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Prevalence and Risk Factors of Bovine Trypanosomiasis in Khartoum State, Sudan

معدل الاصابة بداء المثقبيات والعوامل المؤاثرة علي حدوث الاصابة في ولاية الخرطوم- السودان

Dissertation submitted to the College of Graduate Studies in Partial Fulfillment of the Requirement of Master Degree of Preventive Veterinary Medicine (MPVM)

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DEDICATION

To those who encouraged me from my birth day till now… to those who made the impossible, possible… to those who turned my life into a better life….

MY PARENTS.

MY HUSBAND.

MY KIDS.

Reem Hajo

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Abstract

A cross-sectional study was conducted in cold dry season from November (2014) up to January (2015) in Khartoum state, Sudan to estimate the prevalence of bovine trypanosomiasis and the prevailing species of trypanosomes as well as to assess host related risk factors.

Blood samples collected from 380 randomly selected cattle from 3 locality were subjected to parasitological examination (Haeatocrit Centrifugation Technique (HCT) and Blood smear).Also packed cell volume(PCV) of each animal was measured for anemia estimation .

The overall prevalence was found to be 21.6%. *Trypanosoma vivax* was the predominant species in the area. The Chi-square test showed that there were 13 out of 19 risk factors show statistically significantly association in bovine trypanosome prevalence (p-value≤0.25). These were: Age- chi square results showed significant differences in trypanosome prevalence between adults, young stock and calves ($\chi 2$ = 24.873, df = 2, p-value =0.000). Body condition show significant differences in trypanosome prevalence between good and poor ($\chi 2$= 27.198,df=1,p.value=0.000). Locality ($\chi 2$ = 10.478, df = 2, p.value=.005), using prophylactic treatment ($\chi 2$= 38.050,df=1,p.value= 0.000), presence of vectors in the farm ($\chi 2$= 5.578,df=1,p.value= 0.018), using insecticide for controlling vectors ($\chi 2$= 71.416,df=1,p.value =0.000).

The risk for trypanosomiasis was therefore positively associated with insect apparent density (χ=28.759 df =2, p-value =0.000), presence of other animals in the farms (χ=6.403 df = 1, p-value =.011, prsence water body and irrigations canals (χ=4.855 df = 1, p-value =0.028), As for vegetation (χ=7.166 df = 1, p-value= .007.There were significant differences (χ=9.570

df = 1, p-value =0.002) between trypanosome prevalence proportions in cattle raised under different husbandry practices, the overall mean PCV (χ=7.663 df = 1, p-value= 0.006) and presence of wild life around the farm (χ=16. 694df =1, p-value=0.000). There were six risk factors not significantly associated with trypanosome infection in cattle in our study (p-value > 0. 25) .the sex (p-value=0.348), production type (p-value 0.348), herd size (p-value =0.998), presence of other disease (p-value =0.438), Breed (p-value= 0.605) and Location of life stock market (p-value =.479).

The Multivarte analysis using the Logistic Regression showed that there were 7 potential risk factor significantly associated with trypanosomiasis (p-value $\leq$0.05).These were age,(p-value=0.001), locality(p-value=0.026), using prophylactic treatment (p-value=0.46), using insecticide for controlling insects (p-value=0.002), presence of other species of animals (p-value =0.010),density of insects (p-value= 0.016) and presence of wild life around the farm (p-value =0.018).

Finally, this work showed that trypanosomiasis is an important disease affecting the health and productivity of cattle in the Khartoum State. Hence, attention should be given to this problem so as to improve livestock production and agricultural development in the area.

ملخص البحث

اجريت دراسة استقطاعية علي عدد 380 رأس من الابقار في ولاية الخرطوم، خلال فترة الشتاء الجاف في الفترة من 11نوفمبر 2014م- يناير 2015م. كان الهدف من الدراسة تقدير معدل إنتشار داء المثقبيات في الابقار ومعرفة النوع المسيطر علي الاصابة في الولاية بالاضافة الي التحقق من عوامل الخطر المرتبطة بالمرض.

تم أخذ عينات دم من الابقار المختارة عشوائياً من ثلاثة محليات مختلفة من الولاية وإجراء الطرق التشخصية للفحص (فحص الشريحة بواسطة المجهرو اختبار HCT كما تم اجراء اختبار نسبة حجم مكبوس الخلايا(PCV) لتقدير نسبة فقر الدم للحيوانات المفحوصة) اظهرت الدراسة معدل انتشار لداء المثقبيات في الولاية 21.6% كما وجد ان النوع المسيطر هو *Trypanosoma vivax*.

عند تحليل قيمة عوامل الخطر باستخدام مربع كاي وجد أن هنالك عوامل مرتبطة ارتباط معنوي بالاصابة بالمرض وهي: المحلية (P-value=0.005), العمر (P-value=0.000), حالة الجسم (P-value=0.000), استخدام العلاج للحيوانات المريضة (P-value=0.000), وجود حيوانات من انواع اخرى بالمزرعة (P-value= 0.011), وجود الحشرات بالمزرعة (value=0.018), كثافة الحشرات فى المزرعة (P-value=0.000) ,استخدام المبيدات الحشرية للسيطرة على نواقل المرض (P-value=0.000), وجود حيوانات برية حول المزرعة

(P-value=0.000), كثافة الغطاءالنباتى (P-value=0.007), وجود قنوات الرى وأماكن تجمع المياه (P-value=0.028), نسبة تراكم الخلايا الحمراء (P-value=0.006), نوعية الرعى-P (value=0.002).

لكن توجد عوامل لم تظهر اى علاقة معنوية بالمرض و هي: السلالة (P-value=0.605), نوعية الانتاج (P-value=0.348), موقع المزرعة من اسواق الحيوانات الحية (P-value=0.479, حجم القطيع (P-value=0.998) وجود اصبات بامراض اخرى (P-value=0.438) والجنس(p-value=0.348).

عند تحليل العوامل المرتبطة بالمرض معنويا بواسطة التحليل المتعدد لمعرفة درجة الاتباط وجد ان سبعة عوامل اظهرت اتباطا بالمرض (P-value≤0.05) وهي:العمر(P-value=0.001), المحلية (P-value =0.026), استخدام العلاج للحيوانات المريضة (P-value=0.046), استخدام المبيدات الحشرية للسيطرة على نواقل المرض (P-value=0.002) ,وجود حيوانات من انواع اخرى بالمزرعة (P-value=0.018) , كثافة الحشرات فى المزرعة (P-value=0.016)ووجود حيوانات برية حول المزرعة (P-value=0.018) .

تشير الدراسة الي ان داء المثقبيات من اهم المشاكل الانتاجية في ولاية الخرطوم بالاضافة الي ان النوع المسيطر هو *T.vivax* و هو ينتقل ميكانيكيا.لذلك يجب اعطاء اهمية لهذه المشكلة. من اجل تحسين انتاجية القطعان والتنمية الزراعية في هذة المنطقة.

Introduction

Trypanosomes are protozoan parasites in the family *Trypanosomatidae.* Most trypanosomes are transmitted by tsetse flies. They include *T. brucei gambiense* and *T. brucei rhodesiense* responsible for Gambian and Rhodesian human African trypanosomiasis (HAT) respectively. Other species include *T. brucei brucei*, *T. congolense, T. vivax* and *T. simiae* those are causing Nagana or African Animal Trypanosomiasis (AAT) in tropical Africa between 14^{o} N and 30^{o} S, this area is referred to as the tsetse belt (Ikade, 1986).

Beyond this belt in and outside Africa, animal trypanosomiasis occurs in the form of Surra caused by *T. evansi*, Dourine caused by *T. equiperdum*. In addition, mechanically transmitted *T. vivax* infections occur in livestock in the Caribbean Islands and South America while chagas disease in humans is due to *T cruzi* (Soulsby, 1982).

Glossina species or tsetse flies are virtually confined to the Afro-tropical region, the genus *Glossina* contains 31 species and subspecies, the genus is assigned to three groups, *morsitans, palpalis* and *fusca* group, named after the commonest species in each group (Kettle, 2000).

Animal African Trypanosomiasis (AAT) signs in infected cattle ranged from totally asymptomatic chronic infections to wasting disease with severe haematological alterations, Central nervous system involvement in AAT caused by the *Trypanosoma brucei* subgroup parasites, abortion and death (Losos and Ikede, 1972; Soulsby, 1982).

Diagnosis of the disease is based on realizing clinical signs or symptoms, detection of the parasite using parasitological, serological or molecular techniques.

The most important trypanosome species affecting livestock in Sudan are *T. congolense*, *T. vivax* and *T. brucei* in cattle and *T. evansi* in camels. *T. brucei* and *T. congolense* are confined to *tsetse* infested areas (Karib, 1961; Hall *et al*. 1984; Kheir *et al.* 1993; Hassan, 2003). Animal African Trypanosomiasis due to *T. vivax* infection, which can occur in tsetse areas, has also become established in tsetse-free areas around the Blue Nile in central-western Sudan, where it is transmitted by biting flies (A/Rahman, 2005; Abdalla *et al.*, 2005).

Transmission of the disease is either cyclical by tsetse flies or mechanically by other biting flies like *tabanids* and *Stomoxys* species. Following the separation of South Sudan, the tsetse infested area in Sudan is reduced to about 50,000 km^2 (Hassan *et a.l*, 2011).

Of the seven *Glossina* species reported previously by Lewis (1949), only two species namely *G. fuscipes fuscipes* and *G. morsitans submorsitans* are found in South eastern Sudan and South Darfur (Hassan *et al,* 2011). Seventy species of *tabanids* flies and six *Stomoxys* were reported in Sudan and South Sudan by Lewis, (1953 and 1954).

Control of tsetse and trypanosomiasis in Sudan have been achieved using different techniques, such as bush clearing, game elimination, insecticide spraying, and use of traps and targets. Also in vegetation and selected rocky breeding sites using BHC and DDT was adopted as a method of control which proved to be successful (Abdel Razig *et al*., 1968., Yagi and Abdel Razig ,1969., A/Rahman *et al*, 2010). Using trypanocidal drug treatments have evolved as the principal method for controlling the disease.

Justification

Trypansoma infection restricts the animal production and cause economic losses by the clinical signs of the infection such as restricted growth, abortion, anaemia, treatment cost, and death of the affected animals.

The objectives

- To estimate the prevalence of the disease in Khartoum State.
- To investigate the related risk factors associated with the disease.
- To identify the prevailing species of *trypanosomes*.

Chapter One

Literature Review:

Trypanosomiasis are group of diseases that are caused by members of the genus *Trypanosoma*

1.1. Classification:

Trypanosomes are classified according to Kreier and Baker (1993) as follows:

Kingdom	:	Protista
Phylum	:	Protozoa
Subphylum	:	Sarcomastigophora
Superclass	:	Mastigophora
Class	:	Zoomastigophora
Order	:	Kinetoplastida
Suborder	:	Trypanosomidae
Family	:	Trypanosomatidae
Genus	:	*Trypanosoma*

The genus *Trypanosoma* is divided according to its site of multiplication in the vector and mode of transmission into two main sections: posterior station development (Stercoraria) and anterior station development (Salivaria).

Stercoraria includes members of the following subgenera:-

Subgenus *Megatrypanum*: *Trypanosoma (M.) theileri* is a common parasite of domestic and wild ruminants worldwide. This very large *trypanosome* has a cosmopolitan distribution among domestic cattle, while in Africa it is also found in various antelopes. Generally, infection is cryptic and is usually detected by blood culture; there is no evidence of any specific

pathological changes attributable to *T. theileri* and its effect on the bovine host is controversial (Hoare, 1972). The subgenus also includes *T. melophagium* a common parasite of sheep. *Tabanid flies* have been shown to be the major vector (Hoare, 1972).

Subgenus *Herpetosoma*: The great majority of trypanosomes of subgenus Herpetosoma parasitizes rodents and is not frequently implicated as agents of trypanosomiasis of medical and veterinary importance; it also includes *T. (S.) rangeli* and *T. (Herpetosoma) lewisi (*Stevens and Brisse, 2004)

Subgenus *Schizotrypanum:* the most important species is *T. cruzi* (Stevens and Brisse, 2004) the causal agent of American Human trypanosomiasis (chagas disease).

In Africa, pathogenic trypanosomes infecting livestock belong to the section of the Salivaria. Within this section, parasites are classified into four distinct subgenera namely *Dutonnella, Nannomonas, Trypanozoon* and *Pycnomonas* and furthermore into different species, this classification is based on several criteria such as the parasite's shape, its development in the vector, its host-range and clinical disease in hosts, and genetic markers (Masumu, 2006). The most important species of subgenus *Dutonnella* are *T vivax* and *T uniforme*, Subgenus*: Nannomonas*, important species are *T congolense* and *T simiae*, Subgenus: *Trypanozoon,* important species are *T brucei* (*T b brucei*, *T b gambiense* and *T b rhodesiense*), *T evansi* and *T* equiperdum and in the Subgenus: Pycnomonas important species is *T. suis* (Stevens and Brisse, 2004).

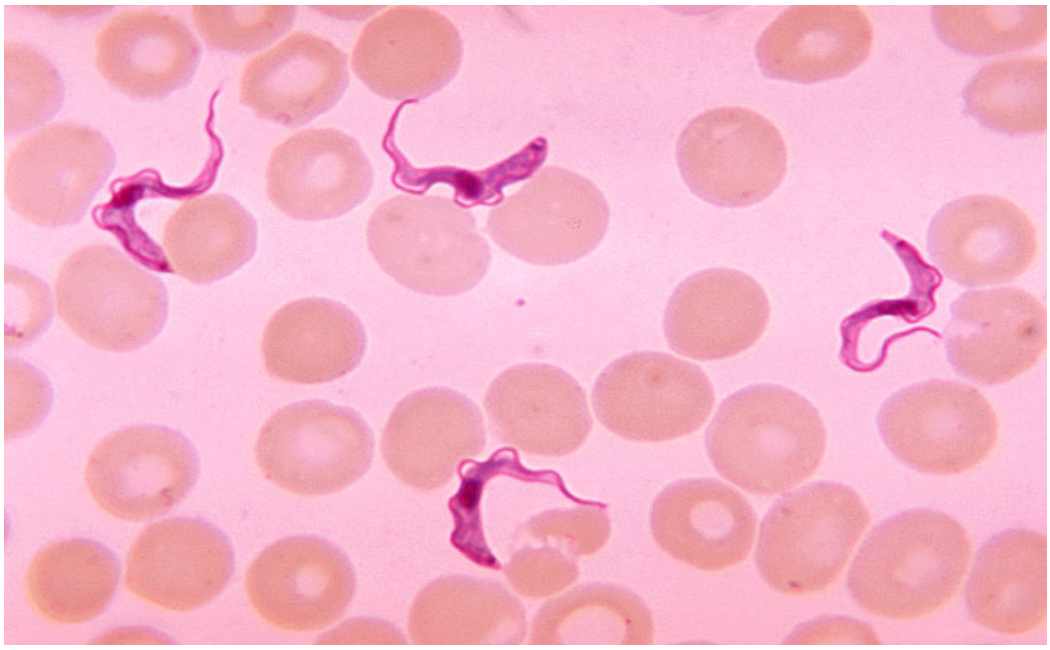

Fig-1 *Trypanosoma vivax* in blood stream forms (Osorio *et al*.,2008)

Fig-2 cattle infected with bovine trypanosomiasis

1.2 The disease:

1.2.1Animal African Trypanosomiasis

Tsetse-transmitted African animal trypanosmiasis is an infectious disease unique to Africa. It occurs in 37 sub-Saharan countries in a total area approaching 10 millon km^2 which corresponds approximately to one-third of the continent's land area. Although *T.vivax* is the main cause of mortality and morbidity in livestock in Africa, together with *T. congolense* they affect livestock health and production in most of the African countries. Moreover, both *trypanosome* species are widely distributed inside and outside the tsetse belts (Hoare, 1972). Concurrent infections can occur with more than one species of trypanosome. *Trypanosomes* can infect all domesticated animals; clinical cases have been described in cattle, water buffalo, sheep, goats, camels, horses, donkeys, alpacas.

1.2.2. Animal Trypanosomiasis in the Sudan

Trypanosomiasis is the most economically important diseases which affect domesticated animals in the Sudan. It has been recognized as an important disease of livestock in this country since the beginning of 20^{th} (Anon, 1907). Trypanosomiasis does not only affect the distribution of cattle, but also the distribution and even the habits of the main tribes (Lewis, 1949). 90% of the 40.8 million head of cattle in the Sudan are at risk of tsetse and trypanosomiasis (Ford, 1976; Mahmoud *et al*., 1991).

In the past trypanosomiasis outbreaks caused heavy losses among cattle herd. Elkarib(1961) reported death that affected shilluk cattle isotherm Sudan were estimate at least 50% of the total cattle owned by that tribe. Even (1948, 1950) attributed these trypanosomiasis outbreaks to mechanical transmission by biting flies mainly *horse flies* and *stomoxyinae* which are

numerous during the rainy season. As far as the epizootology of the disease is concerned, Elkarib (1961) recognize two distinct zones: The tsetse fly-infested and tsetse fly- free zones. The tsetse zone covers an area of about 90000 mile^2 which lies south of the diagonal line extending from a point on the western boarder at 10^0N parallel to a point on the southern border at 32^0longitude. The trypansoma species reported by Elkarib (1961) were *T.congolens*, *T.vivax*, and *T.bruci* in cattle, together with *T. evansi* which causes a serious disease in camals known locally as Guffar and elsewhere as Surra.

Trypanosomiasis is a parasitic disease caused by species of flagellated protozoa belonging to the genus *trypanosome* which inhabit the blood plasma, various body tissues and fluids of vertebrate host. The disease transmitted cyclically by tsetse flies (*Glossina* species), and none cyclically by other biting flies (Tadesse *et al*., 2010).

T. vivax is enzootic in wide areas of Sudan more than 2,000 km away from the known tsetse belt of the country. The transmission of the disease is due to the enormous populations of *Tabanidae* and biting flies. *Trypanosoma* infection restricts the animal production and causes economic losses by the clinical signs of the infection such as restricted growth, abortion, anaemia, treatment cost, and death of the affected animals.

1.3. Transmission:

Trypanosomes transmission is either directly through coitus or indirectly by vectors.

1.3.1. Direct transmission:

Dourine is a venereal transmitted disease caused by *T. equiperdum* that exclusively affects equines and has a wide geographical distribution.

The disease is endemic in North Africa, the Middle East, Eastern Europe, South America and Indonesia (Taylor and Authié, 2004).

1.3.2. Vector transmission:

Insects have a major role in transmission of African *trypanosomes*. In this mode of transmission the life cycle consists of two phases, one occurs in the vector and the other one is in the mammalian host (Uilenberg, 1998). *Trypanosome* transmission by insects is either cyclical, by bugs or tsetse flies, or mechanically by other biting flies.

1.3.2.1. Tsetse flies:

The species of genus *Glossina* or tsetse flies are important blood sucking flies; they transmit cyclically several species of trypanosomes which cause fatal diseases. 30 species and subspecies of the tsetse vectors are found to play an important role in the transmission of Trypanosomiasis. Classification of tsetse, based on morphological criteria, divides the species into three groups, which have different habitat requirements that are thought to reflect their evolutionary history. The *fusca* group flies, tend to occur in the lowland rainforests of West and Central Africa; *palpalis* group species occupy similar forest habitats throughout Africa and also extend into riverine and lakeside forests or the moist areas between such forests; and finally the morsitans group of flies occurs in a variety of savannah habitats lying between the forest edges and deserts. The Gambian HAT is transmitted by the riverine species (*G. palpalis, G. fuscipes* and *G. tachinoides)* tsetse flies, which prefer a humid and dense riverine habitat while the Rhodesian HAT is transmitted by the savannah species (*G. morsitans, G. pallidipes*, and *G. swynnertoni*). An exception is the East African *T. b. rhodesiense* form, which is transmitted by the riverine species *G. fuscipes* (Smith *et al.*,

1998; Waiswa *et a.l,* 2006).

Tsetse fly has been associated with the presence of certain species of wildlife. The important wildlife hosts for most tsetse are Bush buck (*Tragelaphus scriptus*), Warthog (*Pharcochoerus aethiopicus*) and Red River Hog (*Potamocherus porcus*) with reptiles most important for riverine tsetse. While some wild animals such as Zebra (Equus spp), Wilder beast (*Connochaetes taurinus*), Duiker (*Cephalo-phus rufilatus*) and Waterbuck (*Kobus defassa*) are rarely fed upon by tsetse flies (Davies, 1977).

1.3.2.2. Bugs:

In Chagas disease the vectors are species of Triatoma bugs, they have many common names e.g. the kissing bug or assassin bug".

1.3.2.3. Biting flies:

Two species of African trypanosomes have succeeded in adapting themselves to so-called mechanical transmission by various biting flies; there is no biological cycle in these flies, no multiplication, and the fly just acts as a needle. A biting insect passes the blood forms from an infected animal to another in the course of interrupted feeding. The time between the two feeds is crucial for effective transmission because the trypanosomes die when the blood dries. The importance of this mode of transmission is variable from place to place, depending on the numbers of hosts and biting insects present, and also on the species of trypanosome. Large biting insects such as tabanids carry more blood and are more likely to act as mechanical vectors than for example mosquitoes. Tsetse flies themselves can of course also act as mechanical vectors (Ulenberg, 1998). Biting flies such as *Tabnids* and *Stomoxys* spp. can transmit bloodstream trypomastigotes to

mammalian hosts and this has enabled some species of pathogenic trypanosomes to spread widely beyond Africa into Asia and Latin America. This non-cyclical or mechanical means of transmission has been acquired by only two species of trypanosomes. *Trypanosoma evansi*, a member of the Trypanozoon group, has evolved from *Trypanosoma brucei* and lost the ability to develop cyclically in tsetse flies (Brown, 1990).

Tabanids are large stout bodied flies, they included several genera such as (*Tabanus, Haematopota* and *Chrysops*). The female feeds on blood more than 4000 species of *tabanids* have been described in the world (Kettle, 2000). Stable flies (*stomoxys spp*) are blood sucking muscids belonging to the sub family *Stomoxinae,* both males and females fed on blood. In Africa 14 species of *Stomoxys* were reported by Mihok (1995), the most cosmopolitan species among them is S. calcitrans.

Transmission by other means include infection of carnivores with *T. evansi* and T. brucei by ingesting meat or organs from infected animals and transmission of *T. evansi* in Latin America by the bites of vampire bats (Uilenberg, 1998) but those ways were considered to be of less importance.

1.4. Life cycles of trypanosomes:

As for transmission by *tsetse*, Brown *et al*., (1990) thought that cyclical transmission occurs when a tsetse fly feed on an infected animal. The ingested trypanosomes undergo a cycle of development in the fly lasting between eight and thirty five days before infective met acyclic trypanosomes are produced. Development in the vector occurs at different sites according to trypanosome species. Hoare (1972) reported that *T. brucei* development took place in the fly midgut and salivary glands*; T. congolense* in the midgut and proboscis and in *T. vivax*, the development took place wholly in the

proboscis. The infected tsetse fly can be able to transmit the infection throughout its life but not at every feed (Moloo *et al*., 1981). Trypomastigotes from the infected host are taken up by the tsetse fly during the blood meal. In the stomach of the fly, the parasites multiply by simple fission and the morphology changes, the kinetoplast coming to lie just in front of the nucleus and the trypanosome is now called epimastigote (crithidia). Then they penetrate the gut wall and migrate to the salivary gland. Later The Infective trypomastigote (The metacyclic trypanosome) is found in the saliva and the fly remains infective throughout its normal lifespan of several months (Gill and Beeching, 2009). In the mammalian host tsetse flies deposit the metacyclic trypanosomes in the skin of the bitten animal. A swelling (chancre) develops at the site of the tsetse fly bite. The chancre forms leave the skin via the efferent lymphatics and reach the blood stream forms within afew days; however, they cannot be detected in the peripheral blood until approximately 10-16 days post infection (Gardiner *et al.*, 1991).

1.5. Pathology:

1.5.1. Animal Trypanosomiasis:

Different clinical signs exhibited in trypanosomes infections (*T. vivax, T. congolense* and *T. brucei*) were described in different animals. In cattle, in early stage of the disease chancre develops at the site of the fly bite, fever, anaemia which is the most prominent feature of animal trypanosomiasis, enlarged lymph nodes, lethargy and loss of condition, abortion , reduced milk production, nervous and ocular disorders and death. Dourine is usually chronic with symptoms involving the skin like circular raised itchy plaques appear in the shoulder , enlarged lymph nodes, fever and nervous signs like

weakening of hind quarters and staging of the lower lip and at last paralysis and edema of the genital organs and abortion (Gilbert, 1998) .

1.5.1. Anaemia:

The One of the major effects of infection with pathogenic trypanosomes is anemia. In acute infections, PCV falls rapidly due to erthrophagocytosis but an equally rapid recovery takes place following trypanocidal drug treatment. Measurement of anaemia gives are liable indication of disease status and productivity performance although its severity is affected by virulence of the infecting trypanosome species and host factors such as age, nutritional status and breed (Murray and Dexter, 1988).

1.5.2. The Haemorrhagic Syndrome:

Trypanosoma vivax isolated from dairy cattle undergoing a haemorrhagic disease was inoculated into Ayrshire steers. Five of six infected animals experienced brief periods of diarrhea and sublingual and gastro-intestinal hemorrhage. Gastro-intestinal bleeding coincided with markedly reduced numbers of thrombocytes and a high level of parasitaemia in the peripheral blood. Prothrombin times were extended and fibrinogen levels were elevated in infected animals. Plasma paracoagulation tests were positive for the presence of fibrin monomers and/or clottable early fibrin degradation products during the course of infection .Certain strains of *T vivax*, mainly in East Africa, cause a haemorrhagic syndrome in cattle associated with a high parasitaemia, in which haemorrhages, particularly of the gastrointestinal tract, occur. This can result in a high level of mortality (Gardiner, 1989). An acute haemorrhagic syndrome that caused cattle mortality in different villages of Tororo district in Uganda has been reported

by (Magona *et al*., 2008). Clinical cases manifested by bleeding through the ears, severe weight loss, anaemia, weakness and enlarged lymph nodes prior to death. Out of an original population of 844 cattle 295 (35%) had died, *Trypanosoma vivax* was the dominant trypanosome species, constituting 82% of trypanosome infections. This work has provided further evidence on the importance of *T. vivax*-induced acute hemorrhagic syndrome in livestock trypanosomiasis.

1.5. 3. Effects upon fertility:

Ikede et al (1988) reviewed that trypanosomiasis cause several disorders in animals which include irregular menstrual (or oestrus) cycle, infertility, abortion and impotence. Intrauterine infections occasionally occur, resulting in still birth or neonatal mortality.

1.6. Diagnosis:

1.6.1. Parasitological Techniques:

Standard parasitological methods include wet smear, thin and thick stained blood smears, haematocrit centrifugation technique, dark ground phase contrast buffy coat technique, miniature anion exchange centrifugation technique, examination of lymph node biopsies and inoculation of laboratory susceptible animal hosts (Lukins, 1986).

1.6.1.1. Wet blood films and Giemsa-stained:

The examination of wet blood films and thick, thin fixed blood films with the aid of the light microscope have been used as diagnostic methods ever since they were first used to identify the aetiological agents of trypanosomiasis. With the wet blood film, a drop of blood can be examined next to the animal, provided that a microscope is available. Thin and thick blood smears fixed in methanol or acetone and stained with Giemsa may be

used in the laboratory to detect blood parasites and determine the trypanosome species involved, respectively. However, these techniques are not sensitive enough to detect the low parasite levels, characteristic of the disease in large animals (Eisler *et al*, 2004).

1.6.1.2. The Haematocrit centrifugation technique (HCT): *Trypanosoma* infections were detected earlier than other parasitological techniques. It has more efficiency in surveys of trypanosomes that are non infective to laboratory animals like *T. vivax* and some strains of *T. congolense*. The technique includes centrifugation of microhaematocrit capillary tubes containing the blood sample and examination of the buffy coat/plasma junction under the microscope, Woo (1970).

1.6.1.3. Dark ground phase contrast Buffy coat technique:

This was subsequently improved in the buffy coat technique (BCT) by cutting the capillary tube, expressing the buffy coat/plasma interface on a microscope slide and using dark-ground or phase-contrast illumination (Murray *et al.,* 1977). The advantages of these two methods are that diagnostic sensitivity is increased, due to a concentration of parasites following centrifugation, and that at the same time the packed red cell volume (PCV) can be determined as a measure of anaemia. Paris *et al*., (1982) found the BCT to be the most sensitive technique, followed by the HCT, Giemsa-stained thick film, Giemsa-stained thin film and wet blood film.

1.6.1.4. The miniature anion exchange centrifugation technique:

The miniature anion exchange centrifugation technique (MAECT) has been described for field use in the diagnosis of Human trypanosomiasis

using blood samples obtained by finger prick (Lanham *et al.*, 1970, Lumsden *et al.*, 1977). The method proved to be sensitive, quick and able to detect very low parasitaemia of *T. brucei gambiense*, however the teschnique is cumbersome for routine use in veterinary practise. Recently Gutierrez *et al*, (2004) reported that MAECT allow salivarian trypanosomes to be separated from the blood of affected animals. They assessed the MAECT in goats infected with *T. evansi* and concluded that in cases of very low parasitemia in goats, MAECT can be used successfully when other parasite-detection tests have failed.

1.6.1.5 Lymph nodes:

Lymph nodes can be punctured and the fresh aspirate is microscopically examined if enlarged lymph nodes are present, Due to its simplicity and low cost, this technique remains widely applied, particularly in *T. b. gambiense* infections. Sensitivity varies from 40 to 80% and seems to depend on the parasite strain, on the occurrence of other diseases causing lymphadenopathy and on the disease stage (more common in the early stage) (Buscher and Lejon, 2004). Stained lymph node smears were proved to be a very good method for diagnosis of *T. vivax, T. brucei brucei*, and chronic cases of *T. congolense* infections (Mare, 1998).

1.6.1.6 Inoculation of susceptible rodents:

May be more effective for some *trypanosome* species, particularly Trypanozoon spp., than others (Eisler *et al.*, 2004). found far more cases of *T. brucei* infection in Kenya using mouse sub-inoculation than using blood examination but for *T. congolense* infections mouse sub-inoculation revealed only half as many positive animals as were identified using blood examination. Mouse sub-inoculation failed to pick up any *T. vivax*

infections. For this species, sub-inoculation of domestic ruminants (usually sheep or goats on grounds of expense) rather than rodents may be recommended. *T. vivax* may vary in its ability to give rise to parasitaemias in goats, depending on the geographical origins of the parasites, and to maximize the chance of detecting this species of trypanosome it may be necessary to sub-inoculate calves. *T. equiperdum* could be diagnosed by inoculation of vaginal and urethral discharges in rabbit testis (Hunter, 1986).

As for Dourine, microscopic examinations of fresh aspirate of motile trypanosomes are present for a few days only, so that lesions should be examined at intervals. Dourine parasite is rarely found in thick blood films, but is sometimes detectable after centrifuging blood and examining the recentrifuged plasma (OIE, 2008).

1.6.2 Serological Techniques:

Animal trypanosomiasis presents special problems with regard to diagnosis. The clinical signs are not pathognomonic and the standard techniques for the detection of trypanosomes are not sufficiently sensitive. Although significant improvements have been made in diagnosis, a high proportion of infections still remain undetected as the chronic, more common form of the disease, is often aparasitaemic. In the face of these constraints, alternative methods of diagnosis have been developed, most of which are for the detection of antibody responses to the antigens of the infecting trypanosomes (Nantulya, 1990). So more sensitive diagnostics methods including the detection of trypanosome-specific antibodies and antigens have been developed e.g. complement fixation test (CFT), agglutination tests, antibodies and antigens detecting Enzyme linked immunosorbent assay (Ab-ELISA, Ag-ELISA) and immunofluorescent

antibody test (IFAT).

1.6.2.1. The complement fixation test (CFT):

Has been the basis for successful detection of *T. equiperdum* presence in horses, however, cross reactions with sera of horses infected with other trypanosomes may occur. Wassal *et al*., (1991) found that the CFT is less sensitive than antibodies detecting ELISA in Dourine diagnosis.

1.6.2.2. The Card agglutination test:

Has been developed from atest for the diagnosis of human sleeping sickness (Magnus *et al.,* 1978), into a commercial kit for *T. evansi*, CATT test *T. evansi* (Diall *et al*., 1994) for the detection of antibodies to surra (*T. evansi* infection). Serum samples are mixed on a plastic card with fixed and stained trypanosomes as antigen and the test is positive when the antigen agglutinates. A titre can be determined by serial dilutions of the serum. The great advantage of this test is that in principle it is easy to carry out even in the field. Its specificity and sensitivity appear to need further evaluation, and in the experience of the author, reading the test results is not always easy.

1.6.2.3. The indirect fluorescent antibody test (IFAT):

As reviewed by Gardiner *et al.*, (1992) is helpful in detecting trypanosome-infected animals but they are not specific enough to distinguish between infections with *T brucei*, *T vivax* and *T congolense*. However it always failed to detect earlier infections and they added that antibodies can be detected for more than two months following treatment. The technique is achieved by a smear of blood containing fixed trypanosomes to constitute the antigen. The cattle serum to be examined is put into contact with the smear, and immunoglobulins (antibodies) against the trypanosomal antigens attach themselves to the trypanosomes in the smear. Antibodies raised in

laboratory animals, usually rabbits, against cattle immunoglobulins, is applied to the smear, and washed off after being allowed to react. In order to show the presence or absence of the rabbit immunoglobulins, and thus indirectly the presence or absence of specific antibodies against trypanosomes in the bovine serum, the rabbit anti-bovine immunoglobulins in the commercial preparation are conjugated with a fluorescent dye, usually fluoresceine, which can be detected by looking at the smear with ultraviolet light (Delespaux, 2006).

1.6.2.4. Ag-ELISA and Ab-ELISA:

Application of trypanosomiasis diagnosis was performed by many authors. Mutugi *et al*., (1996) evaluated the performance of Ag-ELISA for trypanosomiasis surveys and surveillance, the technique had low sensitivity, however the use of dried blood spots as antibody source, on the other hand performed well and is believed to have potential as a practical diagnostic test in trypanosomiasis surveys. Diall *et al.,* (1996) advised that Ag-ELISA should not be recommended for trypanosomiasis monitoring on its own as the assay suffered from low sensitivity.

Ab-ELISA have the potential in trypanosomiasis surveys to determine the spread of the disease, however its use as a tool to evaluate the effect of tsetse control operations on the transmission of trypanosomiasis will need more research into the dynamics and persistence of trypanosomal antibodies (Mutugi *et al*., 1996). They added that the use of eluted blood spots as an antibody source has certainly improved the sustainability of Ab-ELISA. Rebeski *et al.,* (2000) used stabile crude trypanosomal antigen procoated plates, they concluded that this modified coating procedure improved assay robustness at an acceptable diagnostics proficiency and provide an improved

quality assurance and standardization procedure for the assay, which is required to allow the reliable detection of trypanosomal antibodies. Bossard *et al.*, (2010) developed a diagnostic technique of bovine trypanosomiasis based on HSP70/BiP inhibition ELISA. They found that the technique shows a good sensitivity in cattle experimentally infected with *T. congolense*, with an improved sensitivity in secondary infections. One major advantage, particularly for its further application in national laboratories, is that one single set of reagents and one single procedure are sufficient to apply on different mammalian host species infected with different trypanosome species. Hasker *et al.*, (2010) tried Micro-CATT and ELISA/*T.b. gambiense* for outbreak detection of *T.b. gambiense* human African trypanosomiasis, they concluded that both techniques showed acceptable sensitivity and very high specificity respectively

1.6.3. Molecular techniques for diagnosis and characterization of trypanosomes:

Accurate identification of species, subspecies and subgroups of the genus Typanosoma remains a challenging problem in the epidemiology of the disease in man and animals in tropical Africa. Although many conventional methods of diagnosis and characterization of trypanosomes were used (Lukins, 1986). However with the advent of molecular biology, the specificity and sensitivity of detection increased. PCR-based assays and DNA probes to identify and detect parasites are highly specific and sensitive (Weiss, 1995). DNA based technology is a recent development in diagnosis of animal trypanosomiasis and has a major impact in many areas of veterinary Parasitology (Uilenberg, 1998). Gasser (1999) described the polymerase chain reaction (PCR) as highly sensitive test. However, the use

of PCR in diagnosis of human and animal trypanosomiasis is limited due to its high cost. Nevertheless several molecular methods are applied in trypanosome detection and differentiation at species and strains level. Moreover molecular techniques are also used successfully in *Trypanosoma* drug resistance studies.

1.7. Socioeconomic Aspects of trypanosomiasis:

The Quantification in economic terms of human losses due to disease and the benefits of control is very difficult. The economic impact is made up of direct losses (consisting of loss of production, mortality, abortion), as well as the cost of control (which includes the cost of drugs, their transport to the field site, the salaries of the operators, etc.), it is concluded that trypanosomiasis treatment cost may be used as a tool for assessing the economic impact of African (Elnour *et al.*, 2010).

1.8. Using the Geographic information system (GIS):

The use of remote sensing for the study of disease has grown rapidly in the past decade. Because of the land-use aspects, there is a growing tendency towards the collection of georeferenced data, which may be plotted on maps; computerized versions may be examined in the so-called geographical information systems (GIS). The analysis using the GIS technology has deepened the understanding of the spatial and temporal epidemiology of trypanosomiasis. The GIS is the powerful technology that has been used mainly in map-making and an enormous amount of knowledge can be gained simply by geographical data projection (De la Rocque *et al.*, 2001

1.9. Antigenic Variation:

Antigenic variation in trypanosomiasis is the sequential expression of a series of antigens on the surface of the parasites. In a normal host, the initial population of parasites derived from a single cell uniformly expresses a single membrane surface antigen. Subsequent parasitemias rise and fall in waves, each new parasite population carrying a new membrane surface antigen immunologically distinct to previous antigens (Williams, 1978). The ability of the surface coat of trypanosomes to undergo continuous antigenetic variation results in the escape from the host's immune response. The surface antigens change every 8 ~ 10 days after the trypanosome entered the vertebrate host. Specific antibodies produced by the host are no longer effective owing to the appearance of new variant surface antigens (VSGs) (Xuedong Kang, 2001). Consequently this new VAT will give rise to an increasing parasitaemic waves. The almost unlimited antigenic variation during infection by one single strain of trypanosome and the antigenic strain diversity within each of the several trypanosome species and types are the main obstacles preventing vaccine development (Ulenberg, 1998).

The antigenic variation system in trypanosomes operates at two levels. At the level of the population within the host, there is much imprecision, resulting in a highly varied course of antigenic variation and of infection. This appears to enable an overwhelming evasion of immunity and high chances for transmission to tsetse. At the cellular and molecular level, there is a highly sophisticated set of systems that ensure precision in the expression of only one VSG at a time, and that yield a highly diverse, yet structurally sound, protective surface coat (Barry and Carrington, 2004).

1.10. Immunity:

Trypanosomes have the ability to affect the host immune mechanisms by several ways, leading to formation of great amounts of antibodies, causing different effects on immunity response to different vaccines and evade the host immunity by changing their surface protein (Clarkson, 1976). Gardiner (1989) reviewed the findings of many authors who reported antibodies responses in ruminants towards trypanosome infections, they stated that a great rise in total serum immunoglobins (Ig) especially Ig M levels occurred during trypanosome infections, which might reached ten times the normal values. The main response to trypanosomal infection is antibody production, particulary IgM. Antibody production initially controls parasitaemia, but antigenic variation in the parasites surface antigens means that immune control is incomplete and this leads to successive waves of parasitaemia, which may explain the fluctuating nature of the illness.

It has long been suspected that infection with trypanosomiasis may lead to immunosuppression by impairing the immune response of the infected animal towards several diseases, antigens and animals vaccines. Immunodepression occurs also in the acute stage. Animals affected by trypanosomiasis often develop a lower antibody titre after vaccination against other diseases, and secondary infections which the host would normally control may also crop up during the disease. For example it is common to find considerable numbers of Babesia, Theileria and/or Anaplasma in bloodsmears of animals suffering from AAT, in situations where normal animals are healthy carriers of these tick-borne infections. Trypanosome infections disrupt the balance. Such concurrent diseases may also affect necropsy findings (Boyt, 1981, Ulenberg, 1998).

1.11. Control

1.11.1 Chemotherapy and chemoprophylaxis of AAT:

Chemotherapy in livestock currently depends upon the salt of six compounds, several of which are chemically closely related. These groups are the phenanthridines, isometamidium (Samorin, Trypamidium) and Homidium (chloride salt: Novidium; bromide salt: Ethidium)
and the aromatic diamidine, diminazene (Berenil). After the introduction of isometamidium in 1961 (Berg *et al*., 1961) the development of new trypanocidal drugs has made little progress. This is compounded by the fact that no new drugs have been produced for treatment of animals over the last 40 years, except for melarsomine. The use of the same drugs over such long periods has resulted in the widespread development of drug-resistant strains of trypanosomes (Peregrine, 1994). The incidence of resistance to these drugs is apparently on the increase (Peregrine, 1994) and the main means of controlling the disease is therefore under threat.

1.11.2. Tsetse control:

Control techniques that have previously been used include: stable animals, use of smudge fires and installation of neting to protect dairy cattle ,using sterile insect, habitat alteration ,vegetation clearing, ground and aerial insecticide spraying, application of repellents, trapping and selective game destruction(Maudlin *et al*.,2004).

1.12. Epidemiology:

Bovine trypanosomiasis was firstly recorded in the annual reports of the veterinary department for the year 1904 (Karib, 1961) in cattle arrived to Khartoum from Upper Nile. The author stated that bovine trypanosomiasis is a major problem of animals in Sudan. He indicated that *T. vivax* is the

commonest trypanosome encountered in cattle outside the tsetse belt, while *T. brucei* and *T. congolense* are confined to tsetse area as well as *T. simiae* which was reported in Bahr Elgazal province, South Sudan.

Hall *et al.,* (1983) reported that veterinarians rank trypanosomiasis as a major problems faced by cattle owners in Southern Darfur. They found that Levels of trypanosomal infections in the herds correlated well with their risk of exposure to tsetse being significantly lower at increasing distance from tsetse loci. *Trypanosoma vivax* infections predominated in all herds, increasingly so with increasing distance from tsetse loci. Packed cell volume values could not be used to assist in trypanosome diagnosis at either individual or herd levels or the lack of correlation between anaemia and parasitaemia is suggested as evidence of a degree of trypanosomal tolerance in the Western Baggara cattle.

In Kordofan state a survey was conducted in (1982) in South Kordofan and revealed an infection rate of up to 8.6% in cattle due to *T. vivax* and because no tsetse was encountered in the area, these infections were considered to be due to mechanical transmission as high populations of tabanids were found (A/Rahman *et al*., (1991).

Situation in central Sudan was studied by different authors, Suliman (1992) in Sennar, Homeida (1993) in Abunaama, Abdalla (1996) and Fadl (2000) in Sinja and A/Rahman (2002) in different sites of central Sudan, all infections were due to *T. vivax*. Moreover During the floods of 1988, outbreaks of bovine Trypanosomiasis due to *T. vivax* and *T. evansi* were reported in Khartoum and central states (Anonymous, 1995). Recently Abdalla *et al*., (2005) confirmed that trypanosomiasis still constitutes an important disease of cattle in the central Sudan, taking into account the low sensitivity of the parasitological diagnostic methods and the uncontrolled use

of trypanocidal drugs; the real prevalence of infection is probably substantially higher. They added that the prevalence of trypanosome infections increased substantially during the rainy season (June to October) and remained high during the early dry season (November).

In Eastern Sudan Kheir *et al*.,(1993) recorded the predominance of *T. brucei* and *T. congolense* infection in cattle herds followed by *T. vivax* and referred that to the resistance of both *T. brucei* and *T. congolence* to Ethidium bromide which was used extensively in treatment regime while *T. vivax* in that area was found to be sensitive to the drug.

The outbreak occurred in June 2010 when indigenous cattle, mainly Kenana and Fulani breed types, crossed the national Sudanese border to Ethiopia and Returned (Bashir. S. *et al*., 2011). Parasitological examinations revealed that 43% (91/210) of the affected cattle population was infected with two morphologically distinct trypanosomes. Seventy animals (33.3%) were infected with *T. vivax* and twenty one (10%) with *T. congolense*. In contrast, ITS1-PCR was able to identify four Trypanosoma species namely *T. vivax*, *T. congolense*, *T. simiae* and *T. brucei* in 56.7% (80/141).

Amnia (1997) Studied that 132 cows were sampled in Khartoum state during a period that extended from October 1996 to February 1997. During dry cold season, (October- November). Prevalence of *Trypanosome* species infection in Khartoum using Ab ELISA,was foud *T. vivax*.*T. congolense* and *T. brucei.*

Recently cross sectional study was conducted in Khartoum state in the dry season by (Elhassan, 2013).The result indicated the prevalence of cattle trypansomiasis was 4.8%(13/271). All the *trypansoma* encountered in cattle were *T.vivax.*

In South Sudan 300 blood films were prepared during the dry and wet seasons of 2003 from Thoan, Rubkona, Dandok and Abiemnom villages in Unity State, Elnasri (2005) . Parallel with the parasitic survey a clinical data from three different clinics in Thoan, Dandok and Mirmir were analyzed. A participatory survey also was conducted, using techniques of, participatory mapping, seasonal calendars and proportional piling. The results from the parasitic survey showed only *T. vivax* in all of the positive smears with a rate of 15% in the dry season compared to 6% in the wet season. Comparing the rates of African Animal Trypanosomiasis (AAT) between the breeds and seasons gave the result of 4 % of the samples collected from Nilotic cattle breed during the dry season were positive compared to 13 % positive samples collected from the Baggara cattle breed. This is while 3.13 % samples were positive from the Nilotic breed during the wet season compared to 11.11 % positive samples from the Baggara breed. Most of the fly samples collected was identified as *Tabanus taeniola* and quite few *Atylotus agrestis.*

1.13.1. Vectors of trypanosomes in Sudan:

In Recent studies, entomological and trypanosomiasis surveys were conducted in the Blue Nile area between Admazien and Khartoum, The surveys showed the area to be tsetse free (A/Rahman, 2005). Entomological surveys conducted in Dinder and Gessan localities by both traps and fly rounds showed that the area is tsetse free, the surveyed areas in Kurmuk province included the seasonal streams of Lili, Oos, Muguf and Ora were found infested with *Glossina fuscipes fuscipes*, which was also found in Khor Yabous While *Glossina morsitans submorsitans,* was caught in Khor Jordan area (Salih, 2010)

Seventy species of *tabanids* in Sudan were reported by Lewis (1953); they were distributed over different ecological zones. Tabanids studies in southern Darfur were carried out by Abdel Karim (1980) and Hall et al. (1983). The studies revealed the presence of *T. sufis*, *T. taeniola, A. agrestis, A. fuscipes* and *T. biguttatus*. The same species were reported in south Kordofan, an area that considered providing an ideal place of mechanical transmission studies (A/Rahman *et al*., 1991). The presence of tabanids in central Sudan was investigated by several authors. Suliman, (1992) reported the abundance of tabanids flies in Sennar area. The same findings were confirmed by Homeida (1993), Abdalla (1996) in Abunaama and Umbenin and Sawsan (1997) in Khartoum State. The only species of trypanosome found to infect cattle in this study was *Trypanosoma vivax*, which infected some of the local cattle that had no history of entering tsetse belts. The prevalence of disease varied with the season. High disease prevalence coincided with the periods when *tabanid* and *stomoxys* flies were abundant. The study showed that the months when biting flies were most numerous coincided with trypanosomiasis outbreaks, but even minimal numbers of these flies may cause the cycle of mechanical transmission to continue in stable enzootic conditions (A/Rahman, 2005). Recently Eltahir (2011) and Ali (2011) conducted tabanid studies in Khartoum state, their results ascertained the presence of only four tabanids species namely, *Atylotus agrestis*, *Tabanus taeniola, T. sufis* and *T. gratus*

Six *Stomoxys* species were reported by Lewis (1954) in the Sudan, the most important are *Stomoxys calcitrans* and *S. niger. Sawsan* (1997), Eltahir (2011) and Ali (2011) reported the presence of *Stomoxys* spp. in Khartoum state. Mohammed (1991) reported that peaks of *stomoxys* flies coincided with high trypanosomiasis incidences in different areas of Sudan.

Haematobia flies that are also considered of the mechanical vectors were reported by Lewis (1954) those include *Haematobia irritaus, H. exigua* and *H. minuta,* which are widely distributed in the country.

1.14.The prevalence of the previous study in other country:

Western Ethiopia, in Asosa district, a study was conducted by Shimelis (2011). The prevalence was significantly higher (P<0.05) in older and poor body conditioned animals but didn't vary between sexes and peasant associations. The most common trypanosome species identified were *T. congolense* (72/108, 66.7%) followed by mixed *T. vivax* and *T. congolense* (21/108, 19.4%), *T. vivax* (10/108, 9.3%) and *T. brucei* (5/108, 4.6%).The proportional prevalence of *T. congolenseis* significantly higher (P=0.000) than the other trypanosome species. The mean PCV values recorded were 20.6% in parasitaemic and 25% in aparasitaemic animals with a statistical significant difference (P<0.05).

In East Wollega zone Ethiopia. A study was conducted in the Diga and Sasiga districts to determine the prevalence of bovine trypanosomiasis and its vectors by (Wagari. *et al*., 2012). Out of 386 blood sample tested, 8.55% were positive for trypanosomes. The majority of the infections were caused by *Trypanosoma congolense* (72.73%), followed by *Trypanosoma vivax* (27.27%). There were no statistically significant differences (p>0.05) between districts, altitudes, sexes and ages, but the prevalence was significantly higher (p<0.05) in cattle which were in poor body condition. The mean PCV value of infected animals. Was significantly lower (p<0.05) than that of non-infected animals .dominant flies were *Glossina*, whilst the remaining flies were either *Stomoxys* or *Tabanus*.

In North West Ethiopia. A cross-sectional survey of bovine

trypanosomiasis was conducted in Wemberma district by (Wagari.*et al*., 2012). The prevalence of the disease as determined by buffy coat techinque was 7.81. *Trypanosoma vivax* and *T. congolense* were detected from buffy coat positive samples. Among the total of 30 cases of trypanosome infections detected 24(80%) of the infections were due to *T.vivax* and the rest 6(20 %) were due to *T. congolense.* No statistically significant associations (P>0.05) were observed between the disease and potential risk factors like age, sex and agroecology. However, when the different species of trypanosomes were considered, *T. congolense* infections were found only in the lowland. A significant association was observed (P<0.05) between the disease positivity and body condition score. When the mean packed cell volume of trypanosome infected animals was compared with that of non infected animals, it was significantly lower (P<0.05) in the infected animals, and the reduction was significantly lower (p<0.05) for *T. congolense* infection as compared with *T. vivax* infection.

In North West Ethiopia, a study was conducted by Shimelis (2004). Found that the apparent fly densities were significantly higher (p<0.05) in the late rainy season the dominant species was, *G. m.submorsitans*, *tabanids* and *muscids* respectively than the dry season. In the parasitological survey a total of 1,648 animals, 814 in the late rainy season and 834 in the dry season were examined with buffy coat technique and the prevalence of trypanosomiasis was 17.07% and 12.35% respectively with a significant difference (p<0.05) between seasons. Higher infection rates were found in the lowland areas below 1600 m.a.s.l.(19.87% and 17.62%) than the midland areas ≥1600 m.a.s.l.(13.39% and 6.54%) in the late rainy and dry season respectively with significant difference (p<0.05).The mean PCV values of parasitaemic and aparasitaemic

animals during the late rainy season were 20.7±3.5SD and 26.6±4.3SD ($p<0.001$) while during the dry season were 21.4±3.6SD and 26.6±4.3SD ($p<0.001$) respectively.

Ethiopia. A study was conducted by Wondewosen. *et al.,* (2012).Found that Blood samples were collected from 384 randomly selected cattle to detect the prevalence of *trypanosomes* using buffy coat method. The overall infection rate was 4.43%. The cattle are invariably infected with different species of trypanosome parasite and among these *Trypanosoma congolense* was the commonest (82.35%) followed by co-infection of *Trypanosoma viviax* and *T. congolense* (11.76%), and *T. vivax* (5.88%). Poor body condition animals were significantly highly affected compared to medium and good body condition. The mean PCV value of parasitemic and aparasitaemic animals was recorded as 20.94 and 23.55%, respectively. *G. pallidipes* was the only tsetse fly species caught in the study area along with other biting flies like *stomoxys* and *tabanus*.

In Woreda, Ethiopia. A study conducted by Gebreyohannes.*et al*., (2014). Found that in 384 animals were included in the study a total of 196 male animals examined, 17 animals (8.67%) were positive to Trypanasomiasis whereas from a total of 188 female animals examined 13 animals (6.91%) were positive to Trypanosome. Even though the prevalence of Trypanosomiasis was relatively higher in male individuals than female individuals there was no significant difference between sexes groups ($p>0.05$). The overall prevalence of Trypanosomiasis was 7.81%. *T.vivax*, was the dominant species folloud by *T. connglense*. Statistically significant difference among body condition scoring ($p<0.05$) was observed. The highest prevalence rate was recorded in older animals.

In Northwest Ethiopia. A study conducted by Ayana *et al.,* (2012). The prevalence of Trypanosomiasis was found to be 2.10% and *Trypanosoma vivax* was the only specie identified. Prevalence was slightly higher in females (2.50%) than males (1.70%). Both age groups were infected with trypanosome and the prevalence rate was higher in adult (2.5) than young (0.9) cattle and in good (3.6%) than in poor body condition animals (0.0%) ($P>0.05$).The trypanosome infection significantly influences the PCV and with mean PCV of parasitaemic and aparasitaemic animals being 21% and 28.54% respectively ($p<0.05$).

In Northern Tanzania. A study was conducted by Emanuel *et al*., (2011). Found that a total of 239 indigenous Tanzania short horn zebu cattle at four different villages, and of different ages and sex, were randomly selected and sampled. The overall prevalence of bovine trypanosomiasis was 5%. The prevalence was significantly higher in Mswaki juu village (7%) and lower in Ortukai (3%). Of the positive cattle, 8/12 (66.6%) had infections with *T.vivax* and 4/12 (33.3%) with *T. congolense.* No cases of *T. brucei* were detected. Animals with poor (13.7%) body score were significantly associated with high prevalence of trypanosomes infection than animals with good (2.7%) score. Prevalence of trypanosomes infections was significantly higher in males (8.42%) than in females (2.47%) and increased markedly in cattle aged >4.5 years, with no significant difference among the age group.

In Chena .A Blood samples were collected by Bizuayehu *et al.,* (2012) from 391 cattle, 6.9% of the animals were found positive for trypanosome infection. The trypanosome species observed across the study animals were *T. congolense* (4.89%), *T. vivax* (1.54%), and *T. b. brucei* (0.51%) as single infections. The infection rate of *T. congolense* and *T. b. brucei* varied

significantly ($P < 0.05$). The statistical analysis revealed that no significant difference ($P > 0.05$) in infection rate was found between male (7.79%) and female (5.62%) animals. The prevalence rate in good, medium and poor body conditioned animals were 7.28%, 0.78% and 13.39%, respectively with a statistical significant difference ($P < 0.05$) among them. The mean PCV of the infected animals (17.56%) appeared significantly ($P < 0.05$) lower than the non-infected (25.4%).

Chapter two

Materials and methods

2.1. Study area:-

Khartoum State lies at the junction of the two rivers, the White and the Blue Niles in the North Eastern part of central Sudan. It lies between latitude 15-16 N and longitude 21-24 East with a length of 250 k and a total area of 20,736 km^2 the surface elevation ranges between 380 to 400 m a.s.l.fig-3

Most of Khartoum State falls within the semi-arid climatic zone while the Northern part of it falls within the arid climatic zone. The state is prevailed with a hot to very hot climate with rainy season during the summer and warm to cold dry winter. Rain fall ranges between 100-200 mm at the North Eastern parts to 200-300 mm at the Southern parts with 10-100 mm at the North Western parts.

Temperature in summer ranges between 25C^O-40 C^O during the months of April to June and between 20 C^O -35 C^O during July-October. Temperature degrees continue to fall during the winter period between November-March to the level of 15 C^O -25 C^O.

Khartoum state divided into three cities, built at the convergence of the Blue and White Niles: Omdurman to the northwest across the White Nile, North Khartoum, and Khartoum itself on the southern bank of the Blue Nile (Adel, E,andOmer,k1999)

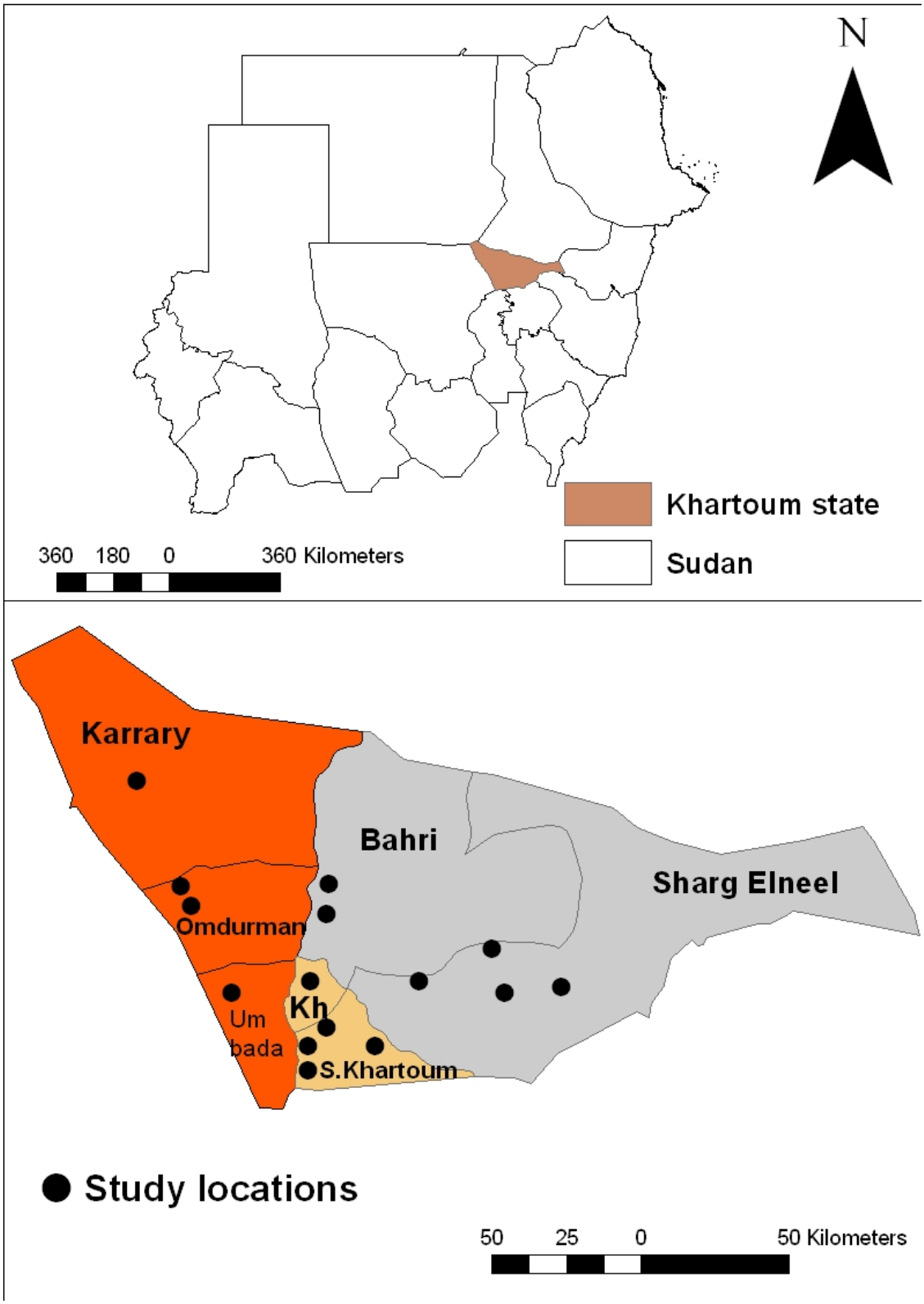

Fig- 3:Maps of Sudan and Khartoum state showing the study area

2.2. Study type:-

The study type is a cross-sectional study to estimate the prevalence of bovine trypansomiasis and investigate the risk factors which associated with the disease and its vector collect samples and data by filling out the questionnaire.

2.3. Sampling Method:

A multistage simple random sampling method was followed to select the study animals based on state, governorates (Khartoum, Khartoum North , and Omdurman), Localities in Khartoum (Jabal Awlia) , (Algrafa), (Soba), Omdurman, (Almoilh),(Karry), and Sharg Elneel (Alselliet region),Almahlb 2), (Eidbabeker).

2. 4.1. Sampling method:

$$\text{Prevalence rate} = \frac{\text{No. of diseased animals with } \textit{trypansoma}}{\text{Total no. of cattle at a particular point in time}} \times 100$$

2.4.2. Sample size determination:

The sample size will be calculating by using the following formula of Thrus feild (2005) the sample size was determined:

$n = (1.96)^2 \times P\blacksquare \times Q\blacksquare / L^2$

n ≡ the sample size

$(1.96)^2 \equiv$ constant

P^ ≡ expected prevalence (56%) (Survey in East Nile 2012).

Q^ ≡ 1- P^

$L^2 \equiv$ allowable error (5%)

n= (1.96)2 × 0.056 × 0.044 /0.0025= 378 samples

The samples were enough to represent the population and to increase the precision of the study and statistical analysis; the totaled samples were

380 samples.

2.5. Sample collection:

Blood samples were collected from each animal; Blood was usually collected in the morning (07.30-11.30AM) by puncture of the jugular vein using a sterile needle. Blood was obtained in dry clean sterile heparinised tube 5 milliliters and was put in cold box with ice and transported to the laboratory (Veterinary Research Institute Laboratories, Soba, Khartoum , Selleit Lab and Ministry of Agriculture, Animal Resources and Irrigation, Department of Animal Health, Hillat kuko lab) as soon as possible for diagnosis. Also estimation of the prevalence of vectors was achieved by using NZI traps in all investigation regions.

2.6. Diagnostic techniques:

2.6.1. Parasitological Examination:

For diagnosis, parasitological methods (Haematocrit Centrifugation Technique (HCT) and thin blood smear stained with Giemsa stain) was used to identify the parasite, also packed cell volume (PCV) of each animal was measured for anemia estimation.

2.6.2. Hematocrit centrifugation technique (HCT):

A capillary tube was filled with blood then sealed from one side using crestaseal, the sealed capillary tube was centrifuged in a microhaematocrit centrifuge (Hawksley and Sons Ltd., England) for four minutes at 12,000 rpm. After centrifugation the capillary tube was placed in a McMaster chamber flooded with water, and the junction of the buffy coat layer and the plasma was examined under a microscope using X10 objective, the capillary tube was rotated from time to time during the examination to ensure that all sides of the tube have been examined (Woo, 1971).

2.6.3. Giemsa stained thin blood smear:

It was prepared from the blood of positive animals with high parasitaemia as follows It was done by placing a drop of blood on a clean slide; another slide (Spreader) was placed at angle of approximately 30˚to the first slides, and drawn back to make contact with the blood droplet. The blood was allowed to run along the edge of the spreader ,which was then pushed to the other end of the slide, drawing the blood out into a thin film. The slide was dried quickly by waving in the air, fixed for three minutes in methanol, and stained for 30 minutes with 10% diluted giemsa stain in buffered water. After staining, the slide was washed gently under tab water and allowed to dry; it was examined under X100 oil-immersion objective len (OIE, 2008).

2.6.4. Packed cell volume (PCV) determination:

Blood was collected on capillary tubes. The tubes were then sealed at one end with crystaseal. PCV was measured in a micro-haematocrit centrifuge (Hawksley and Sons, UK). The capillary tubes were placed in microhaematocrit centrifuge with sealed end outer most. Then the tube was loaded symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 min. After centrifugation, the capillary tubes were placed in a haematocrit reader. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anaemic (OIE, 2008).

2.7. Investigation of risk factors associated with the disease and its vector:

A questionnaire was completed in an interview with the farms owner and

workers to collect information data about selected potential risk factors on the occurrence of trypanosomiasis. A pre-tested structured questionnaire with the primary objective of elucidating the multi factorial background of disease was conduct in an interactive manner at every farm that was visited. Eight localities was visited and just one animal from each herd was examined and then filling out the questionnaire by asking the owner. The individual risk factors attributes was include breed, age, sex, previous history of the disease, body condition, other diseases, and appearance signs of disease. The farm attributes will include the size of the herd, public administration and the type of grazing, milk production, stoking density, vector control, use treatments, and presence of other animals. Then divided these risk factors to categories.

2.8. Data analysis:

The collected raw data and the results of parasitological and hematological examination were entered into a Microsoft excel spread sheets program Statistical analysis was performed using Statistical programme SPSS version 16, and prevalence of trypanosome infection was calculated as the number of positive animals as examined by Giemsa stain of thin blood film and buffy coat method divided by the total number of animals examined at the particular time. Chi-square was used to evaluate the association of different variables with the prevalence of trypanosome infection. (P-value ≤ 0.025) at 25% level of significance were considered significant in all analysis. Multivariable logistic regression model: here we describe the risk factor, number of positive cases, odds ratio, confidence intervals and (p-value ≤ 0.05) at 5% level of significance.

Chapter three

Results

This study estimated the overall prevalence of bovine trypansomiasis in Khartoum, state. Sudan in cold dry season was 21.6% (table.1).

Table .1: Estimated mean distribution of positive and negative animals of bovine trypansomiasis in Khartoum state:

Result	Frequency	Relative frequency
positive	82	21.6
Negative	298	78.4
Total	380	100.0

Distribution of 380 cattle examined for trypanosomiasis in Khartoum State according to potential individual risk factor Table.2:

A total of 380 cattle were sampled from three localities (Khartoum, Khartoum north and Omdurman 37,312 and 31 animals were examined respectively, in eight area Alsellit Alhalfaia, Mahlab2 ,Soba Elmashro Algreaf, Jabal Awlia, Almoalih and Karrary were selected respectively. Regarding age 69 animal (≤ 1year), 104 (2-5years) and 207 (≥5 years were examined. Regarding sex 33male and 347 female were selected .As for breeds cross and local 327 and 53 animals respectively. As for body condition group 225 were good and 155 poor were selected. Tow production

type 33 beef animals and 347 diary animals were selected. Regarding presence of other disease of animals (present and not present) 139 and 241 animals were selected respectively. Two herd size groups <30 and>30 animals 102 and 278 animals were selected respectively. As for Presence of vector 292 owners of animals said vector present and 88 are animals not present. When using prophylactic treatment for the disease 252 animals were treated and 128 animals were not treated. As for presence of other animals in the farm (present and not present) 37 and 343 animals were selected respectively. As for grazing system 98 animals were outdoor grazing while 282 were indoor grazing. From location of live stock market (close to the farm and away from the farm 266 and 114 animals were selected respectively. For density of insects in the farm (low density, moderate and high density) 157,163 and 60 animals were selected respectively. For using insecticide for controlling (yes and no) 224 and 156 animals were selected respectively.

For water body and irrigations canals (present and not present 348 and 32 animals were selected respectively. As for vegetation 327, animals in good vegetation and 53 animals in poor vegetation. Regarding packaed cell volume (263animals were anemic and animals117 were not anemic). 380 animals their owners not using traps for control. As for wild life presence around the farm found and not found 320 and 60 animals were selected respectively. (Table.2.1) and (Table.2.2).

Fige-4: showed prevalence of trypanosomiasis in November, December and Janauary

Fige-5: showed distribution of positive and negative animals of bovine trypansomiasis in three different months Khartoum state

Table .2.1: Distribution of 380 cattle examined for trypanosomiasis in Khartoum State according to potential individual risk factor:

Risk factor	Frequency	Relative frequency (%)	Cumultive Frequency (%)
1.Age (year)			
<1	69	18.2	18.2
2-5	104	27.4	45.5
>5	207	54.5	100.0
2.Sex			
Male	33	8.7	8.7
Female	347	91.3	100.0
3. Breed			
Local	53	13.9	13.9
Cross	327	86.1	100.0
4. Body condition			
good	255	40.8	40.8
Poor	155	59.2	100.0
5. Production type			
Dairy	347	91.3	91.3
Beef	33	8.7	100.0
6. Locality			
Omdurman	31	8.2	8.2
Khartoum	37	9.7	17.9
Khartoum North	312	82.1	100.0
7.Presence of other disease			
Yes	139	36.6	36.6
No	241	63.4	100.0
8.Herd size			
<30	102	26.8	26.8
>30	278	73.2	100.0

Table.2.2distributiin of 380 cattle examined for trypanosomiasis in Khartoum state according to mangemental risk factors

Risk factor	Frequency	Relative frequency (%)	Cumultive Frequency(%)
1.UsingProphylactic treatment			
Yes	252	66.3	33.7
No	128	33.7	100.0
2. Presence of vector			
Yes	292	76.8	76.8
No	88	23.2	100.0
3. Using of insecticide			
yes	224	58.9	58.9
No	156	41.1	100.0
4. Density of in sects			
Low	157	41.3	41.3
Moderate	163	42.9	84.2
High	60	15.8	100.0
5. Presence of other animals			
Yes	37	9.7	9.7
No	343	90.3	100.0
6.water body and irrigation canal			
Yes	348	91.6	91.6
No	32	8.4	100.0
7. Vegetation			
Poor	53	13.9	13.9
Good	327	86.1	100.0
8.Grazing system			
Out door	98	25.8	25.8
In door	282	74.2	100.0
9. PCV			
<24	263	69.2	69.2
>24	117	30.8	100.0
10. Location of live stock Market			
Away	114	30.0	30.0
Near	266	70.0	100.0
11. Wild life			
Yes	320	84.2	84.2
N0	60	15.8	100.0

3. Cross tabulation:

The distribution of positive animals according to the risk factors were Khartoum 4 (10.8%) Khartoum North 77 (24.7%) Omdrman 1 (3.2%) (Distributed in Alselliet, Alhalfaia, Mahlab2, Soba Elmashro, Algreaf, Jabal Awlia, Moalih and Karrary farms. Regarding age 4 (5.8%) animal (≤1year) ,14 (13.5%) (2-5years) and 64 (30.95) (≥5 years were found positive. As for sex, in male 5 (15.2%) and in female 77 (22.2%). For breeds the positive sample in cross breed were 72 (22.0%) and in local breed 10 (18.9%) for body condition positive animals in good body condition was 28 (12.4%) and in poor body condition was 54 (34.8%).In dairy animals there were 77 (22.2%) positive and in beef 5 (15.2%). For presence of other disease 27 (19.4%) positive animals in presence and 55 (22.8%) in not presence. In herd size 60 (21.6%) in >30animas group and 22 (21.6%) <30 animals were positive .In owners not using prophylactic treatment 51 (39.8%) and in using prophylactic treatment 31(12.3%). 50 (17.7%) positive animals in grazing indoor and 32 (32.7%) in outdoor. In presence of other species of animals in the farms present positive animals 14 (37.8%) and not present positive animals 68 (19.8%). For using insecticide for controlling owner say yes positive animals 15 (6.7%) and owner say no using positive animals 67(42.9%). For presence of vectors in the farm in present 71 (24.3%) positive and 11 (12.5%) in not present.

For density of insects in the farm, in low density 13 (8.3%) in, moderate 48 (29.4%) and high density 21 (35.0%). For water body and irrigations canals, present 80 (23.0%) and not present 2 (6.2%). As for vegetation , animals, in good vegetation 78 (23.9%) positive animals and in poor vegetation 4 (7.5%) positive animals .From location of live animals market,

close positive 60 (22.6%)to the farm and away from the farm were 22 (19.3%). For wild life around the farm, not found 1 (1.7%) positive animals and found 81(25.3%). As for packed cell volume (animals were anemic 67(25.5%) were positive and 15 (12.8%) positive in animals were not anemic group (Table.3.1) and (Table.3.2).

Table.3.1: Cross tabulation of trypanosomiasis in 380 cattle examined in Khartoum State according to individual risk factor:-

Risk factor	No. tested	No. positive	Percent (%)
1.Age (year)			
<1	69	4	5.8%
2-5	104	14	13.5%
>5	207	64	30.9%
2.Sex			
Female	347	77	22.2%
Male	33	5	15.2%
3. Breed			
Local	53	10	18.9%
Cross	327	72	22.0%
4. Body condition			
Good	255	28	12.4%
Poor	155	54	34.8%
5. Production type			
Dairy	347	77	22.2%
Beef	33	5	15.2%
6. Locality			
Omdurman	31	1	3.2%
Khartoum	37	4	10.8%
Khartoum North	312	77	24.7%
6. Presence of other disease			
Yes	139	27	19.4%
No	241	55	22.8%
8. Herd size			
<30	102	22	21.6%
>30	278	60	21.6%

Table 3.2 Crosses tabulation of trypanosomiasis in 380 cattle examined in Khartoum State according to mengmental risk factor

Risk factor	No. tested	No positive	Percent (%)
1.Using Prophylactic treatment			
Yes	252	31	12.3%
No	128	51	39.8%
2. Presence of vector			
Yes	292	71	24.3%
No	88	11	12.5%
3. Using of insecticide			
Yes	224	15	6.7%
No	156	67	42.9%
4. Density of in sects			
Low	157	13	8.3%
Moderate	163	48	29.4%
High	60	21	35.0%
5. Presence of other animals			
Yes	37	14	37.8
No	343	68	19.8%
6. water body and irrigation canal			
Yes	348	80	23.0%
No	32	2	6.2%
7. Vegetation			
Poor	53	4	7.5%
Good	327	78	23.9%
8. Grazing system			
Out door	98	32	32.7%
In door	282	50	17.7%
9. PCV			
<24	263	67	25.5%
>24	117	15	12.8%
10. Location of live stock Market			
Away	114	22	19.3%
Near	266	60	22.6%
11. Wild life			
Yes	320	81	25.3%
N0	60	1	1.7%

4. Univariate analysis:

The Chi-square test showed that there were 13 out of 19 risk factors show statistically significantly association in bovine trypanosome prevalence (p-value≤0.25). These were:

Using chi square significant difference in trypanosome prevalence was between adults, young stock and calves ($\chi 2$ = 24.873, df = 2, p-value 0.000 (p-value≤0.25). Body condition show significant differences in trypanosome prevalence between good and poor ($\chi 2$= 27.198, df=1, p-value 0.000). Locality ($\chi 2$ = 10.478, df = 2, p-value.005), when using prophylactic treatment ($\chi 2$= 38.050,df=1,p-value 0.000), for presence of vectors in the farm ($\chi 2$= 5.578,df=1,p-value 0.018), for using insecticide for controlling ($\chi 2$= 71.416,df=1,p-value 0.000).

The risk for trypanosomiasis was therefore positively associated with tsetse apparent densit (χ=28.759 df =2, p-value 0.000), in presence of other animals in the farms (χ=6.403 df = 1, p-value .011). For water body and irrigations canals (χ=4.855 df = 1, p-value 0.028), As for vegetation (χ=7.166 df = 1, p-value .007.There were significant differences (χ=9.570 df = 1, p-value 0.002) between trypanosome prevalence proportions in cattle raised under different husbandry practices, the overall mean PCV (χ=7.663 df = 1, p-value 0.006) .and For wild life around the farm (χ=16. 694df = 1, p-value 0.000) .

There was Six risk factors not significantly associated with of trypanosome infection in cattle in our study (p-value > 0. 25).

Sex (χ=.882p-value=0.348), production type (χ=.882 p-value= 0.348),herd size (χ=.000 p-value 0.998), presence of other disease(χ=.601 p-value=0.438), Breed (χ=.267 p-value=0.605) and Location of live stock market(χ=.501 p-value =.479) (Table 4.1) and (Table 4.2).

Table.4.1: Univariate analysis for risk factor associated with trypanosomiasis in 380 cattle sample in Khartoum State, Sudan

Risk factor	No. tested	Positive Percent (%)	d.f	X^2	P.value
1.Age (year)			2	24.873	.000*
<1	69	5.8%			
2-5	104	13.5%			
>5	207	30.9%			
2. Sex			1	.882	.348
Female	347	22.2%			
Male	33	15.2%			
3. Breed			1	.267	.605
Local	53	18.9%			
Cross	327	22.0%			
4. Body condition			1	27.198	.000
Good	225	12.4%			
Poor	155	34.8%			
5. Production type			1	.882	.348
Dairy	347	22.2%			
Beef	33	15.2%			
6. Locality			2	10.478	.005
Omdurman	31	3.2%			
Khartoum	37	10.8%			
Khartoum North	312	24.7%			
7. Presence of other disease			1	.601	.438
Yes	139	19.4%			
No	241	22.8%			
8. Herd size			1	.000	.998
<30	102	21.6%			
>30	278	21.6%			

Significant p-value ≤0.25

Table 4.2: Univariate analysis for risk factor associated with trypanosomiasis in 380 cattle sample in Khartoum State, Sudan.

Risk factor	No. Tested	Positive Percent (%)	d-f	X^2	p-value
1. Prophylactic treatment			1	38.050	000
Yes	252	12.3%			
No	128	39.8%			
2. Presence of Vector			1	5.578	.018
Yes	292	24.3%			
No	88	12.5%			
3. Using of insecticide			1	71.416	.000
Yes	224	6.7%			
No	156	42.9%			
4. Density of in sects			2	28.759	.000
Low	157	8.3%			
Moderate	163	29.4%			
High	60	35.0%			
5. Presence of other animals			1	6.403	.011
Yes	37	37.8			
No	343	19.8%			
6. water body and irrigation canal			1	4.852	.028
Yes	348	23.0%			
No	32	6.2%			
7. Vegetation			1	7.166	.007
Poor	53	7.5%			
Good	327	23.9%			
8. Grazing system			1	9.570	.002
Out door	98	32.7%			
In door	282	17.7%			
9. PCV			1	7.663	.006
<24	263	25.5%			
>24	117	12.8%			
10. Location of live stock market			1	.501	.479
Away	114	19.3%			
Near	266	22.6%			
11. Wild life			1	16.694	.000
Yes	320	25.3%			
N0	60	1.7%			

Significant p-value ≤0.25

5. Multivariate analysis

The Multivariate analysis using the Logistic Regression showed that there were seven potential risk factor significantly associated with trypanosomiasis (p-value ≤0.05).These were age,(p-value=0.001), locality(p-value=0.026), using prophylactic treatment (p-value=0.46), using insecticide for controlling(p-value=0.002), presence of other species of animals (p-value =0.010), pr essence of wild life around the farm (p-value= 0.018). And density of insects (p-value= 0.016) (Table 5.1) and (Table 5.2).

5. Multivariate analysis of potential risk factor of trypansomiasis in 380 sample in Khartoum State, Sudan

Risk factor	No. tested	Positive (%)	OR	CI 95%	P-value
1.Age (year)					0.001
<1	69	5.8%	Ref	Ref	
2-5	104	13.5%	.133	.039 - .446	
>5	207	30.9%	.346		
2. Body condition					.315
Good	225	12.4%	Ref	Ref	
Poor	155	34.8%	1.439	.708-2.924	
3. Locality					0.026
Omdurman	31	3.2%	Ref	Ref	
Khartoum North	312	24.7%	46.789	.812-2.6983	
Khartoum	37	10.8%	1.261	.056-28.346	
4.Using Prophylactic treatment					.046
Yes	252	12.3%	Ref	Ref	
No	128	39.8%	2.465	1.018-5.970	
5. Presence of vector					846
Yes	292	24.3%	Ref	Ref	
No	88	12.5%	.899	.307-2.630	
6. Using of insecticide					.002
Yes	224	6.7%	Ref	Ref	
No	156	42.9%	3.945	1.677-9.281	
7. Presence of other animals					.010
Yes	37	37.8	Ref	Ref	
N0	343	19.8%	.221	.070-.696	
8.Density of insects					.016
low	157	8.3%	Ref	Ref	
Moderate	163	29.4%	4.86	1.576-15.006	
High	60	35.0%	1.54	.641-3.728	
9. water body and irrigation canal					.495
Yes	348	23.0%	Ref	Ref	
No	32	6.2%	.438	.041-4.542	
10.Vegtation					.398
Poor	53	7.5%	Ref	Ref	
Good	327	23.9%	.091	.000-23.877	
11. Grazing system					.285
Out door	98	32.7%	Ref	Ref	
In door	282	17.7%	1.540	.689-3.400	
12. PCV					.131
<24	263	25.5%	Ref	Ref	
>24	117	12.8%	1.931	.821-4.542	
13. Wild life					.018
Yes	320	25.3%	Ref	Ref	
N0	60	1.7%	.067	.007-.631	

Significant p-value ≤0. 5

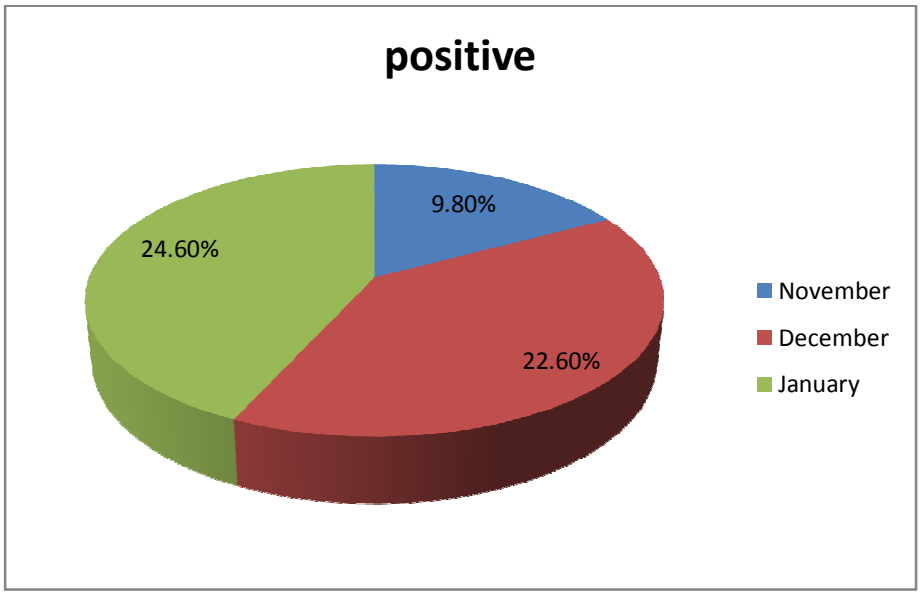

Fig. 4: Estimated mean prevalence of bovine trypansomiasis during cold dry season Khartoum state.

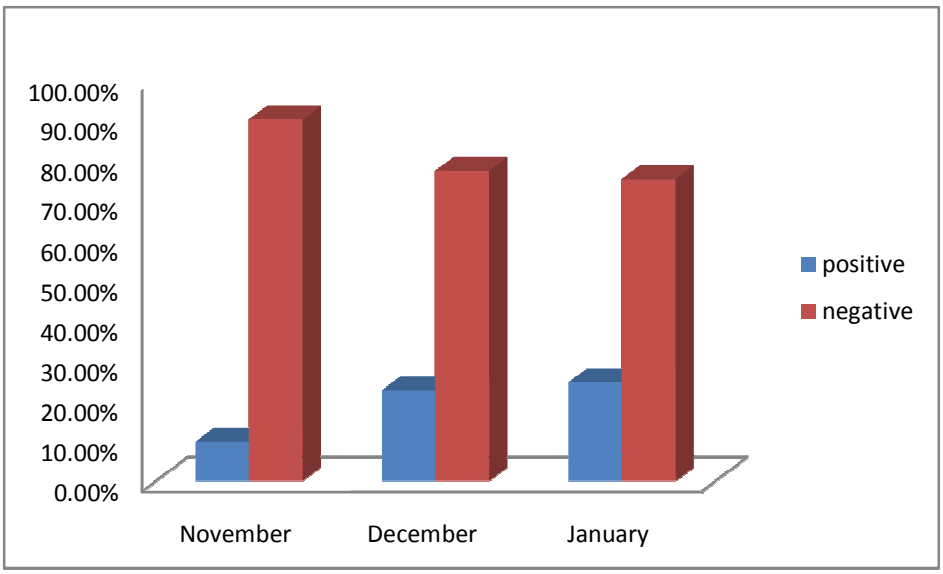

Fig. 5: Estimated mean distribution of positive and negative animals of bovine trypansomiasis in during cold dry season Khartoum state.

Chapter four

Discussion

The results of this study further confirm that trypanosomiasis still constitutes an important disease of cattle in the central Sudan. The prevalence of trypanosomiasis in our study was 21.6% taking into account the low sensitivity of the parasitological diagnostic methods and the uncontrolled use of trypanocidal drugs, the real prevalence of infection is probably substantially higher. Which agree with the finding of Abdalla *et al*., (2005).

In our study the prevalence and risk factors of bovine trypansomiasis in cold dry season (November, December and January), in Khartoum state, Sudan were investigated. *T.vivax* was the only trypanosome species found during the study period Only *Tabanids* (*Tabanus taeniola,Tabanus sufis , Stomoxy* and *Atylotus agrestis*) were observed and described by the informants groups, and using NZI traps. No Tsetse flies have ever been described or observed. This is in agreement with Tafur *et al*.,(2002), Abdalla *et al*.,(2005),Delafosse *et al.,* (2006),Gonzalea *et al.,* (2007), (Abdalla *et al.,*2008),Tadesse *et al*.,(2011),Batista *et al.,(*2012), and Elhassan, (2013).who reported that *T.vivax* constituted the majority of infection in the tsetse free zone. The prevalence of trypanosome infections increased substantially during the rainy season (June to October) and remained high during the early dry season (November). Outbreaks o£ acute *T. vivax* in Sudan cattle have been associated with increased rainfall (Elkarib. 1961; Abdalla and EI Malik, 2003; Abdalla *et al*., 2005).

The prevalence of trypanosomiasis in our study was 21.6%,this result is different from other studies carried out in Khartoum State during the hot

dry season Elhassan, (2013) showed a low prevalence of trypanosomiasis in Khartoum State which was 4.8% ,this result is not different from other studies carried out in Blue Nile area between Admazin and Khartoum where the prevalence in Khartoum was 1% in dry season and 6% in rainy season (Abdelrahman, 2005), and Sinnar, Sudan where the prevalence was 4.4%(Abdalla *et al*.,2008) when compared with the results obtained in the same localities in the late rainy season and early dry season (October to February). Abdel Salam (2005) reported similar findings from the Sinjah area, where he found a positive correlation between trypanosomiasis and the seasonal abundance of biting flie, also high prevalence was 50.3% in Sinnar, Sudan (Abdalla *et al* .,2005), 43% in Blue Nile State, Sudan (Salim *et al*.,2011),and in Western Kenya (Thumi *et al*.,2010). Although in our study low was reported compared to the study that was carried previously in (2012) in East Nile, Sudan 56% (T.T.c department of Veterinary Researcher Institute (VRI) (2012). The relatively low prevalence of trypanosomiasis in our study may be related to vectors distribution and low fly–animal contact. In addition there were parasite and vector control programmes practiced in the area which might also have contributed to the low prevalence.

The *trypanosome* infection in the study area was due to *T. vivax*. This high proportion of *T. vivax* infections is in accordance with the observations made in other tsetse-free areas of Sudan, demonstrating a high level of interaction between cattle and biting flies during the rainy season. (Abdalla and Elmalik, 2003; Abdalla *et al*., 2005; Rahman, 2005). So, the role of mechanical transmission of *T.vivax* in such areas cannot underestimate. Similar conclusions were drawn by Kidanemariam *et al*., (2002) who conducted surveys along the edge of the tsetse-belt in southern Ethiopia.

Although it has been shown that *T. congolense* and *T. brucei* can be transmitted mechanically, the transmission rate is usually low (Mihok *et al*., 1995).

In this study the univariate analysis showed that 13 risk factors were statistically significantly associated with Trypanosome infection in cattle, (p-value ≤0.25) age of the animal, locality , Prophylactic treatment ,body condition, PCV, grazing system, using of insecticide, presence of other animals spp, water body and irrigation canal, density of insect, vegetation and wild life. There was no significant difference in the occurrence of of *trypanosome* infections between local and /crossbreed cattle, sex, herd size, production type, presence of other disease and market

An increase prevalence of *T. vivax* infections in cattle has been noted during rainy season attributed to higher density of biting flies and/or the abundant presence of mechanical vectors, such as *tabanids* and *Stomoxys,* spp mechanical vectors present prevalence (24.3%), in not present (12.5%) there was significant association reported in *trypanosome vivax* and tsetse flies. Abdalla *et al.,* (2008) also found only *Trypanosoma vivax* in infected cattle from tsetse free areas of the Sudan. The difference might have cattle reared in each area.

Trypanosome prevalence differed significantly with age, adults having higher infection rates (30.9%), heifers (13.5%) and calves (5.8%), it showed statistically significant difference (p-value=0.001). Similar observations have been reported from East and West Africa (McDermott and Coleman, 1999). In the Southern Rift valley of Ethiopia, Karanja (1999) noted that adult cattle were 7.5 times at a higher risk of trypanosomiasis as compared to calves. These observations are attributable to the fact that calves are usually tethered around homesteads and hence face a lower challenge. In

addition, young animals are thought to be naturally protected to some extent by maternal antibodies in colostrums from their dams (Fimmen *et al.,* 1982).

The presence of *trypanosome* infections resulted in a significant decline in PCV (<24) 25.5% and (>24) 12.8% p-value (0.000) and body condition the prevalence in good (12.4%) and in poor (34.8%), (p-value= 0.000). *trypanosome* infection in those animals with poor body condition were significantly higher than those in good body condition. This was in agreement with (Abiy, 2002) who reported statistically significant difference between body condition and PCV in infected animals with trypansomiasis. Other factors such as malnutrition or other diseases may also affect the PCV and body condition. This result agree with Tadesse *et al*.,(2010) who found that difference ($p \leq 0.05$) between poor and animals with good to medium in body condition was statistically significant ,Begna *et al*., (2011) who say that animals of various body condition showed statistically significant difference ($p \leq 0.05$) in the prevalence of trypansomiasis These two factors may be related to the debilitating nature of the disease (Radostits *et al*., 2007). In the absence of other diseases causing anaemia, a low PCV value of individual animals is a good indicator of trypanosome infection (Abebe 2005; Marcotty *et al* .2008; Taylor, Coop & Wall 2007). Bitew *et al*., (2011) and Mulaw *et al*., (2011) who say that infection rate in poor body condition animals were significantly higher than good body condition animals ($p \leq 0.05$). This coud be due to the chronic nature of the disease that result in anaemia and decrease of body condition and that lead to emaciation of the animals.

Considering the husbandry practices the prevalence of the disease was significantly higher in animals grazed outdoors 32.7%, than that grazed indoors 17.7%, (p-value=0.002). This result disagree with the findings of

Cherenet *et al*.(2006) and Habtamu (2009). Swallow (1997) reported that, in mixed farming systems where trypanosomiasis is severe that it constrains the number of oxen, it can reduce the average area planted per household by as much as 50%.

Animals treated using insecticides have significantly higher prevalence in animals using 6.7% than those with no insecticide treatment 42.9% (p-value .000). This agree with work carried out in various parts of Africa (Stevenson *et al*.,1991; Bauer *et al.*,1992; Fox *et al*.,1993; Leak *et al*.,1995) recommends insecticide-treatment of cattle as an 124 appealing method of control.

Regarding presence of other species of animals in the farm positive animal if present (37.8%) if not present (19.8%), the study showed there was significant association between Trypanosomiasis and the presence of other species in the farm (P-value = 0.011). These agree with Mustafa, (2004) who find that sheep can act as a potential reservoir in mixed herd.

Also, location of water body and irrigation canal to live stock farm the prevalence (23.0%and6.2%) in present and not present respectively , had a significant association with the disease (p-value =0.028). These act as a source of breeding site to the neighboring farms. Logically, if there is one animal have the disease in the farm all the area around may be at risk when the vector is present.

The risk of trypanosomiasis is also influenced by apparent density and types of vectors in the area. The apparent density of *Stomoxys* and *Tabanus* the prevalence in higher (35.0%) ,moderate(29.4%) and low density (8.3%) statistically significant difference (p-value=0.000). This finding was lower than the report of Solomon and Fitta (2010).

In localities (Khartoum, Khartoum north and Omdurman

(10.8%,24.7%and 3.3%) *trypanosome* positive animals were found respectively, There was statistically significant association between the disease and locality (p-value=0.005) .this result agree with Elhassan (2013) (p-value= 0.084). The apparent geographical variation in prevalence may reflect differences in the levels of cattle management system, regular use of trypanosome chemoprophylaxis, resistance to parasite and distribution and challenges by fly vectors which agree with finding of Specht,E.J.(2008).

Variability in biting flies apparent density location could be explained by the fragmentation of biting flies habitat along riparian vegetation prevalence in good vegetation 23.9% and in poor 7.4% and there was association with disease (p-value= 0.007) described by Rocque *et al* . (2001).

It also should be noted that the vast and systematical use of trypanocidal drugs and chemoprophylaxis treatment was highly associatied with the low prevalence of disease (p-value =0.000) the prevalence in our study animals when using treatment (12.3%) and when not using (39.8%) of trypanosomiasis in the County. This result agree with Kidanemmariam *et al.,* (2002) who reported that chemotherapy and chemoprophylaxis treatment is important against trypanosomiasis . Although resistance to trypanocidal drugs has been documented in Kwale County (Eisler *et al.* , 2000).

When analysis was performed using multivariate analysis using logistic regression there were seven risk factor associated with trypanosomiasis (p-value $\leq$0.05),those were: age (p-value= 0.001), locality (p-value= 0.026), using prophylactic treatment (p-value=0.046), using of insecticide (p-value =0.002), present of other animals spp (p-value 0.016), density of insect (p-value =0.018) and wild life (p-value =0.010).

Conclusion

The study confirms that bovine trypanosomiasis is the one of the parasitic disease distributed in Khartoum state farms. Caused by *T. vivax*, more prevalence was found the presence of *T. vivax* in the study area indicated the importance of mechanically transmitted trypanosome mediated by biting flies in the study area. Bovine trypanosomiasis an important disease of cattle in Khartoum state.

According to the study results in univariate analysis risk factors which were statistically significantly associated with Trypanosome infection in cattle (p-value≤0.25%) include: Age of the animal, locality, Prophylactic treatment, body condition, PCV, grazing system, using of insecticide, present of other animals spp, water body and irrigation canal, density of insect, vegetation and wild life. There was no significant difference in the occurrence of trypanosome infections between local and /crossbreed cattle, sex, herd size, production type, presence of other disease and market. Using multivariate analysis using logistic regression there were seven risk factor associated with trypanosomiasis (p-value≤ 0.05): Age (p-value =0.001), Using prophylactic treatment (p-value=0.046), Using of insecticide (p-value =0.002), Present of other animals species (p-value= 0.016), Density of insect (p-value=0.018), Wild life (p-value=0.010) and Locality (p-value =0.026).

Recommendation

- Trypanosomiasis diagnosis in Sudan should be improved. More attention must be paid to improve diagnostic tools of *trypanosomes* by evaluation and improvement of parasitological tests and introduction of new, suitable and sensitive molecular methods to improve trypanosomiasis diagnosis
- Control efforts in the study area should target mechanically transmitted trypanosome infections to minimize biting flies specially in season of high vector population (vector control and chemotherapy)
- Using of prophylactic treatment for the disease control.
- Proper and strict follow-up of trypanocidal drugs treatment should be done professionals and supervision of the field personnel experts
- Awareness program for the owner about the disease should be promoted.
- Further studies and investigation of the disease in other areas in Khartoum state and other states should be encouraged.

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Appendix I

Frequency table for the distribution of 380 cattle examined for trypanosomiasis in Khartoum State according to potential risk factor.

1.1 Age

Age	Frequency	Relative Frequency %	Cumulative Percent %
<1	69	18.2	18.2
2-5	104	27.4	45.5
>5	207	54.5	100.0
Total	380	100.0	

1.2-Sex

Sex	Frequency	Relative Frequency %	Cumulative Percent%
Male	33	8.7	8.7
female	347	91.3	100.0
Total	380	100.0	

1.3-**Bcs**

Bcs	Frequency	Relative Frequency %	Cumulative Percent
Good	225	59.2	59.2
poor	155	40.8	100.0
Total	380	100.0	

1.4- Production type

Prod. type	Frequency	Relative Frequency %	Cumulative Percent
Milk	347	91.3	91.3
Beef	33	8.7	100.0
Total	380	100.0	

1.5-Herd. Size

Herd size	Frequency	Relative Frequency %	Cumulative Percent
<30	102	26.8	26.8
>30	278	73.2	100.0
Total	380	100.0	

1.6-Prophylactic treatment

Proph. tretment	Frequency	Relative Frequency %	Cumulative Percent
Yes	252	66.3	66.3
No	128	33.7	100.0
Total	380	100.0	

1.7- Breed

Breed	Frequency	Relative Frequency %	Cumulative Percent
Cross	327	86.1	86.1
Local	53	13.9	100.0
Total	380	100.0	

1.8 Using .insecticide

Using insecticide	Frequency	Relative Frequency %	Cumulative Percent
Yes	224	58.9	58.9
No	156	41.1	100.0
Total	380	100.0	

1.9 Presence of Vectors

Vectors	Frequency	Relative Frequency %	Cumulative Percent
Yes	292	76.8	76.8
No	88	23.2	100.0
Total	380	100.0	

1.10- PCV

PCV	Frequency	Relative Frequency %	Cumulative Percent
<24	263	69.2	69.2
>24	117	30.8	100.0
Total	380	100.0	

1.11- Water. Body

Water. Body	Frequency	Relative Frequency %	Cumulative Percent
Yes	348	91.6	91.6
No	32	8.4	100.0
Total	380	100.0	

1.12- Wild life

Wild life	Frequency	Relative Frequency %	Cumulative Percent
Yes	320	84.2	84.2
No	60	15.8	100.0
Total	380	100.0	

1.13- Vegetation

Vegetation	Frequency	Relative Frequency %	Cumulative Percent
good	327	86.1	86.1
poor	53	13.9	100.0
Total	380	100.0	

1.14- locality

locality	Frequency	Relative Frequency %	Cumulative Percent
Khartoum North	312	82.1	82.1
Khartoum	37	9.7	91.8
Omdurman	31	8.2	100.0
Total	380	100.0	

1.15- Density .tick

Density .tick	Frequency	Relative Frequency %	Cumulative Percent
low	157	41.3	41.3
Moderate	163	42.9	84.2
High	60	15.8	100.0
Total	380	100.0	

1.16- Others. Disease

Others. Disease	Frequency	Relative Frequency %	Cumulative Percent
yes	139	36.6	36.4
No	241	63.4	100.0
Total	380	100.0	

1.17- Other. Animals

Other. animals		Relative Frequency %	Cumulative Percent
yes	37	9.7	9.7
no	343	90.3	100.0
Total	380	100.0	

1.18- Grazing.system

grazing.system	Frequency	Relative Frequency %	Cumulative Percent
out door	98	25.8	25.8
indoor	282	74.2	100.0
Total	380	100.0	

1.19-Market

Market	Frequency	Relative Frequency %	Cumulative Percent
away	114	30.0	30.0
near	266	70.0	100.0
Total	380	100.0	

Appendix II

Cross tabulation of trypanosomiasis in 380 cattle examined in Khartoum State according to risk factor.

2.1. Age

Age					
Result		<1	2-5	>5	Total
+ve	count	4	14	64	82
	% within Age	5.8%	13.5%	30.9%	21.6%
-Ve	count	65	90	143	298
	% within Age	94.2%	86.5%	69.1%	78.4%
Total		69	104	207	380
	% within Age	100.0%	100.0%	100.0%	100.0%

2.2. Sex

Sex				
Result		male	Female	Total
+ve	Count	5	77	82
	% within Sex	15.2%	22.2%	21.6%
-ve	Count	28	270	298
	% within Sex	84.8%	77.8%	78.4%
Total	Count	33	347	380
	% within Sex	100.0%	100.0%	100.0%

2.3. Herd size

Herd Size				
Result		<30	>30	Total
+ve	Count	22	60	82
	% within Herd Size	21.6%	21.6%	21.6%
-ve	Count	80	218	298
	% within Herd Size	78.4%	78.4%	78.4%
Total	Count	102	278	380
	% within Herd Size	100.0%	100.0%	100.0%

2.4. Prophylactic Treatment

Prophylactic Treatment				
Result		yes	No	Total
+ve	Count	31	51	82
	% within Prophylactic Treatment	12.3%	39.8%	21.6%
-ve	Count	221	77	298
	% within Prophylactic Treatment	87.7%	60.2%	78.4%
Total	Count	252	128	380
	% within Prophylactic Treatment	100.0%	100.0%	100.0%

2.5. Breed

Breed				
Result		Cross	Local	Total
+ve	Count	72	10	82
	% within Breed	22.0%	18.9%	21.6%
-ve	Count	255	43	298
	% within Breed	78.0%	81.1%	78.4%
Total	Count	327	53	380
	% within Breed	100.0%	100.0%	100.0%

2.6. Using insecticide

Using Insecticide				
Result		Yes	No	Total
+ve	Count	15	67	82
	% within Using Insecticide	6.7%	42.9%	21.6%
-ve	Count	209	89	298
	% within using Insecticide	93.3%	57.1%	78.4%
Total	Count	224	156	380
	% within using Insecticide	100.0%	100.0%	100.0%

2.7. Presence of vector

Vectors				
Result		Yes	No	Total
+ve	Count	71	11	82
	% within Vectors	24.3%	12.5%	21.6%
-ve	Count	221	77	298
	% within Vectors	75.7%	87.5%	78.4%
Total	Count	292	88	380
	% within Vectors	100.0%	100.0%	100.0%

2.8. PCV

PCV				
Result		<24	>24	Total
+ve	Count	67	15	82
	% within pcv	25.5%	12.8%	21.6%
-ve	Count	196	102	298
	% within pcv	74.5%	87.2%	78.4%
Total	Count	263	117	380
	% within pcv	100.0%	100.0%	100.0%

2.9. Water body and irrigation canal

Water Body				
Result		Yes	No	Total
+ve	Count	80	2	82
	% within water body	23.0%	6.2%	21.6%
-ve	Count	268	30	298
	% within water body	77.0%	93.8%	78.4%
Total	Count	348	32	380
	% within water body	100.0%	100.0%	100.0%

2.10. Wildlife

Wildlife				
Result		Yes	No	Total
+ve	Count	81	1	82
	% within wildlife	25.3%	1.7%	21.6%
-ve	Count	239	59	298
	% within wildlife	74.7%	98.3%	78.4%
Total	Count	320	60	380
	% within wildlife	100.0%	100.0%	100.0%

2.11 Vegetation

Vegetation				
Result		Good	poor	Total
+ve	Count	78	4	82
	% within vegetation	23.9	7.5%	21.6
-ve	Count	249	49	298
	% within vegetation	76.1%%	92.5%	78.4%%
Total	Count	327	53	380
	% within vegetation	100.0%	100.0%	100.0%

2.12 Locality

Locality					
Result		K. North	Khartoum	Omdurman	Total
+ve	Count	77	4	1	82
	%within locality	24.7%	10.8%	3.2%	21.6%
-ve	Count	235	33	30	298
	% within locality	75.3%	89.2%	96.8%	78.4%
Total	Count	312	37	31	380
	% within locality	100.0%	100.0%	100.0%	100.0%

2.13 Body condition

Body Condition				
Result		good	poor	Total
+ve	Count	28	54	82
	% within B.cs	12.4%	34.8%	21.6%
-ve	Count	197	101	298
	% within B.cs	87.6%	65.2%	78.4%
Total	Count	225	155	380
	% within B.cs	100.0%	100.0%	100.0%

2.14 Using prophylactic treatment

prophylactic treatment				
Result		yes	No	Total
+ve	Count	31	51	82
	% within proph.tre	12.3%	39.8%	21.6%
-ve	Count	221	77	298
	% within proph.tre	87.7%	60.2%	78.4%
Total	Count	252	128	380
	% within proph.tre	100.0%	100.0%	100.0%

2.15. Density of Insect

Density of Insect					
Result		Low	Mod	High	Total
+ve	Count	13	48	21	82
	% within Den.Insect	8.3%	29.4%	35.0%	21.6%
-ve	Count	144	115	39	298
	% within Den.Insect	91.7%	70.6%	65.0%	78.4%
Total	Count	157	163	60	380
	%within Den. Insect	100.0%	100.0%	100.0%	100.0%

2.16 Others. Disease

Others. Disease				
Result		Yes	No	
+ve	Count	27	55	82
	% within others.dis	19.4%	22.8%	21. 6%
-ve	Count	112	186	298
	% within others.dis	80.6%	77.2%	78.4%
Total	Count	139	241	380
	% within others.dis	100.0%	100.0%	100.0%

2.17 Other Animals

Other Animals				
Result		yes	No	Total
+ve	Count	37	343	380
	% within other Animals	37.8%	19.8%	21.6%
-ve	% within other Animals	62.2%	80.2%	78.4%
	Count	23	275	380
Total	% within other Animals	100.0%	100.0%	100.0%

2.18 Grazing System

Grazing System				
Result		out door	indoor	Total
+ve	Count	32	50	82
	% within Grazing System	32.7%	17.7%	21.6%
-ve	Count	66	232	298
	% within Grazing System	67.3%	82.3%	78.4%
Total	Count	98	282	380
	% within Grazing System	100.0%	100.0%	100.0%

2.19 Market

Market				
Result		Far away	Near	Total
+ve	Count	22	60	82
	% within Market	19.3%	22.6%	21.6%
-ve	Count	92	206	298
	% within Market	80.7%	77.4%	78.4%
Total	Count	114	266	380
	% within Market	100.0%	100.0%	100.0%

Appendix III

Univariate analysis for risk factor associated with trypanosomiasis in 380 cattle sample in Khartoum State, Sudan.

3.1 Age

	X^2	Df	P-value
Pearson Chi-Square	24.873[a]	2	.000
Likelihood Ratio	27.606	2	.000
Linear-by-Linear Association	23.830	1	.000
N of Valid Cases	380		

3.2 Sex

	X^2	Df	P-value
Pearson Chi-Square	.882[a]	1	.348
Continuity Correction[b]	.515	1	.473
Likelihood Ratio	.951	1	.329
Fisher's Exact Test			
Linear-by-Linear Association	.880	1	.348
N of Valid Cases[b]	380		

3.3 Body condition

	X^2	Df	P-value
Pearson Chi-Square	27.198[a]	1	.000
Continuity Correction[b]	25.891	1	.000
Likelihood Ratio	26.904	1	.000
Fisher's Exact Test			
Linear-by-Linear Association	27.127	1	.000
N of Valid Cases[b]	380		

3.4 Production type

	X^2	Df	P-value
Pearson Chi-Square	.882[a]	1	.348
Continuity Correction[b]	.515	1	.473
Likelihood Ratio	.951	1	.329
Fisher's Exact Test			
Linear-by-Linear Association	.880	1	.348
N of Valid Cases[b]	380		

3.5 Herd size

	X^2	Df	P-value
Pearson Chi-Square	.000[a]	1	.998
Continuity Correction[b]	.000	1	1.000
Likelihood Ratio	.000	1	.998
Fisher's Exact Test			
Linear-by-Linear Association	.000	1	.998
N of Valid Cases[b]	380		

3.6 Prophylactic treatment

	X^2	Df	P-value
Pearson Chi-Square	38.050[a]	1	.000
Continuity Correction[b]	36.440	1	.000
Likelihood Ratio	36.296	1	.000
Fisher's Exact Test			
Linear-by-Linear Association	37.950	1	.000
N of Valid Cases[b]	380		

3.7 Breed

	X^2	Df	P-value
Pearson Chi-Square	.267[a]	1	.605
Continuity Correction[b]	.114	1	.736
Likelihood Ratio	.275	1	.600
Fisher's Exact Test			
Linear-by-Linear Association	.267	1	.605
N of Valid Cases[b]	380		

3.8 Using insecticide

	X^2	Df	P-value
Pearson Chi-Square	71.416[a]	1	.000
Continuity Correction[b]	69.290	1	.000
Likelihood Ratio	73.131	1	.000
Fisher's Exact Test			
Linear-by-Linear Association	71.228	1	.000
N of Valid Cases[b]	380		

3.9 Vector

	X^2	Df	P-value
Pearson Chi-Square	5.578[a]	1	.018
Continuity Correction[b]	4.902	1	.027
Likelihood Ratio	6.113	1	.013
Fisher's Exact Test			
Linear-by-Linear Association	5.563	1	.018
N of Valid Cases[b]	380		

3.10 PCV

	X^2	Df	P-value
Pearson Chi-Square	7.663[a]	1	.006
Continuity Correction[b]	6.934	1	.008
Likelihood Ratio	8.244	1	.004
Fisher's Exact Test			
Linear-by-Linear Association	7.643	1	.006
N of Valid Cases[b]	380		

3.11 Water body and irrigation canal

	X^2	Df	P-value
Pearson Chi-Square	4.852[a]	1	.028
Continuity Correction[b]	3.913	1	.048
Likelihood Ratio	6.158	1	.013
Fisher's Exact Test			
Linear-by-Linear Association	4.839	1	.028
N of Valid Cases[b]	380		

3.12 Wild life

	X^2	Df	P-value
Pearson Chi-Square	16.694[a]	1	.000
Continuity Correction[b]	15.326	1	.000
Likelihood Ratio	24.113	1	.000
Fisher's Exact Test			
Linear-by-Linear Association	16.650	1	.000
N of Valid Cases[b]	380		

3.13 Vegetation

	X^2	Df	P-value
Pearson Chi-Square	7.166[a]	1	.007
Continuity Correction[b]	6.235	1	.013
Likelihood Ratio	8.702	1	.003
Fisher's Exact Test			
Linear-by-Linear Association	7.147	1	.008
N of Valid Cases[b]	380		

3.14 Locality

	X^2	Df	P-value
Pearson Chi-Square	10.478[a]	2	.005
Likelihood Ratio	13.494	2	.001
Linear-by-Linear Association	10.288	1	.001
N of Valid Cases	380		

3.15 Density of insect

	X^2	Df	P-value
Pearson Chi-Square	28.759[a]	2	.000
Likelihood Ratio	31.408	2	.000
Linear-by-Linear Association	25.662	1	.000
N of Valid Cases	380		

3.16 Others disease

	X^2	Df	P-value
Pearson Chi-Square	.601[a]	1	.438
Continuity Correction[b]	.417	1	.518
Likelihood Ratio	.608	1	.436
Fisher's Exact Test			
Linear-by-Linear Association	.600	1	.439
N of Valid Cases[b]	380		

3.17 Others animals

	X^2	df	P-value
Pearson Chi-Square	6.403[a]	1	.011
Continuity Correction[b]	5.383	1	.020
Likelihood Ratio	5.673	1	.017
Fisher's Exact Test			
Linear-by-Linear Association	6.387	1	.011
N of Valid Cases[b]	380		

3.18 Grazing system

	X^2	df	P-value
Pearson Chi-Square	9.570[a]	1	.002
Continuity Correction[b]	8.709	1	.003
Likelihood Ratio	9.001	1	.003
Fisher's Exact Test			
Linear-by-Linear Association	9.545	1	.002
N of Valid Cases[b]	380		

3.19 Market

	X^2	df	P-value
Pearson Chi-Square	.501[a]	1	.479
Continuity Correction[b]	.327	1	.568
Likelihood Ratio	.508	1	.476
Fisher's Exact Test			
Linear-by-Linear Association	.499	1	.480
N of Valid Cases[b]	380		

Appendix IV

Multivariate analysis of potential risk factor of trypansomiasis in 380 sample in Khartoum State, Sudan.

	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
				Lower	Upper
Age	2	.001			
age(1)	1	.001	.133	.039	.446
age(2)	1	.018	.346	.144	.834
Body condition (1)	1	.315	1.439	.708	2.924
Prophylactic .treatment (1)	1	.046	2.465	1.018	5.970
Using .insecticides (1)	1	.002	3.945	1.677	9.281
vectors(1)	1	.846	.899	.307	2.630
PCV (1)	1	.131	1.931	.821	4.542
Water. body(1)	1	.495	.438	.041	4.687
Wildlife(1)	1	.018	.067	.007	.631
Vegetation (1)	1	.398	.091	.000	23.877
Locality	2	.026			
Locality (1)	1	.063	46.789	.812	2.698E3
Locality (2)	1	.884	1.261	.056	28.346
Density of insects	2	.016			
Density of insects (1)	1	.006	4.863	1.576	15.006
Density of .insects (2)	1	.333	1.545	.641	3.728
other. animals (1)	1	.010	.221	.070	.696
grazing. System (1)	1	.285	1.540	.698	3.400
Constant	1	.500	.120		

Questionnaires

Name of interviewee…..

Name of farm…….

Date of interview……

1-locality

1. Omdarman ( ) 2. Khartoum ( ) 3. Khartoum North ( )

2- Age (year)

1. <1( ) 2. 2-5 ( ) 3. >5( )

3-Sex

1. Male ( ) 2.Female ( )

4-Breed

1. Local ( ) 2. Cross ( )

5- Body condition

1-Good ( ) 2.Poor ( )

6- Production type

1. Dairy ( ) 2. Beef ( )

7- Presence of other disease

1. Yes ( ) 2. No ( )

8- Herd size

1. <30 ( ) 2. >30( )

9- .Using Prophylactic treatment

1. Yes ( ) 2. No ( )

10- Presence of vector

1. Yes ( ) 2.No ( )

11- Using of insecticide for controlling insects

1.Yes ( ) 2. No ( )

12- Density of in sects

1. Low ( ) 2. Moderate ( ) 3. High ( )

13- Presence of other animals in the farm

1. Yes ( ) 2. No ( )

14- Presence of water body and irrigation canal

1. Yes ( ) 2. No ( )

15- Vegetation

1. Poor ( ) 2. Good ( )

16- Grazing system

1. Outdoor ( ) 2. In door ( )

17- PCV

1. < 24 ( ) 2. > 24 ( )

18- Location of life stock Market

1. Away ( ) 2. Near ( )

19- Wild life

1. Yes ( ) 2. No ( )