Incidence and treatment of Camel trypanosomosis (Guffar)
In Butana area, Eastern Sudan

By

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Dedication

To my family with love....
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ABSTRACT

The incidence of camel trypanosomosis (Guffar) caused by *T. evansi* in Butana plains, Eastern Sudan was surveyed using direct smear, microhematocrit centrifugation technique (MHCT) and card agglutination test for *T. evansi* (CATT/*T. evansi*). In a total of 220 blood and serum samples, 115 (52.2%), were positive by CATT, 72 (32.7%) were positive by the MHCT and 31 (14.1%) were positive by the wet smear preparation. Contingency table and parcel Chi²-test revealed that CATT-*T. evansi* was statistically the most sensitive technique for *T. evansi* followed by the MHCT and lastly the wet smear technique. The percentage packed cell volume (PCV %) differed significantly between the diagnostic techniques used. Thus the wet smear technique detected positive camels with the lowest PCV %. Camels infection rate with *T. evansi* did not differ significantly with sex. However the infection rates differed appreciably with location during the rainy season and between seasons irrespective of the sex of the animals. Treatment of rats infected with *T. evansi* isolates from Butana area with quinapyramine pro-salt made by three different manufacturers revealed that Tryquine (wokharde, India) was the most effective in clearance of parasitaemia with in two weeks. Biquin (Star, Pakistan) and quinapyramine (Nicholas Primal, India) did not clear the parasitaemia in rats during the
same period. The results are discussed in relation to studies leading to control of *T. evansi* in camels using chemotherapy and chemoprophylaxis.
ملخص الстраحة

في هذا البحث تم فحص الجفاف في الأيل في منطقة البطانة التي تقع في شرق ووسط السودان وذلك باستعمال تقنيات المسح الدمومي المباشر وآلي كوت ومكداس الدم وتقنية التلازن الدمومي على الكرت كما تم أيضا فحص أثر دواء الايترسبيد على الجرذان التي تمت عدئتها بعتره من انترBuffer. ووسيط شرقي يقع إلى الشمال من السودان بأفانزاي والتي اخذت من نفس المنطقة.

تم التوصيل في هذه الدراسة إلى أن من أصل 220 عينه تم جمعها وجد فيها 15 عينه إيجابية لاختبار التلازن الدمومي على الكرت 72 عينه إيجابية لاختبار الطرد المركزي الدقيق 31 عينة إيجابية لفحص المسح الدمومي المباشر كما تم أخذ نسبة مكداس الدم ومقارنتها بالعينات الموجهة، أيضا تم مسح للطفل في ثلاث مناطق مختلفه وتم استصحاب الجنس والموسم كما تم المقارنة بين الاختبارات الطفيلة والمصلية وذلك باستخدام التحاليف الإحصائيه بواسطة مربع كاي، كما تم اختبار حساسية الطفل لعقار الانترسبيد في الجرذان وقد حللت احصائيه بواسطة اختبار الاستيودنت والتي اعطت نتائج متفاوتة مما دل على وجود تفاوت في حساسية الطفل للعقار.

اكتست الدراسة ان مرش الجفاف في الأيل في هذه المنطقة متوطن وأن استخدام تقنية التلازن الدمومي على الكرت أكثر الاختبارات المصلية امانا وسهولة في الاستخدام الحقلي، كما اقترح اندخل عقاقير جديد واحتر فاعله لمحاربة انتشار الطفل.
**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, 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 and residues of crops, e. g., Durra or Sorghum spp. (Abu sin, 1988).

Trypanosomosis, caused by *Trypanosoma evansi* is one of the major and most important diseases in camels in the arid and semiarid zone of the world (Boid, *et al* 1985). Camels managed under nomadic pastoralism have higher risk of being exposed to *T. evansi* infection than camels under a ranching system of management (Ngaira, *et al* 2003).

*T. evansi* belongs to the genus *Trypanosoma* which are haemoflagellate protozoa that belong to the order: Kinetoplastida. They
parasitize man, domestic and wild animals causing trypanosomosis. Unlike other parts of the world, in the Sudan, T. evansi is primarily a parasite of camels causing a disease locally known as `Guffar`. 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). Guffar is believed to be transmitted mechanically by biting flies, particularly, horseflies (Diptera: Tabanidae) (Losos, 1986).

Generally, the disease assumes a chronic course resulting in serious morbidity and moderate mortality. Diagnosis is largely based on demonstration of the causative agent by the standard trypanosome parasitological methods. Serological tests have also been developed to detect antibodies (Luckins, 1979).

Since control of the vector flies is not feasible at present, control of guffar is basically through chemotherapy and chemoprophylaxis. The most common drugs used in chemotherapy are still Naganol and Quinapyramine salts (e. g., Antrycide). Due to the development of resistance to the former, Quinapyramine salts remain the drugs of choice in the treatment of T. evansi in camels and experimentally infected laboratory animals. They are also considered as the standard chemotherapeutic agents against which other
drugs are evaluated (Harone, et al, 2003). Considering the number and the economic importance of camels in the Sudan, control of camel trypanosomosis appears imperative. The present study is designed to contribute to the understanding of the prevalence and chemotherapy of camel trypanosomosis in Butana area, one of the traditional camel pastoralist areas in Gadaref State, eastern Sudan.

**Objectives of the study:**

1. - To determine the prevalence of *T. evansi* infection in camels in Butana area.

2. - To compare the sensitivity of direct parasitological diagnostic methods versus the most recent serological diagnostic techniques.

3. - To document the effects of sex, season, and location on the prevalence of *T. evansi* in camels in the study area.

4. - To study the response of *T. evansi* isolates from Butana to treatment with the Quinapyramine salts, namely, Antrycide pro-salt.
CHAPTER ONE

LITERATURE REVIEW

1.1. Historical note:

*Trypanosoma evansi* was the first pathogenic trypanosome discovered by Griffith Evans (1880) in the blood of equines and camels affected by this disease in Punjab State, India (Hoare, 1972). In the Sudan, *Trypanosoma evansi* in camels was identified by Balfour in camels as early as 1904 (Karib, 1961).

1.2. Classification:

The systematic position of *Trypanosoma* among the protozoa and the revised classification of the mammalian trypanosomes proposed by Levine *et al* (1980) and Corliss (1994) is as follows:

Sub kingdom:  **Protozoa** (Goldfuss, 1818; Levine *et al*, 1980)

Phylum:  **Sarcomastigophra** (Honigberg and Balaniuth, 1963; Levine *et al* 1980).

Subphylum:  **Mastigophora** (Diesing, 1866; Levine *et al*, 1980)

Class:  **Zoomastigophorea** (Calkins, 1909)

Order:  **Kinetoplastida** (Honigberg, 1963)

Family:  **Trypanosomatidae** (Doflein, 1901; Grobben, 1905)

Genus:  **Trypanosoma** (Gruby, 1843)
\textit{T.evansi} has almost certainly arisen from \textit{T. brucei brucei} by adaptation to mechanical transmission by biting flies, and remains very closely related to \textit{T.brucei} (Soulsby, 1982). It has lost the capability of being cyclically transmitted by tsetse flies and in that process has become almost monomorphic (Uilenberg, 1998).

1.3. Morphology:

Under the ordinary light microscope with Romanowsky stained preparations \textit{T. evansi} appears as an elongate form of \textit{T.brucei}. The cytoplasm stains blue or purple and the nucleus usually lie near the center or in the anterior half (Soulsby, 1982). The kinetoplast is small and typically occupies a sub-terminal or marginal position in the body at the tapering posterior end. Both kinetoplast and nucleus stain red (Losos, 1980). The organism bears a single flagellum (Karina and Amanda, 2001).

1.4. Geographical Distribution:

Pathogenic trypanosomes cause disease in all species of domesticated livestock throughout many of the tropical and sub-tropical regions of the world. In Africa, \textit{T.brucei}, \textit{T. vivax} and \textit{T.congolense} occur wherever the tsetse fly vector is found. (Luckins, 1988). However, \textit{T. evansi} is the most widely distributed of the pathogenic animal trypanosomes, occurring in a variety of different ecological regions in Africa, Latin America and Asia.
(Dia et al, 1997; Monzon et al, 1995). The enzootic areas of camel trypanosomosis that extend across the Sudan between latitude 13° N and 18° N are well known as tabanid areas and are avoided as much as possible in years when rainfall is heavy (Kheir & Majid, 1999).

1.5. Hosts:

1.5.1. Domestic host:

In Africa and South America there is very little evidence to suggest that domesticated livestock other than camels and horses are clinically affected by *T.evansi*. There are, however, reports from the Sudan of serological evidence of infection in goats and sheep (Boid et al, 1981) and in cattle from Brazil (Franke et al 1994). Sheep and goats are known to carry symptoms of chronic infection of *T. evansi* for up to a year or longer (Malik and Mahmoud, 1978, cited by Kheir & Majid, 1999), and since camels may often be herded in close proximity to sheep and goats, these animals have been suggested as natural reservoirs of *T. evansi* infection (Boid et al, 1981).

In Asia, a much wider range of hosts is involved including Bactrian and dromedary camels, cattle, buffalo, horses and pigs (Silva et al 1995 Pathak et al, 1993; Partoutomo et al, 1994; Balakrishnan, 1994) cases of *T.evansi* in dogs in Asia were reported by Losos & Ikede (1972).
1.5.2. Wild hosts:

Numerous species of wild animals have been shown to be susceptible to *T. evansi* infections which caused severe disease and death (Hoare, 1972; Morales *et al.*, 1976; Losos, 1986). Such infections were found in captive tigers and other large felines from India and Sumatra (Raju and Swaminath 1947), in deer from Mauritius (Adams & Lionnet, 1933), and in wild dogs from South America (Curasson, 1943; Rodriguez, 1956).

1.6. Epizootiology:

1.6.1. Transmission:

1.6.1.1. Mechanical transmission:

Camel trypanosomosis has been reported to be transmitted mechanically from camel to camel by a number of species of haematophagous biting flies including the following genera *Tabanus*, *Stomoxys*, *Lyrosia* and *Haematobia* (*Diptera*). (Rutter, 1967.; Scott, 1973). The role of tabanid flies in mechanical transmission of the disease was first described by Rogers (1901). In the Sudan they play an important role in the mechanical transmission of animal trypanosomosis (Karib, 1961). They were also considered as major cause of the seasonal migration of cattle from the south to north during the rainy season (Kheir, *et al.*, 1995). Lewis (1954) reported seventy species of Tabanid flies in the Sudan and Yagi
(1968) added four more species to those recorded by Lewis. The main tabanid species found throughout the country and involved in animal irritation together with mechanical transmission of pathogens are Atylotus agrestis, A. fuscipes, Tabanus toeniola, T. sufis, T. biguttatus, T. gratus, Philoliche. magretti, Ancala latipes, and A. Africana (Razig and Yagi, 1975).

Lewis (1953) reported two species of Stomoxys in the Sudan namely Stomoxys calcitrans, and S. nigra. Mohamed (1991) showed that the peak of Stomoxys spp. coincides with high trypanosomosis incidence. Arachnid ticks of the genera Hyalomma, Dermacentor and Rhipicephalus have been suggested as vectors of T. evansi, although Kirmse and Taylor- Lewis (1978) considered that they are either not involved in, or are inefficient at transmitting T. evansi (Boid, et al 1985). In south and central America T. evansi is also transmitted by the vampire bat (Demodus rotundus) which also acts as a carrier (Hoare, 1972).

1.6.1.2. Cyclical transmission:

Salivarian trypanosomes, which include the important agents of African trypanosomosis, are spread mainly by at least 30 species and subspecies and races of tsetse flies which infest vast areas of tropical Africa (Losos, 1986). Parasites in the blood stream of the infected mammal are
taken by the fly as it feeds and they undergo a cycle of development and division in the gut until the infective or metacyclic trypanosomes are produced (Uilenberg, 1998). The incidence of trypanosomosis in animals is high during or after the rainy season due to the growth and proliferation of tsetse flies during this favorable season. However, a high transmission index was not necessarily linked to a high fly infection rate and vice versa (Wilson et al., 1972). The tsetse infested area in the Sudan is estimated at 300,000 Km² in the south-western part of the country (Razig and Yagi, 1973). Lewis (1949) recognized seven *Glossina* spp in the Sudan. These are *G. morsitans submorsitans*, *G. fuscipes*, *G. fusca*, *G. fuscipleuris*, *G. longipenis*, *G. pallidipes* and *G. tachenoides*. So far there has been no evidence to suggest the biological transmission of *T. evansi* by any organism (Uilenberg, 1998)

### 1.7. Clinical signs:

Trypanosomosis due to *T.evansi* is a chronic wasting disease characterized by intermittent fever (38.5 – 40.1°C), anemia, fluctuating parasitaemia, emaciation, weakness with paler mucous membrane and dry scruffy coat (Syakalima, 1992). The animal stands with its nose somewhat depressed and head hanging forward. The eyes turn dull and half closed with considerable amount of tears (Karram et al., 1991). In addition it was observed that 100% of infected camels stared at the sun (Abo-shehada et al.,
Hematological studies revealed that in trypanosomosis-affected animals there was severe oligocythemia with significant decrease in both hemoglobin and packed cell volume (Karram et al., 1991).

*T. evansi* infection can be manifested in both acute and chronic forms; the acute form of the disease is characterized by progressive anaemia, high fever, anorexia, loss of condition and often rapid death (Rutter, 1967 Haroun et al., 2000). The chronic form which is more common shows relapsing parasitaemia with or without pyrexia, emaciation, oedema of the abdomen and legs, abortion and death in some animals (Haroun et al., 2000). Production losses occur due to lower milk and meat yields in adults (Richard, 1979). Abortion, premature birth and an inability to feed young, all greatly reduce the reproductive potential in affected herds (Yagil, 1982).

Chronically–infected animals may survive for three to four years; the disease in this form is characterized by anemia, emaciation, recurrent fever, disappearance of the hump, atrophy of the thigh muscles, oedema of the dependant parts, corneal opacity, diarrhea and sexual excitement (Singh et al., 1980). Other clinical changes recorded in experimentally–inducted chronic infections include debility, alopecia, keratinization, depletion of sub–cutaneous fat and facial oedema (Raisinghani et al., 1980). Also an infected animal suffers from hypoglycaemia (Igbokwe, 1994).
Animals which recover from the acute disease are often clinically normal with low numbers of circulating trypanosomes. In chronic infections too, low numbers of trypanosomes are present but parasitaemia often increases with physiological stress. Localization of organisms in the tissues results often in low grade parasitaemia (Losos, 1980). The low and intermittent parasitaemia as a result of antigenic variation in trypanosome population (Jones and Mckinnell, 1984) will interfere with detection of parasites even in acute infections (Murray et al, 1977).

1.8. Clinical Pathology:

The main pathological features include degenerative and necrotic changes involving various organs (Raisinghani et al., 1980; Haroun et al, 2000) and in camels, anemia is described cytologically as macrocytic (Jatkar & Purohit. 1971) and etiologically as hemolytic (Raisinghani et al. 1981). Raisinghani et al (1981) carried out haematological studies on experimental camels in India over a period of one year. He showed reductions in haemoglobin, packed cell volume and serum levels of calcium, potassium and sodium. Increases in reticulocytes, eosinophils and organic phosphate, fluctuated reciprocally with bouts of parasitic and non parasitic phases. The level of blood glucose also falls with increasing parasitaemia (Jatkar & Singh, 1971).
Naturally–infected camels were found to have higher serum protein and gamma–globulin levels but lower albumin and Beta-globulin levels than uninfected animals (Boid et al., 1980). The level of IgM in both naturally and experimentally–infected animals increased by as much as five times the pre–infection level. These values may remain high despite drug treatment.

1.9. Necropsy findings:

Postmortem findings are non specific. A known history and findings such as generalized muscular waste, pale and watery muscles, serous atrophy of fat, swollen lymph nodes, presence of oedema and evidence of anaemia indicate infection (Schwartz, 1992).

1.10. