has been confirmed in most regions of the world both farm and wild animals(Kuttler, 1984), and hence the aneamia of the infected animals(Splitter). Between 35-40% of *A.ovis* are found in the central or submarginal part of the erythrocytes while between 60-65% found in the marginal part of erythrocytes (Shompole*etal*., 1989).

The symptom that occur with Anaplasmosis in sheep and goats were usually subclinical, but clinical manifestation may be triggered by malnutrition and other stress condition such as infection with another blood parasite. The most consistent findings include, fever, depression, weakness, pale mucous membranes, difficulty breathing and increased heart rate(Ray *etal* 2007).

1.8. Economic impact of Anaplasmosis:

Keeping in view the economic impact of the anaplasmosis can be expressed in terms of mortality, loss of production(including live weight gain, milk production and draught potential), the cost control and in some cases, restriction placed on the movement of the animals(Norval*etal*.,1991). As in the case of other areas of management, there are few reliable estimates of economic loss resulting from anaplasmosis. Infestation generally causes local irritation resulting in wound, which predispose the host to attacks by blowflies, scare worms and secondary bacterial infection (Soulsby, 1982).

Another aspect of economic impact of ticks is heavy losses in live weight gain, which is attributed to tick infestation (Mohammad, 2003). Anaemia caused by loss of blood imbibed by ticks is another important impact of ticks on livestock industry knowing the amount of blood taken by a particular vector is important (Rechav*etal*., 1994). On the other hand, many ticks transmit serious disease agents such as protozoa, bacteria, rickettsia and viruses (Anon, 1979).

1.9. Diagnosis of Anaplasmosis:

History, clinical signs and post-mortem lesion are suggested of Anaplasmosis infection, and cannot be regarded as pathognomonic for the disease until to make an

accurate diagnosis. Hence laboratory conformation is necessary for accurate diagnosis of the disease .Whole blood , serum and DNA are the commonly examined directly or

indirectly for the presence of *Anaplasma*. Laboratory diagnostic techniques of Anaplasmosis as general divided into three main categories : parasitological, serological and molecular methods .

1.9.1. Parasitological techniques :

Parasitological techniques provides directed evidence of anaplasma infection. It includes; microscopic examination of ethanol fixed Giemsa stained thin blood smears, to identify of the *A.*ovis and determine the parasitemia of the infected animal (Yoshihara *etal*.,2003; Siddiki*etal*.,2009).

1.9.2. Sreologicaltechniques :

Serological diagnosis provides indirect evidence of anaplasma infection by demonstration specific antibody or antigens. The adopted serological tests include; competitive enzyme-linked immune sorbent assay (CELISA) and indirect fluoscent antibody assay (IFA) (Sandor*etal*., 2007).

1.9.3. Molecular techniques :

The principle of molecular test is the demonstration of nucleotides, that are specific for *Anaplasma* species or even type or strain. A positive result indicates infection with the species for which the sequences are specific, as parasite DNA will not persist for long in the host after live parasites have been eliminated. The molecular technique in use is the polymerase chain reaction (PCR) (Sandor*etal*., 2007).

1.10. Control of Anaplasmosis:

Vector control ensures that incidence of the disease kept within manageable limits. The two commonly method are application of chemical acaricides. However , the cost of and acaricides makes the use of acaricides a less attractive option . Moreover , the problem of acaricides resistance is a further dimension in acaricideapplication besides pollution and effects on the non-target organisms(Badria*etal*., 1997).

Chemotheraby of sick animal is widely use. It is used under veterinary supervision. In conjunction with acaricidal control, for some measure of pre-immunity to be established against the disease. Drugs can be used during the period of initial

exposure in a method known as chemo-immunization (Ilembade, 1991). However, the method can be expensive and may be create problem. Immunization using live blood vaccines has been used only under experimental condition (IIembade , 1991). In endemic countries, control of anaplasmosis rather than eradication is the only realistic option, especially where the infection of non-bovine reservoirs and variety of vector species are existed. Active control of Anaplasmosis is achieved by immunization, chemoprophylaxis and vector control (Vos ,1991) .Control of tick population using chemical acaricides is carried out by dipping or spying. This method of control is very costly, less efficient and ecologically undesirable (Latif and Pegram, 1992). A major problem facing chemical control is the ability of ticks to develop resistance against acaricides (Wharton et al.1970; Luguru*etal.*, 1984). Biological control depends on using natural tick enemies such as parasitoids, pathogens and predators (Mwangi*etal*., 1991) in addition to host resistance and anti-vaccines. The latter is expected to reduce on acaricides and other method (Willadsen*etal*., 1995). The reliance on any single method of tick control often causes problems or lead to breakdown in the all control system.

One world during theout break the sick animal should not be moved to avoid animal collapse and then death. In addition to that healthy animals should be moved away from the sick ones and provide adequate protection for the susceptible one (Richey, 2003).

1.11. Vaccination :

World wide vaccination is economical and effective means to Anaplasmosis . For effective vaccination it is recommended to administer an initial vaccination in the first year in form of 2 doses given 4 week apart scheduled so that the second dose is given at least 2 week before the vector season begins. Additional boosters at least every year should be applied2week before the next vector seasonto provide adequate protection (Kocan*etal*.,2007) .

Although there are different type of vaccines. These vaccines induce protective immunity that reduce disease severity but does not prevent persistent infected with *Anaplasma* (Richey, 2003).

1-Live Anaplasmavaccines :

This attenuated vaccines by irradiation and passaged through deer and sheep. This vaccine affords solid protection against virulent challenge (Corrier*etal*.,1985).

2- Non-living vaccines :

Non-living vaccines overcome difficulties inherent in the production, transportation and use of live vaccines. Anaplasma vaccine based on lyophilized preparation of organisms is administered with adjuvant. Yearly boosters of vaccine are recommended and the level of protection afforded is dependent upon the nature of the challenge isolate (Plamer*et.al*,1989) .

3-Subunit vaccines :

The vaccine strain used comprised several sub-population with variable biological and genomic characteristics (Carson *etal*., 1990).

1.12.Treatment of Anaplasmosis in sheep and goats :

Usually livestock owners are not concerned about the control of Anaplasmosis, when the clinical signs are noticed. The animal is almost over the acute infection stage and suffering from anemia. Subsequently the treatment of the affected animals is required. It is recommended that a single treatment with long acting LA-200200mg/ml oxytetracycline at the rate of 9mg/BWthis does will be affective rather than repeated treatment with a lower concentration of oxytetracycline. Blood transfusions may be indicated in severe cases (Whittier *etal*., 2009).

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study Area:

The study was carried out in Khartoum state which lies between longitudes 31.5-34E^{o} and latitude 15-16N^{o} in an area of 28165 square kilometers. It is located in the central part of the Sudan bordered by Nile state to the North, North Kordofan and White Nile state to the west ,Gedarf and Kassala states to the east and el-Jazira to the South direction.

The state is consists of three big cities namely; Omdurman City, Khartoum City and Khartoum North City. The state has desert and semi-desert climate and receives little in-frequent rain with an average less than 300mm per year, and is characterized by three distinct seasons; cold-dry from November to February, hot-dry from March to June and hot-wet season from July to October; The highest temperatures occur during March and May, ranging between 35-43C^{o} and the lowest temperatures occur during December and February ranging between 15-28C^{o}.

2.2. Study Design:

A cross section survey was conducted to determine prevalence of Anaplasmosis in sheep and goats and also to investigate the risk factors could be associated with the disease. Using Multistage Simple Random Sampling program the study areas were selected; at first level splited up Khartoum state into the two cities Khartoum North and Omdrman. In the second level, East Nile and Ombeda were randomly selected. In the third level, selected the sample from 2area East Nil and Ombeda.

