

بسم الله الرحمن الرحيم

**Sudan University of Science and
Technology
College of Graduate Studies
Prevalence and Risk factors of camel
trypanosomosis in Butana area Gedarrif
State**

نسبة انتشار وعوامل الخطر لمرض المثقبيات في الجبال في
البطانة ولاية القضارف

**A Thesis Submitted for a Partial
Fulfilment of Award of Master Degree in
Veterinary Preventive Medicine
(MVPM)**

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Abstract

Across sectional study was carried to determine the prevalence of camel trypanosomiasis (surra) or (Guffar), and Risk factor association with occurs of the disease together with determination the packed cell volume (PCV) in the disease animal Butana area Gedarrif State from March to Jun 2015. Blood samples were collected from randomly selected 270 camels. thin smears stained by Giemsa- and the Buffy coat were used for the detection of trypanosomes. Among these, 14 (5,1%) samples were positive for *Trypanosome evansi*. A higher prevalence was Buffy coat as compared thin will smears 11(4%) and 14(5,1%) by Giemsa's stain and Buffy coat respectively . A Higher significant prevalence detected in males than female . A Higher prevalence was found in Butana area , Gedarrif and in Showak Positive sample ware found in Arabi 9(8.4%), Bushary was 2(2.4%) and 3(10.3%) in Anafi .as the age differed significantly among age group and higher prevalence in (>11years).the large heard size showed higher prevalence as compared to, moderate and small heard size the positive samples in good body condition were 3(3.3%), in the moderate was 5 (5.4%) and 6(6.7%) were poor body condition .prevalence in breeder camel was 10 (5%) and 4(5.7%) detected in the camel market . the packed cell volume (PCV) of the infected camels was lower (less than 20%) than that of the negative or or uninfected animals. and the Most of the camels that were not infected with trypanosomes and had PCV values less than 20%

المخلص

اجريت هذه الدراسة لتحديد نسبة الاصابه لمرض التربانوسوما في الابل (سييرا) او (الجفار) والعوامل المساعدة على حدوث المرض وتأثير المرض علي حجم الدم المتراص في الفتره من مارس الي يونيو 2015 في منطقة البطانه ولاية القصارف.

270 عينة دم اختيرت عشوائيا تم فحصها بواسطة المسحه الرقيقه بصبغه جمسا وبوفي كوت

لتشخيص (5.1%) 14 عينه كانت إيجابيه وتربانوسوما إيفنساوي هو النوع الوحيد الذي تم

تشخيصه. أعلى نسبة إصابه كانت بواسطه بوفي كوت مقارنة مع المسحه الرقيقه (4%) 11 و

14(5.1%) بواسطة جمسا وبوفي كوت علي التوالي .

وجد ان هنالك فرق معنوي بين الذكور مقارنة بالإناث وأعلي إصابه كانت في الذكور. وأعلي

اصابه كانت في البطانه ,القصارف والشواك علي التوالي اما بالنسبه للسلاله (8.4%) 9 عينه

إيجابيه كانت في العربي (2.4%) 2 في البشاري و(10.3%) 3 في العنابي اما العمر فقد وجد ان

هنالك فرق معنوي بين المجموعات العمريه وأعلي اصابه كانت في عمر اكبر من 11 سنه. القطيع

الكبير اعلي إصابه مقارنة بالمتوسط والصغير وهنالك فرق معنوي بينه وبين المرض .

اما في حالة الجسم (3.3%) عينه إيجابيه كانت في الجيد والمتوسط (5.4%) وفي الفقير كانت

(6.7%) بالنسبه لمصدر الحيوانات كانت في المولد العينات الايجابية (5%) والمشتري من

السوق (5.7%) 4.

في حجم الدم المتراص في الإبل المصابه كان اقل من 20% مقارنة بالابل الغير مصابه وفي اغلب

الاحيان كان حجم الدم المتراص في الابل الغير مصابه بالتربانوسوما اقل من 20% ويعتقد انها

مصابه ببعض الامراض التي تؤثر علي حجم الدم مثل الطفيليات الخارجيه (القراد, القمل الحلم)

والطفيليات الداخليه (طفيليات المعده والامعاء).

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INTRODUCTION

The One-humped (dromedary) camel (*Camelus dromedarius*) is the most numerous animal in the semi-arid and arid tropical areas of Africa. In the Sudan, the camel population exceeds 3.1 million head (Anon, 2000). In fact about 20% of the world camels are found in the Sudan. Here((Abo Sin, 1988).), Camels in the Sudan are normally found in northern parts of the country about the 13°N parallel. They are concentrated mainly in the north western parts of Kordofan and Darfur regions and in the eastern region. (Osman (1987)

Sudan is the second most densely camel-populated country in the world (after Somalia). According to the 1977 livestock census, the number of camels was put at 2.5 million. However, this figure may be far below the current number. (Osman (1987).

Camels are important source of meat, milk and hair, and are used for transportation and drought power, they are also exported to north Africa and the middle east thus contributing a significant proportion to the gross national product (GNP). Their main owners are pastoralists in northern Sudan, and although many are still nomadic, an increasing number are settling as agro-pastoralists into the large agricultural schemes in eastern Sudan, in such schemes they keep camels and small ruminants on the products e.g., Durra or sorghum spp; (Abo Sin, 1988).

Trypanosomiasis are a group of closely related diseases of man and animals caused by single-celled haematoparasitic protozoan parasites called trypanosomes. They are transmitted biologically by flies belonging to the genus *Glossina* (Tsetse flies) and mechanically by

haematophagous flies, the most important of which belong to the genera *Tabanus* and *Stomoxys*. Different forms of the disease occur over a large area, producing varying degrees of morbidities and mortalities resulting in heavy economic losses in lives and wealth, (DFID, 2004).

Camel trypanosomiasis, also called surra, caused by *Trypanosoma evansi*, is the main agent of disease prevalent in most areas where camels are found (Richard, 1976). Although other species of trypanosomes like *Trypanosoma congolense*, *Trypanosoma brucei* and *Trypanosoma vivax* have also been isolated from camels, their role in camel trypanosomiasis

is insignificant compared to *Trypanosoma evansi* (Mahmoud and Gray, 1980; ELamin *et al.*, 1998). The disease is the most important single cause of economic losses in camel rearing areas, causing morbidity of up to 30.0% and mortality of around 3.0% camels in Ethiopia (Njiru *et al.*, 2001; Tekle and Abebe, 2001)

This trypanosoma was discovered in India more than a hundred years ago by Evans (1880), who detected it in horses, mules and camels with a disease locally called "surra". Subsequently, numerous reports of trypanosomiasis in horses and camels were recorded in North Africa, the Americas and Eurasia. Many different scientific names for the parasite were used, Hoar *ca.* (1956).

Trypanosoma evansi is transmitted mechanically by haematophagous biting flies. No developmental stage in a vector has been demonstrated which differentiates the parasite from *brucei*. Tabanids (horseflies) play the major role in transmission,

while *Stomoxys spp.* and *Lyperosia spp.* may also transmit. An interrupted feed upon an infected host leaves the fly hungry. Whenever it moves to another host, it can establish a new infection through its trypanosome-contaminated mouthparts. Trypanosomes remain infective on the proboscis for a short period only. The parasite replicates in camels, horses, donkeys, dogs, cattle, water buffaloes and even elephants. Equines and dogs are very susceptible and usually die after an acute course of the disease. Dogs may also become infected by eating meat from a trypanosome-infected carcass (Leese 1927)

Direct life cycle with no intermediate host. Agent is transmitted from animal to animal mechanically by hematophagous flies, including *Tabanus spp.* and *Musca spp.* Also *Lyperosia*, *Stomoxys* and *Atylotus* genera. Tabanids (horse flies) are the most significant vectors. Vampire bats in South and Central America are hosts, reservoirs and vectors of *T. evansi*; they transmit *T. evansi* mechanically in their saliva, and may develop high parasitaemia which may kill the bat. Recovered bats serve as carriers. Carnivores may become infected after ingesting infected meat. Transmission in milk and during coitus has been documented (OIE 2013)

In the Sudan *T. evansi* infection of camels is known as Guffar (Babeker *et al.*/2014)

1-2 Objective of the study:

1\To estimate the prevalence of camel trypanosomosis in Butana area Gedarrif State.

2\ Investigate the risk factors association with the disease.

Chapter One

Literature Review

2-1The Trypanosome:

2-2Etiology

Trypanosomosis are a large group of diseases caused by obligated flagellate blood parasites; that infect members of every vertebrate class (Hoare, 1972). The Trypanosomes are found in blood and ortissues of vertebrate host causing Trypanosomosis in human and animals(FAO, 1994). "Surra" is an animal disease occurring in Africa, Asia, and Latin America ,caused by *Trypanosoma evansi*. The parasite is transmitted by biting flies such as Tabanidae and Stomoxys species, as well as by Vampire bats including Desmodus rotundus (Hoare,1972).

2-3 Morphology

The morphology of trypanosome in Giemsa-stained blood smears are distinguished by their size, shape, position of kinetoplast, size of kinetoplast, position of nucleus, the attachment and length of flagellum. In wet mounts the type of motility and locomotion are also used for differentiation (Losos, 1986).

2-4 Classification of Trypanosomosis

The Trypanosoma is classified as a Flagellate Protozoa from the genus

Trypanosoma of the Family Trypanosomatidae which belong to the Order Kinetoplastida of the class Zoomastigophora. The Zoomastigophora is classified under the Phylum Sarcomastigophora. (Soulsby 1982).

The genus Trypanosoma is further divided into two sections: The Salivaria; which contains the subgenera, Duttonella, Nannomonas, Pycnomonas and Trypanozoon. The other section is the Sterocoraria which include three subgenera, Megatrypanum, Herpetsoma and

Schizotrypanum. Each of the two sections contains many species, and here in the following is a summarized classification assumed from Soulsby (1982) (**Table 1**).

Recent studies based on isoenzymatic differences and molecular techniques, resulted in new subdivisions of the species of trypanosomes into several types. (Uilenberg, 1998).