2.3. Sample Size:

The sample size was calculated according to formula of Thrusfield (2007).

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

Where:

n = Sample size

$(1,96)^2$ = constant

P_{exp}= expiated prevalence

d =desired accuracy level at 95% confidence interval

The expected prevalence was calculated depended on previous study, they found that the prevalence of anaplasmosis was 8.33% (Nasreen*etal*.,2016).

n =$[(1.96)^2$ (0.08)(0.92)]/0.0025 = 113 animals. To increase the precision of the study the sample size was completed till 150 samples.

2.4. Sample Collection:

Whole blood samples were collected from 150 randomly selected sheep (80 samples) and goats (70 sampls) found in Alseleate in East Nile and Ombeda localities , respectively . Whole blood from jugular vein was collected in EDTA.K3 tube and stored in container with ice pack, cold water and transported to laboratory for diagnosis of blood parasitis.

2.5. Diagnostic of Anaplasmosis:

2.5.1. Thin Blood Smear Technique:

The thin blood smears were made, as per method described by (Murray *etal*., 1977) air dried smears were fixed in absolute methyl alcohol for 2-3 minutes. The slide were immersed in 10% Giemsa's stain for20-25 minutes and washed with tape water to remove excess stain. After air drying the slides were examined under oil immersion objective lens (100x) for detection and identification of *A.ovis*.

2.6. Questionnaire Survey:

A questionnaire was filled via interview to collect information regarding the selected potential risk factors that might be associated with the prevalence of Anaplasmosis in sheepand goats. The questionnaire contained three types of factors ;

animal factors such as breed ,gender and age. Farm factors such as grazing system and farm hygiene. Environmental factors such as temperature and humidity.

2.7. Data Analysis :

Data derived from the questionnaire and laboratory examination were entered and preserved in Microsoft Program Excel program and then entered into using SPSS Microsoft Program version 1,0. The data were analysis by simple descriptive analysis using frequencies and cross tabulation.

Univariate analysis was used to estimate the significance of the association between the risk factors and the disease using the Chi-square(X^2), degree of free dame(df)and P value ($P<0.25$).

CHAPTER THREE

RESULTS

3.1. Overall prevalence rate of Anaplasmosis in sheep goats in Khartoum State:

During this survey which was carried out in the Khartoum State , 150 samples was collected, among these samples (70) from goates and (80) from sheep . The overall prevalence of Anaplasmosis was 12%, 12.9% in goats and 11.3% in sheep (Table 1) .

Table 1: The prevalence rate of Anaplasmosis in sheep and goats in Khartoum State:

Speices	No. tested	No. of positive	Infection %
goats	70	9	%12.9
sheep	80	9	%11.3
Total	150	18	12%

The prevalence rate of Anaplasmosis in sheep and goats based on farms:

Using Gemsa-stained thin blood smears, the result revealed that 3 out of 17 (17.6%) , 2 out of 15 (13%) , 2 out of 18 (11%) and 2 out of 20(10%) of goats were infected with Anaplasma in Abdalrahman Farms, Hosham Farms, Alatrack Farms and Ali Farms, recpectively. Regarding the samples obtained from sheep , the result revealed that 3 out of 25 (12%), 1 out of 25(4%) and 5 out of 30(16%) of sheep were infected with *Anaplasma* in Hosham Farms , Alatrack Farms and Ganndahar market , recpectively . Among these localities ,ShargElneel goats scored the highest infection rate . However, the Chi-square analysis (P-value=0.594) showed no significant association between anaplasmosis infection rate and the localities (Table 2).

Table 2: The prevalence of Anaplasmosis in sheep and goats in Khartoum State, Sudan:

Table 2: The prevalence of Anaplasmosis in sheep and goats in Khartoum State, Sudan:

Speices	Locality	The Farms	No.tested	No.+ve	Infection %	Chi-square
goats	ShargElneel	Abdalrahman Farms	17	3	17.6%	
		Hosham Farms	15	2	13%	X^2=0.284
		Alatrak Farms	18	2	11%	
	Ombada	Ali Farms	20	2	10%	P=0.594
	Total		70	9	12.9%	
Sheep	ShargElneel	Hosham Farms	25	3	12%	df = 1
		Alatrak Farms	25	1	4%	
	Ombada	GanndahaMarkt	30	5	16%	
	Total		80	9	11.3%	
Total			150	18	12%	

3.2.Risk factors contributed to Anaplasmosis in sheep and goats :

3.2.1. Theprevalenc of Anaplasmosis in sheep and goats according to the herd size :

The examined animal in these study were obtained from three groups of herd size, small herd size, medium herd size and larg herd size. The obtained result showed that 2 out of 15(13.3%) of animals from small herd size, 11 out of 105 (10.5%) of animals from medium herd size and 5 out of 30(16.7%) of animals from larg herd size were infected with the *Anaplasma* . High infection rate was reported in the animals from medium herd size . The Chi- square tested showed no significant difference (P=0.646) between the infection and herd size (Table 3).

3.2.2. The prevalence of Anaplasmosis in sheep and goats according to the speices :

The examined animlsfrom the various localities were classified based on speices. out of80 sheep and 9 out of70 goats were infected with *A .ovis* , with prevalence rate of 11.3% and 12.9% respectively . No significantly association (0.763) between the infection with Anaplasmosis and the speices of the animal (Table 3).

3.2.3.TheprevalenceofAnaplasmosisofsheepandgoatsaccordingtotheanimalage:

The examined animals from the various localities were classified based on age into

two groups, young (0-1years) and old (over 1 years) animals . The results showed that 3 out of 34(8.8%) and 15 out of 116(12.9%) were infected with *A .ovis*recpectively .

No significantiy (P=0.517) different between the infection rate and age of and (Table 3) .

3.2.4. The prevalence of Anaplasmosis of sheep and goats according to the sex:

The examined sheep and goats from of various localities were classified based on sex. The result showed 8 out of 83(9.6%) of female and 10 out of 67(14.9%) of male were infected with*A.ovis*. No significant association (P=0.322) between the infection rate and gender of animals (Table 3) .

3.2.5. The prevalence of Anaplasmosis in sheep and goats according to the body condition :

The examined animals in these study were classified according to the animal body condition into medium, good and very good body condition .The infection rate was higher in animals that had either (medium or good and very good) body condition compared with those had poor body condition 11.6% , 15.4% and 14.3% respectively .The chi-square tested showed there was no significant (P=0.954) association between the body condition and *A.ovis* infection rate(Table 3).

3.2.6. The prevalence of Anaplasmosis in sheep and goats according to the grazing system :

The higher *A.ovis* infection rate was reported in the herd grazing outdoor(16.7%) compared with the herd grazing indoor (10.8%) .However, these observations have no significant association(P=0.773) with the infection with *A.ovis* (Table 3).

3.2.7.The prevalence of the Anaplasmosis in sheep and goats according to the season :

A higher *A.oivs* infection rate (11%) in cold season compered with hot season (14%). No significant association (P=0.284) with the infection rate and season (Table 3) .

All examined animals in this study from various localities and farms were presented insect and ticks in farms and used acarecide to control of ticks infection (12%). No

significant association between the infection of *Anaplasma* and percent of insect,

present of ticks in farm and use acarecide to control of ticks infection.

Table 3: Univariate analysis of risk factors associated with Anaplasmosis infection in sheep and goats (n=150) in Khartoum State:

Risk factors	No.Tested	No. +ve(%)	df	X^2	P-value
Locality			1	0.28	0.594
East Nile	100	11(11%)		4	
Ombeda	50	7(14%)			
Herd size			2	0.87	0.646
Medium	105	11(10.5%)		5	
Small	15	2(13.3%)			
Larg	30	5(16.7%)			
Speices			1	0.01	0.763
Goats	70	9(12.9%)		9	
Sheep	80	9(11.3%)			
Age			1	0.42	0.517
Young	34	3(8.8%)		0	
Old	116	15(12.9%)			
Sex			1	0.98	0.322
Male	67	10(14.9%)		1	
Female	83	8(9.6%)			
Body condition			3	0.32	0.954
Poor	7	1(14.3%)		9	
Medium	129	15(11.6%)			
Good	13	2(15.4%)			
Using prophylactic treatmentfor disease			-	-	-
Yes					
No	150	18(12%)			
Sick animal in farm		18(12%)	-		-
Yes	150	18(12%)		-	
No					
Grazing system			1	0.77	0.379
Indoor	120	13(10.8%)		3	
Outdoor	30	5(16.7%)			
Farm hygien			1	0.77	0.379
Good	120	13(10.8%)		3	
Poor	30	5(16.7%)			
Season			1	0.28	0.594
Cold Season	100	11(11%)		4	
Hot Season	50	7(14%)			
Present of insect			-		-
Present	150	18(12%)		-	

Not present					
Present of tick in farm Present Not present	150	18(12%)	-	-	-
Use acarecide to controlling Use Not use	150	18(12%)	-	- 	-

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.CHAPTER FOUR

DISCUSSION

The research of sheep and goats anaplasmosis is rare and little literature is available. This study was designed to report the prevalence of anaplasmosis in sheep and goats in Khartoum Stat in Sudan. In this study, 150 blood samples from sheep and goats were collected from December 2016 to April 2017 from 5 farms in Khartoum stat to identify prevalence of anaplasmosis.

The present study showed some epidemiological aspect of ovine and caprineanaplasmosis. The occurrence of anaplasmosis at two regions i.eEest Nile and Ombada is influencd various factors like environment and presence of vector.

In this study, the prevalence of anaplasmosis by microscopy was 11.3% in sheep , 12.9% in goat and 12%in total.

In other study like Nasreen*etal*.(2016), the prevalence of anaplasmosis by microscopy was 13.89% in sheep, 8.33% in goat and by ELISA 23.89% in sheep, 20.56% in goat similar results recorded byRazmi*etal*. (2006) 47.5% in sheep and 80.3% by microscopy.

In this study the infection rate ofanaplasomosis in goat higher than sheep because No. of positive is similar and No. of tested animals in sheep higher than goat. No significant association between the infection with Anaplasmosis and all risk factors because samples size is small (150samples) divided into 80 samples in sheep and 70 samples in goat and diagnostic technique was used microscopic examination of thin blood smears preparation only was not specific test compare with ELISA and PCR test.

Females (sheep) were more susceptible (24.56%) than males (22.72%) in Nasreen*etal*, 2016. In this study males are more susceptible (14.9%) than females(9.6%) compare with other farms.

The present result were similar to the Razmi*etal*, (2006), Nasreen*etal*,(2016)

reported that in animals greater than one year of age anaplasmosis is predominantly in

autumn and winter.

Tick vector population in the farms of Khartoum state is present during sample collection and this may be the reason of comparatively the prevalence rate of infection in the region.

CONCLUSION

This research is not the first epidemiological study of Anaplasma infection in sheep and goats from Khartoum State in Sudan and it is concluded that anaplasmosis is prevalence in Khartoum State.

In view of our findings, anaplasmosis in sheep and goats due to*A.ovis* is prevalence in Khartoum State farms. The study also concluded that the difference in loacalities were the most important factors of the disease.

RECOMMINDATION

Therefore based on the above conclusion the following points are forwarded as recommendation:

1- Wide studies are needed to know the epidemiology of *Anaplasmaspp* infection in sheep and goats in Sudan by using advanced serological and molecular techniques, mainly to define which species is involved.

2-Extention service and training programs aiming at creation of awareness about the importance and prevention of anaplasmosis in sheep and goats.

3-Some epidemiological risk factors associated with anaplasmosis prevalence in the present study need confirmation in further studies.

4-Tick control.

5-Vaccination.

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Appendix 1

Table: Frequency distribution of 150 animals examined for anaplasmosis according to potential risk factors in Khartoum State:

Risk factor	Frequency	Relative frequency %	Cumulative frequency%
Locality			
ShargElneel	100	66.7	66.7
Ombeda	50	33.3	100.0
Herd size			
Small	15	10.0	10.0
Medium	105	70.0	80.0
Larg	30	20.0	100.0
Species			
Goats	70	46.7	46.7
Sheep	80	53.3	100.0
Age			
Young	34	22.7	22.7
Old	116	77.3	100.0
Sex			
Male	67	44.7	44.7
Female	83	55.3	100.0
Body condition			
Poor	7	4.7	4.7
Medium	129	86.0	90.7
Very good	13	8.7	99.3
Using prophylactic treatment for disease			
Yes	150	100.0	100.0
No			

Grazing system Indoor Outdoor	 120 30	 80.0 20.0	 80.0 100.0
Farm hygien Good Poor	 120 30	 80.0 20.0	 80.0 100.0
Season Cold Season Hot season	 100 50	 66.7 33.3	 66.7 100.0
Present of insect Present Not present	 150	 100.0	 100.0
Present of tick in farm Present Not present	 150	 100.0	 100.0
Use acarecide to controlling Use Not use	 150	 100.0	 100.0

.**Table : Cross tabulation of anaplasmosis infection in 150 animals classified according to the potential risk factors in Khartoum State:**

Risk factor	**Number tested**	**Number+ve**	**%Percent**
Locality			
ShargElneel	100	11	11%
Ombeda	50	7	14%
Herd size			
medium	105	11	%10.5
Small	15	2	13.3%
Larg	30	5	16.7%
Speices			
Goats	70	9	12.9%
Sheep	80	9	11.3%
Age			
Young	34	3	8.8%
Old	116	15	12.9%
Sex			
Male	67	10	14.9%
Female	83	8	9.6%
Body condition			
Poor	7	1	14.3%
Medium	129	15	11.6%
Very good	13	2	15.4%
Using prophylactic treatment for disease			
Yes	150	18	12%
No			
Sick animal in farm			
Yes	150	18	12%
No			
Grazing system			
Indoor	120	13	10.8%
Outdoor	30	5	16.7%
Farm hygien			
Good	120	13	10.8%
Poor	30	5	16.7%
Season			
Cold Season	100	11	11%
Hot Season	50	7	14%
Present of insect			

Present Not present	150	18	12%
Present of tick in farm Present Not present	150	18	12%
Use acarcide to controlling Use Not use	150	18	12%

Appendix 2

Questinnair to investing of the prevalence and risk factors of the Anaplasmosis of the sheep and goats in Khartoum State, Sudan:

1-Questinnair n( )

2-Locaty

ShargElneel ( ) Ombeda ( )

3-Herd size

Small ( ) Medium ( ) Larg ( )

4-Species

Goats ( ) Sheep ( )

5-Age

Young(0-1years) ( ) Old (over 1 year) ( )

6-Sex

Male ( ) Female ( )

7-Body condition

Poor ( ) Goog ( ) Very good ( )

8-Using prophylactic treatment of disease

Yes ( ) No ( )

9-Sick animals in farm

Yes ( ) No ( )

10-Grazing system

Indoor ( ) Outdoor ( )

11-Farm hygien

Good ( ) Poor ( )

12-Season

Cold season ( ) Hot season ( )

13-Present of insect

Present ( ) Not present ( )

14-Present of ticks in farm

Present ( ) Not present ( )

15-Use acarecide to controlling

Ues ( ) Not use ( )