Table (1) Summarized classification of the Trypanosomes Souls by (1982)

Phylum	Sarcomastigophora (Honigberg and Balmuth, 1963)
Subphylum	Mastigophora (Diesing, 1866)
Class	Zoomastigophora (Calkins, 1909)
Order	Kinetoplastida (Honigberg, 1963)
Suborder	Trypanosomatina (Kent, 1880)
Family	Trypanosomatidae (Ement and Grobden, 1905)
Genus	Trypanosoma (Kent, 1880)
Section	Anterior Station Group (Hoare, 1964)
Salivaria	
Subgenus	Duttonella (Chalmers, 1918)
Species	<i>T. (D) vivax</i> (Zieman, 1905) <i>T. (D) uniforme</i> (Bruce et al. 1911)
Subgenus	Nannomonas (Hoare, 1964)
Species	<i>T. (N) congolense</i> (Borden, 1904) <i>T. (N) simae</i> (Bruce et al., 1911)
Subgenus	Pycnomonas (Hoare, 1964)
Species	<i>T. (P) suis</i> (Ochmann, 1905)

Subgenus	Trypanozoon (Luhe, 1906)
Species	<i>T. (T) brucei</i> and the subspecies <i>T. (T) bruceibrucei</i> (Plimmer and Bradford, 1899)

	<i>T. (T) bruceigambiense</i> (Dutton, 1902) <i>T. (T) bruceirhodesiense</i> (Stephen and Fantham, 1910) <i>T. (T) bruceievansi</i> (Steel 1885) <i>T. equiperdum</i> (Doflein, 1901) <i>T. equinum</i> (Voges, 1901)
Section Sterocoraria	Posterior Station Group (Hoare, 1964)
Subgenus s	Megatrypanum (Hoare, 1964)
Species	<i>T. (M) theileri</i> (Laveran, 1902) <i>T. (M) iragilaphi</i> <i>T. (M) ingens</i>
Subgenus s	Herpetsoma (Doflein,1901)
Species	<i>T. (H) lewisi</i> <i>T. (H) muscle</i>
Subgenus s	Schizotrypanum (Chagas, 1909)
Species	<i>T.(S) cruzi</i>

2-5 Morphology and biology of *Trypanosoma evansi*

Trypanosoma evansi is morphologically identical with, and indistinguishable from, slender forms of other members of the subgenus *Trypanozoon*. Akinetoplastic populations are relatively

common, particularly after drug exposure (Killicd-Kendrick R (1964). Ray H.N *et.al.* (1960). It can be distinguished from *brucei* by isoenzyme electrophoresis (Gibson *.Cet .al* (1983)). *T. evansi* is not restricted to the blood stream. Like other members of the subgenus *Trypanozoon*, it enters tissue compartments or other body fluids; it may cross the blood-brain barrier (Leese (1927). or enter the joint fluids (Rttcher and Schillinger, unpublished results), thus being less accessible to chemotherapy. This situation is comparable to chronic *brucei* infections in mice Jennings F.W. (1977) or to the late stage of human sleeping sickness.

2-6 Life cycle and transmission

Replication of the trypanosome occurs by longitudinal binary fission both in the host and in the vector with the flagellum and kinetoplast dividing together (Liu-Liu *et al.*, 2005), but in the non cyclically transmuted *T. evansi* developmental stages were not observed in any of the mechanical vectors. Consequently a procyclic or insect stage (epimastigotes) does not exist in *T. evansi* which is attributed to lack of maxi circles in the kinetoplast DNA (Ellie *et al.*, 1999) . The noncyclical transmission of trypanosomes is aided by biting flies and thus, in the absence of *Glossina*, the transmission is maintained in the ecosystem. Biting flies, such as *Tabanids* (horse flies), *Stomoxys* and *Hippoboscids* transmit *T. evansi* mechanically through their mouthparts when they feed on more than one host within a short interval because

the trypanosomes remain infective for only a short period (Evans et al., 1995).

2-3 Epidemiology of the camel Trypanosomosis

disease is endemic in Africa, Asia and South America and in addition to camels it is reported in other species of domesticated livestock Enwezor, F. et al(2005)

Trypanosoma evansi causes a trypanosomosis known as 'surra'. It affects a large number of wild and domesticated animal species in Africa, Asia, and Central and South America. The principal host species varies geographically, but camels, horses, buffalos and cattle are particularly affected, although other animals, including wildlife, are also susceptible. It is an arthropod-borne disease; several species of haematophagous flies, including Tabanids and Stomoxes, are implicated in transferring infection from host to host, acting as mechanical vectors *Trypanosoma evansi* lacks the genes necessary for mitochondrial (OIE Terrestrial Manual 2012)

Direct life cycle with no intermediate host Agent is transmitted from animal to animal mechanically by hematophagous flies, including *Tabanus spp.* and *Musca spp.* Also *Lyperosia*, *Stomoxys* and *Atylotus* genera. Tabanids (horse flies) are the most significant vectors Vampire bats in South and Central America are hosts, reservoirs and vectors of *T.evansi*; they transmit *T. evansi* mechanically in their saliva, and may develop high parasitaemia which may kill the bat. Recovered bats serve as carriers Carnivores may become infected after ingesting infected meat

Transmission in milk and during coitus has been documented (OIE Technical Disease Cards 2013).

2-3-1 The camel Trypanosomosis:

Camel trypanosomosis (surra), caused by *Trypanosoma evansi*, is the most important single cause of morbidity and mortality in camels. The disease, transmitted non-cyclically by other haematophagous flies is endemic in Africa, Asia and South America, and in addition to camels other species of domesticated livestock are affected. Because of the wide geographic range of surra (Felicia Nneka Chizoba Enwezor *et al.* 2005)

2-3-2 The camel Trypanosomosis in Sudan:

In the Sudan, By far the most important protozoan disease of camels in Sudan is trypanosomiasis, especially infection with *T. evansi*. According to Karib (1961). camel trypanosomiasis was first recorded in 1904. By 1908 the disease had been diagnosed in Kordofan province north of the 12° parallel, the whole of the White Nile province and the area between Suakin and Kassala along the border with Eritrea. Darfur province was not annexed to the British colony until 1916. Mahmoud (1980)

At present camel trypanosomiasis is widespread in all of the camel breeding areas, occurring in acute and chronic forms. In the acute form the disease is almost always fatal. In the chronic form

there is usually loss of production and bodily condition, and anaemia. Mahmoud(1980)

T.evansi is primarily a parasite of camels causing a disease locally known as Guffar.(Babeker *et,al* 2014) The disease is common in Kordofan and Darfur states in the west, Kassala, Gadaref and Red sea states in the east and to a lesser extent in central Sudan, in the Gezira, Sennar, Blue Nile and Khartoum states (karib, 1961))Then successive reports about to evaluate a simple PCR-based technique for field diagnosis of *T.evansi* infection in camels from Eastern and Western regions of the Sudan(Nahla *et.a/*2011). and other reported The incidence of camel trypanosomosis (Guffar) caused by *Trypanosoma evansi* (*T.evansi*) in Omdurman west plain, western Sudan was surveyed using direct smear, Micro Hematocrit Centrifugation Technique (MHCT) and Card Agglutination Test for *T.evansi* (CATT/*T.evansi*). (Babeker *.et,a/*2014) recently, reports the effects of trypanosomes on camel packed red cell volume (PCV) as depicted from field surveys to determine the prevalence of camel trypanosomosis in the Sudan(Ibrahim1, *et al* 2011)

2-3-3 Tabanus Distribution in Sudan

Transmission is reported to be exclusively mechanical, carried out by a number of species of haematophagous biting flies, including *Tabanus* and *Stomoxys*. Mahmoud and Gray found a definite correlation between the seasonal outbreak of *T. evansi* infections and the increase in number of tabanids during the rainy

season in the Sudan (June-October). Yagi and Razig found that *Tabanus taeniola* and *Tabanus buguttatu* are prevalent throughout the year. The prevalence of tabanid species throughout the year ensures permanent possibility of infections with *T. evansi*, with outbreaks occurring during the rainy season. Malik and Mahmoud found that cattle, sheep, goats and donkeys undergo a protracted infection which may result in a carrier state, and these species may act as reservoir hosts. Although camels do not frequently come into close contact with cattle in the Sudan, they do come into contact with sheep, goats and equines. This situation has now changed as a result of the recent drought, so that wherever cattle are kept in northern Sudan, camels also exist, thereby increasing the possibility of contracting the infection with *T. evansi* and other trypanosomes. Yagi and Abdel Razig reported in (1972) that *Tabanus taeniola* was prevalent throughout the year, while *Atylotus agrestis* have a predisposition to appear at the end of the rainy season. *Atylotus fuscipes* has the same ecological preferences as *A. agrestis* and usually appears at the same season. It was also mentioned that *Tabanus biguttatus* was also common throughout the year, while *Ancala latipes* occurs during the rainy season together with *Philoliches magrettii*, but its flight season was very short. Suliman (1992) reported that seven species were collected from Sinnar area including: *Tabanus Taeniola*, *T. gratus*, *T. biguttatus*, *T. sufis*, *Atylotus agrestis*, *A. fuscipes* and *Philoliches magrettii*. He stated

that the first three were found in all of the surveyed areas (Central State) while *T. biguttatus* was found

2-3-4 Stomoxys Distribution in Sudan

Lewis, (1953) reported two species of *Stomoxys* in Sudan.

Stomoxys

calcitrans and *S.nigra*. Like the Tabanidae they are widely distributed in

Sudan. (AbdelRahman, 1994) confirmed these species in both Blue Nile and Khartoum States.

2-4 Pathology of the cametrypanosome

2-4-1 Course of infection and risk factors

Incubation period in horses, mules, donkeys and camels varies from 5-60 day. The sequel to infection with the trypanosomes is not always a disease, some may affect self cure, but some individual animals may come down with the disease of different stage. In camel trypanosomosis, the period between initial infection and the onset of clinical signs is extremely variable, but generally ranges between 5 and 60 days - although longer periods (such as 3 months) have been recorded. The interval between infection and the demonstration of parasites in the blood is usually less than 14 days (AHA, 2005). Factors that affect the incubation period include the initial infective dose (equivalent to the number of infective insect bites), and stress.

Stress occurs in late pregnancy and early in lactation in animals that are more susceptible (Getahun and Demeke, 1998). Intercurrent infections (helminthosis), also stressful, may accentuate the severity of the disease. Trypanosoma tolerance may also be reduced by low plane of nutrition or when animals have to trek for long distances in search of water and pasture in the dry season. This is especially common in the nomadic pastoral communities. Surra affects camels of all ages with a higher incidence of disease in sub-adult camels shortly after weaning (Evans et al., 1995).

The disease occurs both in chronic and acute form (Gutierrez et al., 2004). The chronic form of the disease is most common and is likely to be associated with secondary infection due to immunosuppression (Njiru et al., 2001).

The disease causing significant losses in camels, the disease, Surra,

manifests itself usually as a chronic infection characterized by weight loss, anemia, infertility and abortion (Luckins, 1988; Lohr, et. al., 1986). The maintenance of good health and productivity in herds is of vital importance to nomadic herders. The most important protozoan parasitic disease is camel trypanosomosis (Surra) which causes severe disease throughout Africa. Trypanosomosis in camels causes considerable economic losses due to a decrease in milk and meat, premature births and abortions (Boidet al., 1985). Affected camels show fever, anorexia, marked generalized edema and deteriorate rapidly and die; the chronic form of *T. evansi* infection is characterized by

progressive loss of body weight ,intermittent high fever, marked generalized muscular atrophy, pale mucous membranes and occasionally abdominal edema. Affected camels also may exhibit a characteristic sweet odour due to an increase of urinary ketones (Schwartz and Dioli, 1992). Trypanosomiasis in camels occurs both in chronic and acute forms (Wilson et al., 1983). The chronic form is most common and may present an association with secondary infections due to immunosuppression caused by *T. evansi* infection, which complicates clinical diagnosis .Clinical signs recorded as emaciation, intermittent fever, anemia, lacrymation, corneal opacity, diarrhea and edema of the dependent parts (Chaudhary and Iqbal, 2000) is insufficient knowledge for diagnosis, while detection of parasites in the blood is difficult because parasitaemia is intermittent (Mahmoud and Gray, 1980). Consequently, there is a need for alternative lesions are non specific and may include:

emaciation of the carcass ,anaemia ,petechial haemorrhages on some internal organs ,hydrothorax and ascites ,enlarged lymph nodes and spleen

and conjunctivitis have been reported in dogs, Neurological signs have been reported in horses, dogs, cattle, deer, water buffaloes and captive Himalayan black bears. Horses in South America frequently develop ataxia, with gradually progressive paresis of the hindquarters accompanied by muscle atrophy. Other neurological signs such as head tilt, circling, hyperexcitability, blindness, proprioceptive deficits and paddling movements have

also been seen. Infertility, abortions and/ or stillbirths have been documented in buffalo, camels and horses. Testicular lesions in camels and experimentally infected goats suggest that, in some species, male fertility might also be impaired. *T. evansi* causes leukopenia, which may result in immunosuppression

2-4-1 Course of infection and risk factors

The gross lesions tend to be nonspecific, and may include wasting or emaciation of the carcass, subcutaneous edema, signs of anemia, enlargement of the spleen and lymph nodes, and petechiae on some internal organs. Muscle atrophy may be noted, particularly in the hindquarters. Hydrothorax and ascites are sometimes seen. The lungs may also be affected; congestion, consolidation, edema, emphysema, hemorrhages and pneumonia have been reported. Cardiac lesions including hydropericardium, pericarditis and evidence of cardiomyopathy or myocarditis occur in some animals.

In some horses with neurological signs, the cerebral hemispheres may be swollen and the gyri flattened. There may be severe edema and malacia, with the white matter becoming yellow, gelatinous and friable. Subpial hemorrhages may also be present. (Murrina, Mal de Caderas, Derrengadera, Trypanosomosis 2009)

2-4-3 Morbidity and Mortality

Camels living in north eastern Africa may have infection rates of 20–70%. Case fatality rate in untreated horses and camels is

nearly 100%. The disease is the most important single cause of economic losses in camel rearing areas, causing morbidity of up to 30.0% and mortality of around 3.0% (Negrenwaet al., 1993; EGBE-Nyiwi and Chaudry, 1994; Pacholer et al., 2001; Njiru et al., 2002)

2-5 Diagnostic techniques

The diagnosis of *Trypanosoma* infection is based on clinical signs and on

the demonstration of the parasites by direct or indirect methods.

The classical direct method for the diagnosis of trypanosomosis led to the

original discovery of the parasite. It is still employed for examining blood or lymph node material, but rarely with extracts of other tissues. In the Tsetse belt where many *Trypanosoma* species are found, a specific identification on blood smears by microscopy is very difficult.

2-5-1 Direct methods

The simplest techniques are examination of wet, thick or thin films of fresh blood, usually obtained from the earvein, jugular vein or the tail. Amongst the direct examination techniques, stained thin blood films are generally regarded as more specific but less sensitive than the other two. The actual specificity and sensitivity of these techniques is directly dependent on the volume of blood actually examined and the skill and experience of the microscopist. (OIE Terrestrial Manual 2013)

2-5-1-1 Wet blood films

A small drop of blood is placed on to a clean glass slide and covered with a cover-slip to spread the blood as a monolayer of cells. This is examined by light microscopy (x200) to detect any motile trypanosomes. But still it will not be enough to identify the species of the trypanosome properly.

The method is simple, inexpensive and gives immediate results. Depending on the trypanosome size and movements a presumptive diagnosis can be made of the trypanosome species. Final confirmation of the species is made by the examination of the stained preparation.

The diagnostic sensitivity of the method is generally low but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the RBCs before examination using a haemolytic agent such as sodium dodecyl sulphate (OIE Terrestrial Manual 2013)

2-5-1-2 Thick blood films

These are made by placing a drop of blood (5–10 μ l) on a clean microscope slide and spreading it over an area of approximately 2 cm in diameter, using the corner of another slide. The thickness of the resultant film should be such that, when dry, the figures on a wristwatch dial can just be read through it. The film is dried thoroughly by rapidly waving in the air and, without fixation, is dehaemoglobinised by immersion in distilled water for a few seconds and dried before staining. A dry smear should be kept dry and protected from dust, heat, flies and other insects. It is

stained for 30 minutes with 4% diluted Giemsa stain in phosphate buffered saline, pH 7.2. Staining time and stain dilution may vary with stain and individual technique. Therefore, it is important to start with the manufacturer's directions and to vary staining time and stain concentration to obtain the optimal result. The stained smear is then washed with buffered water and examined at ×500 to ×1000 total magnification. The method is simple and relatively inexpensive, but results are delayed because of the staining process. Trypanosomes are easily recognised by their general morphology, but may be damaged during the staining process. This may make it difficult to identify the species. ((OIE Terrestrial Manual 2013))

2-5-1-3 Stained thin smears

A drop of blood is placed 20 mm from one end of a clean microscope slide and a thin film is drawn out in the usual way. The film is air-dried briefly, fixed in methyl alcohol for 2 minutes and allowed to dry. The smears are then stained by Giemsa. This technique permits detailed morphological studies and identification of the trypanosome species. Rapid staining techniques also exist like Field's stain. The same technique can be used with Lymph node biopsies.

Usually, both a thin and thick smear is made from the same sample. Thick smears contain more blood than thin smears and, hence, have a higher diagnostic sensitivity. Thin smears on the other hand allow

Trypanosoma species identification.(OIE Terrestrial Manual 2013).

2-5-1-4 Haematocrit centrifugation

The probability of detecting trypanosomes in a sample from an infected animal depends largely on the amount of blood examined and the level of parasitaemia. The amount of blood examined with direct examination techniques is low and parasites are often very scanty in the blood of an infected animal. Both of these factors contribute to the low sensitivity of direct examination techniques. Sensitivity can be improved by increasing the volume of blood to be examined and by concentrating the trypanosomes.(OIE1013)

In the mild clinical or sub clinical cases (carriers) with low parasitaemia in which it is difficult to demonstrate the parasites concentration methods become necessary.

Blood is collected (70 µl) into heparinised capillary tubes (75 x 1.5 mm),

which are then sealed at the dry end and centrifuged, sealed end down. Then capillary tube is placed under microscope and the buffy coat junction where the trypanosomes will be concentrated is checked for trypanosomes. The buffy coat can also be placed on a slide and checked under dark field microscope. (Wernery *et al.*, 2001)

2-5-1-5 Animal inoculation

Laboratory animals may be used to reveal subclinical (nonpatent) infections in domesticated animals. *Trypanosoma* spp. has a broad spectrum of infectivity for small rodents, and so rats and mice are often used. In studies of *T. evansi* infections in camels, comparisons have been made between thick blood film examinations and rat or mouse inoculation methods; the latter animal inoculation gave 15.2% and 17%, respectively, more positive results than thick smears alone. However, even mouse inoculation is not 100% sensitive (Monzen *et. al.* 1990). Sensitivity of this in-vivo culture system may perhaps be increased by use of immunosuppressed laboratory animals. Drugs such as cyclophosphamide or hydrocortisone acetate and X-ray irradiation or splenectomy are used for this purpose. (Monzen *et al.*, 1990)

2-5-2 Serological tests

Methods to detect specific humoral antibodies to trypanosome antigens include complement fixation (CF), indirect haemagglutination and precipitation tests. These have not been applied in large-scale surveys. More recently, indirect fluorescent antibody, ELISA and card agglutination tests (CATT) have been employed. Extensive evaluation of ELISA and CATT has been carried out.

2-5-2-1 Indirect fluorescent antibody test

The test is used to detect trypanosomes antibodies. It has proven to be sensitive test but it has the disadvantage of that it can only be carried out in laboratories and the procedure is rather long and complicated as well as some extent subjective(i.e. titration but different operators may give somewhat different results). (G.Uilenberg, 1998)

2-5-2-2 Enzyme-linked immunosorbent assay

An immunodiagnostic method based on a direct sandwich Enzyme linked Immuno Sorbent Assay (ELISA), using monoclonal antibodies, has been examined in a number of African laboratories for its suitability for monitoring tsetse control and eradication programmes. Generally, the direct sandwich ELISAs for the detection of trypanosomal antigens in serum samples have proved to be unsatisfactory with respect to diagnostic sensitivity when compared with traditional parasitological methods such as the dark ground/phase contrast buffy-coat technique. Consequently, antigen-detection systems exploiting various other direct, indirect and sandwich ELISA systems and sets of reagents are being developed to improve diagnosis. In addition, an existing indirect ELISA for the detection of antibodies has been improved and is being evaluated in the field in order to detect cattle that are or have been recently infected with trypanosomes. (De Rebeskiet *al*, 1999).

2-5-2-3 Card agglutination tests

It is well known that certain predominant variable antigen types (VATs) are expressed in common by different strains of salivarian trypanosomes from different areas. On this basis, a field test for the diagnosis of Gambian sleeping sickness, the card agglutination test - CATT/*T. bruceigambiense* - was developed at the Laboratory of Serology, Institute of Tropical Medicine, Antwerp. For the diagnosis of *T. evansi* infections, a similar test system has been developed. CATT/*T. evansi*. (Nantulya, 1995)(Van den Bossche *et al.*, 1999)

2-5-3 Detection of trypanosomal DNA

During the past few years, several research centres have been working on the development of polymerase chain reaction procedures for the detection of minute amounts of trypanosomal DNA sequences. PCR assays for diagnosis of trypanosome infection in cattle were evaluated for their ability to detect trypanosome DNA in blood spots samples collected from cattle in four different provinces from the Bolivian lowlands and the results compared with those obtained with standard parasitological Micro Haematocrit Centrifugation Technique (MHCT) and stained smears and serological methods (Card Agglutination Test for *T. evansi* (CATT), and Antibody ELISAs for *T. vivax* and *T. congolense*). Kappa agreement analysis showed a significant agreement between PCR assays and results from parasitological methods but there was no agreement when PCR was compared with serological

assays. Some samples from *T. vivax* smear positive animals were negative by PCR, therefore modifications to the PCR assay conditions were undertaken to try to improve agreement between PCR and parasitological assays. Changes in the template DNA concentration or the use of an alternative primer set resulted in improvements in the PCR detection rate, but not all the parasitologically positive samples were detected by PCR. Results from PCR assays for *T. vivax* and *T. evansi* were combined with results from parasitological and serological assays to provide information on prevalence rates for the four provinces from where the samples were obtained. (Gonzales *et.al*, 2003)

So far the method is still being evaluated and may be in the future it will

be widely used in the field.

2-6 Control

2-6-1 Parasite control

2-6-1-1 Immunization

Due to the fact of that the trypanosome has genes that can code for many different surface - coat glycoprotein and change its surface coat to evade the antibodies it has been very difficult to produce vaccine immunization from trypanosome; lots of researches have been done but still no vaccine (Mare, 1998, Uilenberge, 1998).

2-6-1-2 Chemotherapy:

The use of drugs for the prevention and treatment of trypanosomiasis has been important for many decades, but the

rapidity with which the trypanosomes have developed resistance to each drug introduced has tremendously complicated this approach to control the disease. In spite of this, some of the older chemoprophylactic drugs such as the Quinapyramine derivatives Antrycide and Antrycide Prosalt are still used and give effective protection against *T. b. Brucei* infection in horses, camels, and cattle for up to 3 months. The drug Pyrithidium bromide (Prothidium and AD2801) is useful in the prophylaxis of *T. vivax* and *T. congolense* infections in cattle, sheep, and goats and can give protection for up to 6 months. The most widely used of the newer chemoprophylactic drugs (and also the least expensive) is Isometamidium chloride. This drug, in use for over 20 years and sold under the trade names Samorin, Trypamidium, and M&B is effective for the prophylaxis of all three African animal trypanosomes, and gives protection for 3-6 months. The development of resistance to this drug has been reported in both east and West Africa. Homidium bromide has also been found to be an effective chemoprophylactic drug in Kenya, and the newly introduced arsenical Cymelarsan is effective in treatment of *T. b. Brucei* infection.

A very widely used chemotherapeutic drug is Diminazine Aceturate

(Berenil), which is effective against all three African animal trypanosomes. The Isometamidium drugs are also excellent chemotherapeutic agents as are the quaternary ammonium trypanocides Antrycide, Ethidium and Prothidium. While

Trypanocidals are used in trypanosomiasis control, as chemotherapy and for chemoprophylaxis (although they are time-consuming and expensive), there is a growing concern that their future effectiveness may be severely curtailed by widespread drug resistance. In addition to the 11 countries (Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, the Sudan, the United Republic of Tanzania, Uganda, and Zimbabwe) reported by Peregrine (1994), the Central African Republic (Kongo) (Finelle and Yvone, 1962) and Zambia (Mubanga and Sinyangwe, 1997) should be included. In eight of the 13 countries, multiple resistances have been reported.

Here in Sudan drug resistance has been reported against Humidium (Ethidium Bromide) for *T. congolense*, *T. vivax* and *T. brucei* (Abdel Gadiret, *al.*1981).

For Human trypanosomiasis the WHO has recommended a two phased treatment according to the disease stage, first phase treatments:

- **Suramine:** discovered in 1921, it is used in treatment of the initial phase of *T. b. rhodesiense*. There are certain undesirable effects, especially on the digestive tract.
- **Pentamidine:** discovered in 1941, it is used in treatment of the initial phase of *T. b. gambiense* sleeping sickness. In spite of a few undesirable

effects, it is well tolerated by patients. Future production is guaranteed by an agreement between WHO and Aventis. Then when the disease enters the neurological phase comes the second phase treatments:

- **Melarsoprol:** discovered in 1949, it is at present the only drug available on the market to treat the advanced stage of sleeping sickness, no matter which parasite is the cause. It is the last arsenical derivative in existence.

The undesired effects are drastic; they include reactive encephalopathy (a hyperacute neurological complication of an allergic nature) - often fatal -

in 3% to 10% of cases; those who survive the encephalopathy suffer

serious neurological sequelae. Furthermore, there is considerable resistance to the drug, rising to 30% in parts of central Africa.

- **Eflornithine:** this molecule, which was registered in 1990, is the alternative to melarsoprol treatment. It is effective only against *T.b.*

gambiense. The regimen is strict and hard to apply. Production ceased in

1999. Aventis company gave the licence to WHO, which has undertaken

several initiatives to seek a new source of production.
(Burchmore, 2004)

2-6-2 Vector control

The nature of the life cycle, with few offspring per female and a large investment per offspring, means that a tsetse population cannot survive under sustained regular mortality above natural levels. It has been calculated that an extra 4% mortality of females per day over a sustained period will cause¹⁷ extinction of a tsetse population. While it is possible to achieve eradication of tsetse in particular areas, these attempts often fail in the long-term because of reinvasion of tsetse-flies from adjacent regions. It is up to individual countries, or countries working together, to ensure that tsetse-flies are controlled sufficiently or eradicated. "The availability of the technology to reach a successful conclusion is usually not the limiting factor" (Nevill2003).

An essential part of a control program is to understand the biology and ecology of the *Glossina* species involved. Especially important is to understand their movement, density and distribution and to use trapping methods to monitor what is going on as the control campaign progresses.

The main control methods are as follows.

2-6-2-1Removal of vegetation:

In savanna areas, larvi position occurs in shaded places, so one control method is to remove trees and bushes so one is just left with grass. This method was used quite extensively with success in the past but is labourintensive and requires that there be reslashing of vegetation on an annual basis. The method fell into disuse with the advent of insecticides. However,removal of vegetation for fire wood and urbanization has sometimes achieved the same effect

2-6-2-2 Killing of wild animals

The object here is to remove reservoirs of infection in the wild animal populations. This method was used extensively in the past.

2-6-2-3 Spraying of insecticides

Two main approaches have been used:

- (a) Spraying of residual insecticides that persist in the environment for at least 2-3 months.
- (b) Spraying of non-residual aerosols that kill adult tsetse at the time of spraying but which must be repeated at regular intervals in order to kill newly emerged adults. Both ground and aerial application methods have been used. Aerial methods are expensive but have been used with success. For instance, in

Zululand (northern KwaZulu-Natal, South Africa), between 1946 and 1953, the savanna tsetse-fly species *Glossina pallidipes* was totally eradicated mainly through the use of aerially applied DDT and BHC. However, consider the detrimental environmental effects of using these residual insecticides. In addition, the tsetse-fly problem still remains in Zululand due to two other cryptic species *G. austeni* and *G. brevipalpis* (Nevill, 2003). Ground spraying of residual insecticides can be a feasible and economical control strategy if it is applied to selected sites where there are concentrations of tsetse-flies

2-6-2-4 Trapping

A number of different traps have been developed for capturing tsetse-flies in large numbers.

a. The NG2G trap was designed for economy, simplicity and efficiency.

It was optimised for the tsetse *Glossina pallidipes* in Kenya following the

development of the F3 and Epsilon trap for this species in Zimbabwe. It

performs well for many tsetses, and for tabanids, but it is a poor trap for

stable flies. The NG2F with the large blue "wing" split into equal wings

on both sides of the trap body is now more popular.¹⁹

b. Biconical traps for the Riverine Tsetse that was the first practical cloth

trap designed for tsetse in West Africa. It is an efficient trap for most riverine tsetse. It has been used for fly surveys for many years, even though it is a poor trap for tabanids and stable flies, and most savannah tsetse. It is a difficult trap to sew due to the use of complicated inner screens and its conical shape.

c. The Vavoua trap was designed as an economical alternative to the pyramidal trap for large-scale control of riverine tsetse. There are many similar designs that employ hanging screens. These designs are efficient for both riverine tsetse and stable flies. Traps like the Vavoua are straight forward to sew and assemble. They can be adapted to hang from simple wooden supports or trees. Trap styles with this open concept are generally poor for tabanids and savannah tsetse.

d. Canopy for Horse Flies & Deer Flies. This is a large trap that evolved from the original Manitoba trap developed for horse flies in Canada.

Traps based on the concept of a large, dark canopy (with or without a suspended, shiny black ball) are often used for tabanids in temperate environments. A commercial option is the horsefly trap. A simple, practical design for the control of *Tabanus nigrovittatus* is the Green head Box trap. For research purposes, large Malaise traps are often used for catching tabanids, especially deer flies *Chrysops*.

e. In the USA, stable flies are often sampled with sticky "traps". A commercial adaptation of the cylindrical Alsynite trap. It uses disposable sticky sleeves attached to a special type of fibreglass panel. Some of these traps are impregnated with insecticides to insure the death of the fly.

2-6-2-5 Sterile insect technique (SIT)

This method involves breeding of male *Glossina* which are sterilized using radiation and then released at regular intervals, thus swamping the population with males that are unable to fertilize females successfully.

Glossin aausteni has been successfully eradicated from Zanzibar using this method. During this campaign, 60,000+ irradiated male flies were

being released per week. From 1995-1996, 5.5 million sterile males were released in total. To get this number of males per week involved rearing a colony of 700,000+ female flies (Dyck et al. 1997). All of these control methods has been criticized either as non environmentally friendly techniques (killing of wild animals, spraying of insecticides and removal of vegetation) or very expensive such as the sterile male technique. (Saini, 2003)

A new approach for the fly control has been adopted recently which is the concept of community participation in the fly control. The approach has been called the community based tsetse control. (Barrett, 1998)

Locals are trained on how to make their own fly traps and how to work together with the scientists to eradicate the fly from their areas. The greater community involvement in project design might help to avoid inappropriate and non sustainable interventions. Specifically, the high level of collective and sustained action that is often expected of

communities in tsetse control projects needs to be compared with the methods that people are already using to control trypanosomosis. This comparison of options is particularly relevant when private, individual action to control the disease is well established and widespread. (Catley,2000)

The trypanosomosis is responsible for significant reductions in the number of red blood cells(RBC), hemoglobin and packed cell volume (PCV) the PCV as an indicator of anemia. (Bourdichon, 1998)

Anaemia is regarded as the main pathological feature of trypanosomosis (Ikedeet *al.* 1977; Ismail 1988 and Rahman *et al.* 1997). In most instances, PCV alone is used to determine the degree of anaemia. Higgins (1986) reported the range of normal camel PCV to be 24% to 42%. Similar studies, however, on small number or in a few camel herds were conducted in the Sudan by Salaheldinet *al.* (1979) and, Musa and Mukhtar (1983). The latter authors reported a mean PCV of 30.0% with a range of 25-34% from 96 Sudanese camels in Tambool area. More recently, the effect of camel age on PCV values was recorded by Omer *et al.* (2006). They reported PCV% of 26.69 ± 3.25 for suckling calves and 24.87 ± 2.63 for weaned calves. Seasonal variations on the haematological values of 100 camels had been recently reported by Nawalet *al.* (2006). The haematological studies carried out so

far on the camel-in their natural pasture- in the Sudan are far from being complete. More baseline data is needed on the haematology of normal and infected camels.

Parasitic infections especially trypanosomosis are one of the major constraints hindering camel industry in the Sudan (Mahmoud and Gray 1980; Losos 1986; Shommein and Osman 1987; Majid 1998).

A prevalence of 2.04%, and 1.12% in Gedarrif and Kassala were reported by Dafalla (1988).

Although anaemia is not itself pathognomonic it remains, however, one of the most important indicators of animal trypanosomosis (Demeke 2003). The ability of trypanosome-infected animal to control development of anaemia is a criterion of trypanotolerance and is measured by the PCV levels (Trail *et al.*, 1992).

A warning was foreseeable the 2007), so effective treatment at this stage of PCV will help the animal to maintain productivity. Finally, close follow-up of the trend of PCV, level of parasitaemia and productivity will allow for strategic and effective use of trypanocides in trypanosomosis control (Mbwamboet *al.*, 2007). They also use PCV values of less than 25% as indicator to improve the sensitivity of mouse inoculation technique. This paper reported the prevalence of "Guffar" in geographically different areas in the Sudan during which the range and mean \pm SD of PCV values of infected and non-infected camels were recorded and compared for significances. The use of PCV as an indicator for

camel trypanosomosis in the Sudan was discussed. (Ibrahim1, A. M.*et al.*2011).

In Ethiopia

Acros-section study was conducted to determine the prevalence of camel trypanosomosis and assess the distribution and dynamics of the vectors responsible for transmission of the disease in five localities of Fntale district from September 2008 to January 2009. Parasitological examination was conducted using the microhaematocrit centrifugation technique (MHCT) and examination of Giemsa stained blood smears. The only species of trypanosome identified was *Trypanosoma evensi* with a prevalence of 4.7% by MHCT and 4.4% by blood smear. The prevalence was higher in male (6.8%) than female(4%) camels. With regard to age, calves(less than 2 years of age) were negative; the prevalence is high (7.7%) in young camels (between 3-4 years of age) and 4% in adult camels (older than 4 years of age). However, the difference in prevalence between sex and age groups was not statistically significant ($P>0.05$). The prevalence using blood smears was found to be different between different localities; the highest being 7.8% for Kobo and the lowest 2% for Harokersa. The mean packed cell volume (PCV) of *Trypanosoma evensi* positive camels (22.43%) was significantly lower than that of negative camels (28.13%) ($P<0.05$). More than 99% of the biting Iflise captured from the study area were If liesunder the genus *Stomoxys*, while a few others such as *Tbanus chrysops* and *Lyperosia* were also captured. The highest fly count was

recorded in September whilst the lowest was recorded in December. The current findings should not be generalized for all camel producing areas of the country or for all seasons in the same area. The prevalence of *trypanosoma evansi* might be higher during the rainy season when the fly population (*Tabanus*) is expected to be high. Therefore, detailed studies should be carried out involving different seasons and the relative importance of different vectors in transmission of the disease in different ecologies.

In south western Ethiopia

An across-sectional study was carried out to determine the prevalence of camel trypanosomiasis (*surra*) in Delo-Mena district, Bale Zone, Oromia region, south western Ethiopia from September to December 2004.

Blood samples were collected from randomly selected 395 camels. Wet film and Giemsa-stained blood smears were used for the detection of trypanosomes. Among these, 72 (18.22%) samples were positive for

Trypanosoma evansi (*T. evansi*), the only *Trypanosoma* species identified. A higher infection was found in males (20.25%) as compared to females (17.72%). However, there was no statistically significant difference in prevalence between sex categories ($p > 0.05$). Highest 27.63% infection was noted in age group > 4 years, followed by 14.54 and 10.52% in 1 to 3 years and 3 to 4 years old camels, respectively. There was a statistically significant difference ($p < 0.05$) in susceptibility among age

groups. These results seem to indicate that *T. evansi* infection has a relatively low prevalence in the study area. There is a need of further study on the distribution and seasonality of the disease and its vectors in order to establish control measures in affected herds and avoid dissemination of the disease. (TodewosKassaTadesseEgule and Hassen 2011).

In Egypt

two local strains of dromedaries' camels (Sudanese and Maghrabi) are different from each other in some morphological, physiological properties and their origin. *Trypanosoma evansi* was well studied in imported and local Sudanese camels. No published studies exist on trypanosomiasis in Maghrabi camels originating from North Africa, despite serious epidemics which have occurred in recent years. For the first time an epidemiological survey was carried out from May 2012 to July 2013 in Northern-West Coast, Egypt to assess prevalence of *T. evansi* infection in Maghrabi dromedary camels. Some mainly parasite-related epidemiological factors that may play a role in the distribution and impact of trypanosomiasis were determined with special attention to the specific epidemiological situation found in the study area. Blood film examination (B. film) and polymerase chain reaction (PCR) using two specific primers (RoTat 1.2 and TBR1.2) based assays optimized for the detection of *T. Evansi* were evaluated. Out of 249 camels examined for trypanosomiasis: 52 (20.9%), 164 (65.9 %) and 186 (74.7%) were positive by B. film, PCR based RoTat 1.2 and PCR based TBR 1.2, respectively.

PCR based TBR 1.2 showed the highest sensitivity values, whereas PCR based RoTat 1.2 was the most specific tool. Age and sex are likely to be risk factors for trypanosomiasis in camels. The majority of trypanosomiasis in Maghrabi camel is apparently caused by a single *T. Evansi* infection in the study area. It is concluded that *T. evansi* was prevalent in all districts sampled at high levels regardless of the test used. Our findings coincide with our previous suggestion of the presence of *T. brucei* and *T. equiperdum* infections at the borders of Egypt. *T. evansi* reveals a big problem that needed to be re-evaluated. (Safaa M. Barghash, et al 2014).

In Kenya

Point prevalences and animal-level risk factors for *Trypanosoma evansi* infection were investigated in a cross-sectional study that involved 2 227 camels from eastern and central parts of Kenya. The screening tests used were haematocrit centrifugation technique (HCT), mouse inoculation and latex agglutination (Suratex®). All camels were screened with HCT, while 396 and 961 of them were, in addition, screened with mouse inoculation and Suratex® tests, respectively. Parasitological and Suratex® test results were used in parallel to determine the number of camels exposed to *T. evansi* infections. Statistical analyses were conducted using Statistical Analysis Systems.

Parasitological and Suratex® test results in parallel were dependent variables in multivariable logistic regression models that determined risk factors for *T. evansi* infection. Herd-level clustering was corrected with general estimation equations. The prevalences were 2.3 % and 19.6 %, using parasitological and Suratex® tests, respectively, and 21.7 % when both tests were used in parallel. There was a positive association between the screening tests (McNemar's test = 104.8, P = 0.001) although the strength of association was low (Kappa = 0.2; 95 % CI: 0.1 - 0.3). Before accounting for herd-level clustering, dry season (OR = 1.5; 95 % CI: 1.0, 2.1) and nomadic pastoralism (OR = 1.8; 95 % CI: 1.1, 3.2) were associated with increased odds of a camel being exposed to *T. evansi* infection compared to wet season and ranching, respectively. Following this correction, only nomadic pastoralism was significantly associated (OR = 3.1; 95 % CI = 1.0, 14.4) with *T. evansi* infection compared to ranching. It is concluded that camels managed under nomadic pastoralism had higher risk of being exposed to *T. evansi* infections than camels from ranching systems of management. (J.M. Ngaira B. BETT et al 2002)

And In Eastern Ethiopia

A cross-sectional study was conducted from October 2008 to June 2009 with the objective of estimating the prevalence of camel trypanosomosis (surra) and identifying the species of

trypanosome involved in Jijiga Zone. The method employed was the Buffy coat examination under microscope. The overall prevalence of camel trypanosomosis was found to be 3.9% (14/362), and the only species identified was *Trypanosoma evansi*. The prevalence of *Trypanosoma evansi* was not significantly ($P < 0.05$) varied between males (3.6%) and females (3.9%) camels. Higher prevalence of trypanosomosis observed in adult camels (4.5%), and no young camel (0 to 4 years) was found positive. The mean PCV was significantly ($P < 0.05$) lower in parasitaemic camels (24.7%) than in aparasitaemic camels (28.9%). The result of his study showed that camel trypanosomosis is prevalent in Jijiga zone and it is a disease of major economic importance in the area. (Tadesse A et al 2012).

Chapter Tow

Materials and Methods

3-1 Study area

The study area was Butana area, the Northern of Gedarrif state this region far about 160 K.M. Form Gedarrif. around Four State Eastern Kassala Western East Gezira southern River Nile Khartoum Western El-Showak is a research station that belongs to the Camel Research Centre (CRC). It is a focal point for camel pastoralists in Butana area. Being a collection point, it becomes an important camel market in the region. Butana is situated well within the arid zone of the Eastern Sudan and occupies an area of approximately 120000 km² and lies between latitude 13° 4' N to 17° 50' N and longitude 32° to 36° E. Most of the Butana is series of flat easily flooded plains interspersed by few hills. The prevailing climate is warm in summer which extends most of the (March-October) and includes the rainy season (June-September). The vegetation composed of *Aristida spp.* (Gow) *Cymbopong on nervatus* (Nal); *Acacia mellifera* (Kitir); *Calotropis procera* (Usher); *Capparis deciduas* (Tunduub) and a variety of grasses (Abdalla, 1985). Normally the camels and their owners move /migrate in search for water and grasses eastward to the Ethiopian borders, where the Tsetse flies *Glossina fucipes* is report

3-4 Questionnaire execution

Apre-tested structured questionnaire with the primary objective of elucidatingthe multifactorial background of disease was conducted in an interactive manner at every farm visited. Five herds were visited in each village and 15animals from each herd were examined and the questionnaire was filled out by asking the owner. The individual risk factors attributes included breed, age, sex, previous history of the disease, body condition, other diseases, appearance signs of disease. The farm attributes included the size of the herd, hygiene and health practiced , public administration and the type of grazing, , stoking density, vector control ,use treatments and source of the animals.

3-5 Study type:

The study type used was cross-sectional study to estimate the prevalence of camel trypanosomosis, and disease and associated risk factors, The effect of the disease upon packed cell volume (PCV) was so investigater

3-6 Sampling methods:

The probability sampling methods were used to selected the animals. Firstly the using multistage sampling, three localities were selected from the 12 localities of the State. Where Then from each locality two administration units were selected and then subunit (villages) were selected from each unit. Finally,

animals were selected by using simple random sampling from each village. The prevalence was calculated using the formula described by Martin *et al.*(1987)

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

Total no. Of camel at a particular point in time

Sample size determination:-

The sample size was calculated by the formula:-

$$N = \frac{4P*Q*}{L^2}$$

N = sample size ,

P = expected prevalence ,

Q = **(1-P).**

L = desired absolute precision. (Martin ,et ,al ,1987)

From the previous studies the samples size was calculated, from Tadewoss *et al*(2011), according to the study on prevalence of camel trypanosomosis (*Trypanosoma evansi*,)The prevalence was estimated about 4.4% so the sample size estimated was 67.4 to increase the precision of the study , the sample size was multiplied by 4 so the number of sample became 270 sample (Thrusfeild ,2005)

$$N = \frac{4 \times (0.044) \times (0.0956)}{(0.0025)} = 67.4 \text{ animal samples}$$

67.4 x 4 = 270 animal samples

3-7 Blood Sample Collection:

Whole blood samples from 270 camels were collected by jugular vein puncture into 5 ml ethylene tetra-acetic acid (EDTA) coated vacutener tubes, kept in cooler boxes and transported immediately to the Gedarrif laboratory for processing.

3-7-1 Giemsa's stain:

The Thin blood smears were made, as per method described by Murray *et al* 1977) Air dried smears were fixed in absolute methyl alcohol for 2-3 minutes. The slides were immersed in Giemsa's stain for 20-25 minutes and washed with tap water to remove excess stain. After air-drying the slides were examined under oil immersion objective lens (100x) for detection and identification of *Trypanosoma* species based on their morphological characters

3-7-2 Microhaematocrit centrifugation technique (Woo method):

Two heparinised capillary tubes (7.5×1.5mm) were filled with blood (about 70µl) from each camel Jugular vein puncture. A drop

of blood was placed on microscopic slides, covered with coverslip (22×22mm) and examined under microscope for the presence of the motile trypanosome using ×10 eye piece and ×40 objective lenses. The capillary tubes were then sealed at one end with “plasticel” and centrifuged using haematocrit centrifuge (SH 120, Shanghai Surgical Instruments factory) for four minutes at 12000 revolutions per minute (rpm). The PCV was recorded using haematocrit reader and then the buffy coat was examined for parasites following the method of Murray *et al.* (1977) and Basaznew Bogale,*et al* (2012).

3-8 Packed Cell Volume (PCV):

The estimation of anaemia was simple and reliable estimated by measuring packed cell volume (PCV) the blood was put in heparinised tubes (3/4full) sealed on one end with cristaseal Amicrohaematocrit centrifuge was used according to Woo(1970) to calculate PCV . the length of the pack red blood cells column was expressed as a percentage volum of blood using PCV reader (Uilenberg1998 and Tegegne2004) Animal with PCV lees than 20% were considered anaemic(Ibrahim1, .*et al.*2011)

3-9 Data analysis:

The overall prevalence was calculated based on positive result, divided by the total number of animal examined at particular time in this study the collected data of the laboratory examination outcome were entered into Microsoft excel spread

sheets (Microsoft office excel 2007)and then transferred to SPSS(version16,0copyright(c)spssIC1989 -2007)for analysis .First the data were analyzed by simple descriptive statistic using frequencies relative frequencies and cumulative frequencies and cross tabulation , To estimated the strength and statistical significance of association between risk factor and disease aunivariate analysis was used in aunivariate analysis chi -square test was used all potential risk factor were significant(p-value <0.25) in the univariat analysis entered for further multivariate analysis using logistic Regression. in the logistic Regression risk factor with(p-value 0.05)was considered significsnt association with disease

Chapter Three

Results

From a total of 270 animals sampled 14(5,1%)were positive.the over all of prevalence of camel Trypanosomisis Butana area, in Gaderrif State, was(5,1%)show in table (2)

(Table2) Distribution of trypanosomisis in 270 camel examined in Butana area Algedarrif State

Results	Frequencies	cumulative frequency%
Positive	14	5,1
Negative	256	94,9
Total	270	100,0

from a total of 270 camel As shown in table (2) examined by direct microscopic by Giemsa's stain and Buffy coat the prevalence detected was 11(4%) and 14(5,1%) by Giemsa's stain and Buffy coat respectively.

table (3) Distribution of Trypanosomiasis in 270 camel examined in Butana area by Giemsa's stain and Buffy coat according to risk factor

Test	Positive No.	Prevalence %
Giemsa's stain	9	3,3
Buffy coat	5	1,85
Total	14	5,1

Sample were selected from three localities Gedarrif, El-Showak and Butana (122, 35 and 113) respectively. three breed were selected Arabi Anafi and Bushary(106, 29 and 81) respectively . In sex group (27 Male 243 Female) were selected .Regarding age

15(1-5)years 127(5-10)years 128(>11)years were examined .three body condition group (90 animal good,91animal Moderate and89animal poor)were selected .Three heard size (39large,197modarat and 34small) were selected .Two source of the animal (200 breeder and 70 market) were used Regarding pack cell volume 35animals were anaemia PCV values less than(20%) and 235 animals were not anaemic table(5)
All animal examined were grazing out door

Table (4) Distribution of 270 camel examined for Trypanosomiasis according potential risk factors

Risk factor	Number tested
Locality	
Gedarrif	122
Showak	113
Butana	35
Total	270
Sex	
Male	27
Female	243
Total	270
Breed	

Arabi	106
Bushary	81
Anafi	29
Total	270
Age	
1-5	15
5-10	127
>11	128
Total	270
Heard Size	
Large	39
Moderate	197
Small	34
Total	270
Body Condition	
Good	90
Moderate	91
Poor	89
Total	270
Source of Animal	
Breeder	200
Market	70
Total	270

PCV values in non-infected and infected camels:

Showed difference in the level between PCV values less than 20% in non-infected and infected camels due to *T.evansi* table (5)

Table (5) The distribution of 270 camel with and without anaemia

Result	Number
Anaemic	35
Non Anaemic	235
Total	270

The percentage packed cell volume (PCV %)

T.evansi infected camels showed a low grade anaemia, which is the main feature of camel trypanosomosis (Fatihu et al., 2000). Death due to trypanosomosis is usually a result of severe anaemia, and animals that are capable of compensating the reduction in PCV and erythrocytes indices during the course of infection often survive

The distribution of anaemic animal with infection in localities were in Gedarrif 6% , Showak 6% and Butana 2% . and Anaemic animal with non infection were Gedarrif 11% , Showak 6% and Butana 4% . .

Most of the camels that were not infected with trypanosomes and had PCV values less than 20%, may be infected with gastrointestinal parasites particularly Haemonchus longistipes. Some Fasciola spp and Schistosoma spp .

Table (6)The distribution of anaemic animal with infection and anaemic with non infection in localities

Area	NO. tested	Anaemi c with infectio n%	Anaemi c with non infectio n	Rang with infectio n	Rang with non infection
Gedarri f	122	6	11	16-20	20-31
Showa k	35	6	6	18-19	20-35
Butana	113	2	4	17-18	20-33
Total	270	14	21		

The distribution of positive samples according the risk factor were 6(5.3%) in Butana ,6(4.9%) in Gedarrif and 2(5.7%)in Showak As for breed ,the positive sample in Arabi was 9(8.4%),Bushary was 2(2.4%) and 3(10.3%)in Anafi .As for Sex 3(11%)in males and 11(4.5%)was in female, for age group the prevalence was 1(6.6%)in 1-5 years ,3(2.3%)in 5-10 years and 10(7.8%) in >11years .In heard size was 7(17.9%)in large heard in Moderate was 5(2.5%) and 2(5.8%) was in small heard size .in body condition positive sample in good body condition was

3(3.3%), moderate was 5 (5.4%) and 6(6.7%) was poor body condition. In sources of animals in breeder 10 (5%) and 4(5.7%) was in market camel (table 7)

Table(7) Distribution of positive camel examined for Trypanosomiasis according potential risk factor

Risk factor	Number tested	Number positive
Locality		
Gedarrif	122	6
Showak	113	6
Butana	35	2
Total	270	14
Sex		
Male	27	3
Female	243	11
Total	270	14
Breed		
Arabi	106	9
Bushary	81	2
Anafi	29	3
Total	270	14
Age		
1-5	15	1
5-10	127	3
>11	128	10
Total	270	14
Heard Size		

Large	39	7
Moderate	197	5
Small	34	2
Total	270	14
Body Condition		
Good	90	3
Moderate	91	5
Poor	89	6
Total	270	14
Source of Animal		
Breeder	200	10
Market	70	4
Total	270	14

Table (8) Distribution of 270 camel examined for trypanosomiasis in Butana area according to potential risk factor:

Risk factor	Frequency	Percent	Cumulative Frequency
Locality			
Gedarrif	122	41.5	41.9
Showak	35	44.9	87.0
Butana	113	12.9	
Total	270	99.3	100.0
	272	100.0	

Sex			
Male	27	9.9	10.0
Female	243	89.3	100.0
Total	270	99.3	
		100.0	
Bred			
Arabi	160	58.8	59.3
Bushary	81	29.8	89.3
Anafi	29	10.7	100.0
Total	270	99.3	
	272	100.0	
Age			
1-5	15	5.5	5.6
5-10	127	46.7	52.6
>11	128	47.1	100.0
Total	270	99.3	
	272	100.0	
Heard Size			
Large	39	14.3	14.4
Moderate	197	12.5	27.0
Small	34	72.4	100.0
Total	270	99.3	
		100.0	
Body Condition			
Good	90	32.7	33.0
Moderate	91	33.1	66.3
Poor	89	33.5	100.0
Total	270	99.3	
	272	100.0	

Source of Animal			
Breeder	200	73.5	
Market	70	25.7	
Total	270	99.3	
	272	100.0	

Table(9)Cross tabulation of *trypanosomiasis* in 270 camel examined in Butana area according to potential risk factor:-

Risk factor	No. Tested	No. Positive	Percent%
Locality			
Gedarrif	122	6	5.3
Showak	35	6	4.9
Butana	113	2	5.7
Total	270	14	
Sex			
Male	27	3	11
Female	243	11	4.5
Total	270	14	
Breed			
Arabi	160	9	8.4
Bushary	81	2	2.4
Anafi	29	3	10.3
Total	270	14	
Age			

1-5	15	1	6.6
5-10	127	3	2.3
>11	128	10	5.8
Total	270	14	
Heard Size			
Large	39	7	17.9
Moderate	197	5	2.5
Small	34	2	5.8
Total	270	14	
Body Condition			
Good	90	3	3.3
Moderate	91	5	5.4
Poor	89	6	6.7
Total	270	14	
Source of Animal			
Breeder	200	10	5
Market	70	4	5.7
Total	270	14	

Univariate analysis

chi-square test showed that there were three out of seven risk factor statistical significant (p-value <0.25) these breed (p-value.890), age (p-value.141), heard size (p-value .000) and sex (p-value.143)table(10)

Table(10)Univariate analysis for risk factor associated with trypanosomiasis in 270 camels sample in Butana_ area Gedarrif State

Risk factor	No. Tested	No. positive	d .f	Chi	p-value
Locality			2	.014	0980
Gedarrif	122	6			
Showak	35	6			
Butana	113	2			
Total	270	14			
Sex			1	2.143	.143
Male	27	3			
Female	243	11			
Total	270	14			
Breed			2	2.849	.241
Arabi	160	9			
Bushary	81	2			
Anafi	29	3			

Total	270	14			
Age			2	3.923	.141
1-5	15	1			
5-10	127	3			
>11	128	10			
Total	270	14			
Heard Size			2	15.765	.000
Large	39	7			
Moderate	197	5			
Small	34	2			
Total	270	14			
Body Condition			2	1.084	.582
Good	90	3			
Moderate	91	5			
Poor	89	6			
Total	270	14			
Source of Animal			1	.054	.817
Breeder	200	10			
Market	70	4			
Total	270	14			

Multivariate analysis

Multivariate analysis using logistic Regression showed that there were three risk factor will significant association with trypanosomiasis (p-value< 0.05) These were age (p-value .006) ,Heard size(p-value.013) and sex (p-value.000)

Table(11)Multivariate analysis for risk factor associated with trypanosomiasis in 270 camels sample in in Butana_ area Gedarrif State

Risk factor	OR	C.L	p-value
Breed			
Arabi	Ref	Ref	.318
Bushary	.694	.077- 6.213	.744
Anafi	.240	.037-1.541	.133
Age			
1-5	Ref	Ref	.006
5-10	.089	.007-1.135	.063
>11	.037	.005-.293	.002
Heard size			
Large	Ref	Ref	.013
Moderate	10.871	3.520-500.830	.095
small	41.989	.914-129.313	.003
Sex			
Male	Ref	Ref	.165
Female	.230	.029-1.830	.000

Chapter Four

Discussion

Camel trypanosomosis is a disease of major economic importance in many countries of Africa, Asia and South America. Because of the wide geographic range of surra, its control has attracted international attention, vector control seems not the solution for surra as a range of non-related biting flies should be targeted, each with its own biology, while unlike tsetse flies most other flies are proliferate breeders, and as such vector populations are difficult to control. Anaemia is a major component of the pathology of surra and of African trypanosomosis. Trypanosomiasis is a disease affecting the immune system of the host animal. *T. evansi* as purely extracellular parasites are permanently confronted with the multiple components of the host's immune system ranging from innate to adaptive immunodefences (Eyob E 2013.)

In the Sudan, *T. evansi* is primarily a parasite of camels causing a disease locally known as Guffar. (Babeker 2013.) The disease is common in Kordofan and Darfur States in the west, Kassala, Gadaref and Red sea States in the east and to a lesser extent in central Sudan, in the Gezira, Sennar, Blue Nile and Khartoum States (karib 1961). (Babeker 2013.)

The causative agent of disease in camel was *trypanosome evansi* this agrees with Eyob *et .al* (2013), Babeker. *et .al*(2013),

(Basaznew Bogale et al.(2012,)and ,Nahla and and Ali e .al. (2011).

In our study prevalence and risk factor of camel trypanosomosis were investigated .The prevalence in our study was (5.1%), this result is not different from other studies carried out by Dafaalla(1988), who surveyed camel trypanosomosis in Gedarif, Showak, Kassla, and New Halfa.And Atarhouchet al.,(2003) and Desquesneset al.,(2008).

However this prevalence is low compared with other studies in Sudan and different countries which was (14.1%) West Omdurman in Sudan(Babeker et al/2013.), (18.22)in Eastern and Western regions of the Sudan(Nahla O. and Ali et al .2011)and was(18.22)in Southwest Ethiopia(BasaznewBogale et al,2012) The different in *Trypanosomosis* prevalence between our study and different studies may be due to different risk factor association with Trypanosomosis like mechanical transmission, environment factor which affect the vectors, control strategy applied in the area, variation climatic conditions and wet season which effect the vector breeding and parasitemia and the method of examination or identification the parasite (sensitivity of the test).

The different prevalence between Giemsa's stain and Buffy coat infected animal may be in the initial period difficult to demonstrate the parasites because low parasitaemia. In most hosts *T. evansi* can induce mild clinical or subclinical carrier state infections with low parasitaemia in which it is difficult to

demonstrate the parasites. In these circumstances, concentration methods are necessary, as they increase the sensitivity of microscopic examination(OIE 2012).show that in table (3).

In this study univariuat analysis showed that four risk factor were statistical significant association with Trypanosomiosis (p-value< 0.25)

The distribution of prevalence of Trypanosomiosis in localities was revealed that the infection rate was(5.3%)in Butana ,(4.9%) in Gedarrif and(5.7%)in Shwak this risk factor was not significant with Trypanosomiosis (p-value .980).

In this study breed was inveshgalid the infection rate was(8.4%) in Arabi ,(2.4%) in Bshary and Anafi was (10.3%)this risk factor was statistically significant (p-value.141), the higher rat was in Anafi this result could be due to this breed it used for transportation and worker than other breed.

In this study the infction rate was 3 (11%) in male and 11 (4.5%)in female there was significant association between Trypanosomiosis and sex(p-value .143)

Herd size in this study showed an infection rate of 17.9%in large herd size ,moderate was2.5% and 5.8%was in small herd size there was highly significant association between Trypanosomiosis and herd size (p-value .000)

In this study body condition was investigated .the infection rate was 3.3% in good body condition ,5.4%was in moderate and 6.7% was in poor body condition .this risk factor was not statistical significant with disease in other study this risk factor was found

statistically significant , This result agrees with Tadesse *et al.*(2010) who found that different (p-<0.05) between poor and animal with good to medium that body condition was statistically significance , and Begna *et al.*(2011) also stated that animal of various body condition showed statistical significant difference (p-0.05) in the prevalence of trypanosomiasis , Bitwet *et al.*(2011) set that infection rate in poor body condition were significantly higher than good body condition animal (p-0.05) Mulow *et al.*(2011) found the prevalence was statistically significance higher (p-<0.05) in poor body condition animal, this could be due to the chronic nature of the disease that resulted in anaemia and decrease of body condition and lead to emaciation of the animal In this study source of the animals was investigated , the infection rate was 5% in the breeder and in market was 5.7% this risk factor was found not statistically significant with disease. Age in this study showed an infection rate 6.6% of the calves (1-5 years) , 2.3 in the young (6-5 years) and 7.8 was in old (>11 years) the old animal it more effected than other age this result was statistically significant with disease this agrees with Basaznew Bogale *et al.*(2012).

In multivariate analysis using logistic regression there was three potential risk factor with significant association with typanosomiasis (p-value<0.05), there was significant association between Trypanosomiasis and sex (p-value .006) which agrees with , Tadevos Kassa *et al.*(2011) who found that although the number of male camels examined was lower, due to the low number of

breeding male animals kept by pastoralists in the study area and Basaznew Bogaleet, *al.* (2012) who found higher infection rate in males than females, this could be due to the fact that female camels were kept in house while males were used for work all the time and subjected to graze out in the field. However, other studies in Asia have reported sex females were observed to be more susceptible to the disease than males counterparts. This record might be due to stress during pregnancy and lactation, which could decrease resistance in female camels and render them more susceptible to *T. evansi* infection

Regarding herd size the study showed there was significant association with trypanosomiasis (p-value .000) this agrees with Bhutto, *et al* Gadahi (2009) set that Infection rate according to herd size was highest prevalence in herds possessing more than 20 animals more than herds possessing 11 to 20, 6 to 10 and 1 to 5 animals, respectively these could be attributed to that most of large herds size were located in area with insect species known as disease vector and more fly attack.

Also age showed significant difference in prevalence between age groups where a higher infection rate was recorded in older camels. The higher prevalence in old camels at this study might be due to heavy stress through their use for transportation of goods from one place to another and poor management.

However, Pathak *.et.al* (1995) reported that all camels were equally susceptible to trypanosome infection regardless of breed and age.

Atarhouchet *al.*(2003) reported that infection rate the increase with age up to maximum in the 7-10 years old age.

The infected camels had PCV lower than 20% The mean PCV of all infected camels was also less than 20%.

Conclusion

In conclusion, the study revealed that camel trypanosomosis is prevalent in Butana area at relatively low levels during the study period in March to April, using parasitological techniques. The findings in this study might not reflect the real situation because the sensitivity of parasitological techniques in the diagnosis of *Trypanosoma evansi* has been reported to be low and most of the time it the disease is under-diagnosed

In univariate analysis breed ,age, sex, and herds size of animals categories have shown significant association with seroprevalence of camels Trypanosomosis .

In multivariate analysis of presumed risk factors indicated age , sex and herds size as a major risk factors associated with camels Trypanosomosis .

for all camel producing areas of the country or for all seasons in the same area. The prevalence might be higher during the rainy season when the fly population (Tabanus) is expected to be high.

Recommendations

-Therefore, detailed studies should be carried out involving both dry and rainy seasons and the relative importance of various biting flies in transmission of the disease in different ecologies.

- More sensitive diagnostic methods such as PCR could give a higher prevalence.

-The disease causes a significant impact on the camel production and economic growth of the study area by affecting health and productivity of camels.

-we need of further study on the distribution and seasonality of the disease and its vectors in order to establish control measures in affected herds and avoid dissemination of the disease.

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Appendix (1)

Frequency table for the distribution of 270 camels examined for trypanosomiasis according to potential risk factors :

number of infection

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-ve	256	94.1	94.8	94.8
	+ve	14	5.1	5.2	100.0
	Total	270	99.3	100.0	
Missing	System	2	.7		
Total		272	100.0		

name of locality

		Frequen cy	Percent	Valid Percent	Cumulative Percent
Valid	elbutan	113	41.5	41.9	41.9
	a elgadari	122	44.9	45.2	87.0
	f elshowa	35	12.9	13.0	100.0
	k Total	270	99.3	100.0	
Missing	System	2	.7		
Total		272	100.0		

breed

		Frequen cy	Percent	Valid Percent	Cumulative Percent
Valid	arabi	160	58.8	59.3	59.3
	bushar	81	29.8	30.0	89.3
	y anafi	29	10.7	10.7	100.0
	Total	270	99.3	100.0	
Missing	Syste m	2	.7		
Total		272	100.0		

age of animal

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1-5	15	5.5	5.6	5.6
	5-10	127	46.7	47.0	52.6
	>11	128	47.1	47.4	100.0
	Total	270	99.3	100.0	
Missing	System	2	.7		
Total		272	100.0		

herd size

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	larg	39	14.3	14.4	14.4
	small	34	12.5	12.6	27.0
	modrat	197	72.4	73.0	100.0
	Total	270	99.3	100.0	
Missing	System	2	.7		
Total		272	100.0		

grazing

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid outdoor	270	99.3	100.0	100.0
Missing System	2	.7		
Total	272	100.0		

body condition

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid poor	89	32.7	33.0	33.0
good	90	33.1	33.3	66.3
moderate	91	33.5	33.7	100.0
Total	270	99.3	100.0	
Missing System	2	.7		
Total	272	100.0		

sex of animal

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	male	27	9.9	10.0	10.0
	female	243	89.3	90.0	100.0
	Total	270	99.3	100.0	
Missing	System	2	.7		
Total		272	100.0		

source of animals

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	breeder	200	73.5	74.1	74.1
	market	70	25.7	25.9	100.0
	Total	270	99.3	100.0	
Missing	System	2	.7		
Total		272	100.0		

Appendix (2)

Across tabulation for the distribution of 270 camels examined for trypanosomiasis in Butana area, Gedarrif State according to potential risk factors :

Number of infection * name of locality

Crosstab

			name of locality			Total
			elbutan a	elgadar if	elshowa k	
number of infection	-ve	Count	107	116	33	256
		% within number of infection	41.8%	45.3%	12.9%	100.0%
		% within name of locality	94.7%	95.1%	94.3%	94.8%
		% of Total	39.6%	43.0%	12.2%	94.8%
Total	+ve	Count	6	6	2	14
		% within number of infection	42.9%	42.9%	14.3%	100.0%
		% within name of locality	5.3%	4.9%	5.7%	5.2%
		% of Total	2.2%	2.2%	.7%	5.2%
Total		Count	113	122	35	270
		% within number of infection	41.9%	45.2%	13.0%	100.0%
		% within name of locality	100.0%	100.0%	100.0%	100.0%
		% of Total	41.9%	45.2%	13.0%	100.0%

Number of infection * breed

Crosstab

			breed			Total
			arabi	bushary	anafi	
number of infection	-ve	Count	151	79	26	256
		% within number of infection	59.0%	30.9%	10.2%	100.0%
		% within breed	94.4%	97.5%	89.7%	94.8%
		% of Total	55.9%	29.3%	9.6%	94.8%
	+ve	Count	9	2	3	14
		% within number of infection	64.3%	14.3%	21.4%	100.0%
		% within breed	5.6%	2.5%	10.3%	5.2%
		% of Total	3.3%	.7%	1.1%	5.2%
Total	Count	160	81	29	270	
	% within number of infection	59.3%	30.0%	10.7%	100.0%	
	% within breed	100.0%	100.0%	100.0%	100.0%	
	% of Total	59.3%	30.0%	10.7%	100.0%	

number of infection * age of animal

Crosstab

			age of animal			Total
			1-5	5-10	>11	
number of infection	-ve	Count	14	124	118	256
		% within number of infection	5.5%	48.4%	46.1%	100.0%
		% within age of animal	93.3%	97.6%	92.2%	94.8%
		% of Total	5.2%	45.9%	43.7%	94.8%
	+ve	Count	1	3	10	14
		% within number of infection	7.1%	21.4%	71.4%	100.0%
		% within age of animal	6.7%	2.4%	7.8%	5.2%
		% of Total	.4%	1.1%	3.7%	5.2%
Total	Count	15	127	128	270	
	% within number of infection	5.6%	47.0%	47.4%	100.0%	
	% within age of animal	100.0%	100.0%	100.0%	100.0%	
	% of Total	5.6%	47.0%	47.4%	100.0%	

Number of infection * herd size

Crosstab

			herd size			Total
			larg	small	modrat	
number of infection	-ve	Count	32	32	192	256
		% within number of infection	12.5%	12.5%	75.0%	100.0%
		% within herd size	82.1%	94.1%	97.5%	94.8%
		% of Total	11.9%	11.9%	71.1%	94.8%
	+ve	Count	7	2	5	14
		% within number of infection	50.0%	14.3%	35.7%	100.0%
		% within herd size	17.9%	5.9%	2.5%	5.2%
		% of Total	2.6%	.7%	1.9%	5.2%
Total	Count	39	34	197	270	
	% within number of infection	14.4%	12.6%	73.0%	100.0%	
	% within herd size	100.0%	100.0%	100.0%	100.0%	
	% of Total	14.4%	12.6%	73.0%	100.0%	

Number of infection * grazing

Crosstab

			grazing	Total
			ooldor	
number of infection	-ve	Count	256	256
		% within number of infection	100.0%	100.0%
		% within grazing % of Total	94.8%	94.8%
	+ve	Count	14	14
		% within number of infection	100.0%	100.0%
		% within grazing % of Total	5.2%	5.2%
Total	Count		270	270
	% within number of infection		100.0%	100.0%
	% within grazing		100.0%	100.0%
	% of Total		100.0%	100.0%

Number of infection * body condation

Crosstab

			body condation			Total
			poor	good	modrat	
number of infection	-ve	Count	83	87	86	256
		% within number of infection	32.4%	34.0%	33.6%	100.0%
		% within body condation	93.3%	96.7%	94.5%	94.8%
		% of Total	30.7%	32.2%	31.9%	94.8%
	+ve	Count	6	3	5	14
		% within number of infection	42.9%	21.4%	35.7%	100.0%
		% within body condation	6.7%	3.3%	5.5%	5.2%
		% of Total	2.2%	1.1%	1.9%	5.2%
Total	Count	89	90	91	270	
	% within number of infection	33.0%	33.3%	33.7%	100.0%	
	% within body condation	100.0%	100.0%	100.0%	100.0%	
	% of Total	33.0%	33.3%	33.7%	100.0%	

Number of infection * sex of animal

Crosstab

			sex of animal		Total
			male	female	
number of infection	-ve	Count	24	232	256
		% within number of infection	9.4%	90.6%	100.0%
		% within sex of animal	88.9%	95.5%	94.8%
		% of Total	8.9%	85.9%	94.8%
	+ve	Count	3	11	14
		% within number of infection	21.4%	78.6%	100.0%
		% within sex of animal	11.1%	4.5%	5.2%
		% of Total	1.1%	4.1%	5.2%
Total	Count	27	243	270	
	% within number of infection	10.0%	90.0%	100.0%	
	% within sex of animal	100.0%	100.0%	100.0%	
	% of Total	10.0%	90.0%	100.0%	

Number of infection * source of animals

Crosstab

			source of animals		Total
			breeder	maraket	
number of infection	-ve	Count	190	66	256
		% within number of infection	74.2%	25.8%	100.0%
		% within source of animals	95.0%	94.3%	94.8%
		% of Total	70.4%	24.4%	94.8%
	+ve	Count	10	4	14
		% within number of infection	71.4%	28.6%	100.0%
		% within source of animals	5.0%	5.7%	5.2%
		% of Total	3.7%	1.5%	5.2%
Total	Count	200	70	270	
	% within number of infection	74.1%	25.9%	100.0%	
	% within source of animals	100.0%	100.0%	100.0%	
	% of Total	74.1%	25.9%	100.0%	

Appendix (3)

Univariate analysis for association between trypanosomiasis t and pontential risk factors in Butana area, Gedarrif State , using Chi - squart

Chi-Square Tests for the Locality

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.041 ^a	2	.980
Likelihood Ratio	.041	2	.980
Linear-by-Linear Association	.000	1	.986
N of Valid Cases	270		

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 1.81.

Chi-Square Tests for the breed

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.849 ^a	2	.241
Likelihood Ratio	2.790	2	.248
Linear-by-Linear Association	.101	1	.750
N of Valid Cases	270		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.50.

Chi-Square Tests for the age

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.923 ^a	2	.141
Likelihood Ratio	4.187	2	.123
Linear-by-Linear Association	2.089	1	.148
N of Valid Cases	270		

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is .78.

Chi-Square Tests for the herd size

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.765 ^a	2	.000
Likelihood Ratio	11.593	2	.003
Linear-by-Linear Association	14.667	1	.000
N of Valid Cases	270		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.76.

Chi-Square Tests for the grazing

Chi-Square Tests

	Value
Pearson Chi-Square	. ^a
N of Valid Cases	270

a. No statistics are computed because grazing is a constant.

Chi-Square Tests for the body condition

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.084 ^a	2	.582
Likelihood Ratio	1.134	2	.567
Linear-by-Linear Association	.137	1	.711
N of Valid Cases	270		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 4.61.

Chi-Square Tests for the sex

Chi-Square Tests

	Value	df	Asymp. Sig. (2- sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.143 ^a	1	.143		
Continuity Correction ^b	1.013	1	.314		
Likelihood Ratio	1.698	1	.192		
Fisher's Exact Test				.154	.154
Linear-by-Linear Association	2.135	1	.144		
N of Valid Cases ^b	270				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 1.40.

b. Computed only for a 2x2

table

Chi-Square Tests for the source of the animals

Chi-Square Tests

	Value	df	Asymp. Sig. (2- sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.054 ^a	1	.817		
Continuity Correction ^b	.000	1	1.000		
Likelihood Ratio	.053	1	.818		
Fisher's Exact Test				.762	.513
Linear-by-Linear Association	.054	1	.817		
N of Valid Cases ^b	270				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.63.

b. Computed only for a 2x2

table

Appendix (4)

Questionnaire to Sarivay Epidemiology study of Brucellosis in camels
trypanosomosis in Butana area , Algardarrif State

(A) General characteristics

Date-----			serial
NO-----			
(1) Owner Name-----		(2) Phone No	
(3) Location-----		(4) Locality	
(5) Education level :			
Illiterate	Primary		Secondary
graduate			
(6) Herd Size :			
<10	10 - 20		> 20
(7) Camels sex :			
All male	All female		mixed
Yeas		No	

(B) Individual Camels factors

(1) Age:
 > 5 (manthes) 5- 10 year >
 10year

(2) sex:
 Male female

(3) Berrd:
 Arabi Bushary Anafi

(4) Boody condition :
 Good Moderate
 poor

(C) Managemental factors:
 (1) Operation type :
 Intensive Semi- intensive
 extensive

(2) Proudction type :
 Milk Meat both
 racing

(3) Housing :
 Open closed
 semi- closed

(4) living near the forest :
 Yeas

(5) Awariness of fetus and fetal membranes disposal :
 Yeas No

(6) Presence of biting flies(Tabanus and Stomoxys)
 Yeas No

(7) Source of new Camels :
 Herd Purchase

(8) useing the Insecticies

	Yeas	No
(9) using Mosquito net		
	Yeas	No
(10) Previous of trypanosomosis		
	Yeas	No
(11) Veterinary Supervision:		
	Yeas	No
(12)Trust in vets :		
	Yeas	No
(13) Ethical treatment :		
	Yeas	No
(14) Awariness of trypanosomosis		
	Yeas	No
(15) Herd (fever , pale mucous membranes , abodominal edema)		
	Yeas	No
(16)Reporductive Disorders (abortion , in fertility)		
	Yeas	No
(17) contact with Wild animals:		
	Yeas	No
(18) Type of Breeding:		
	Within herd	from out
side		

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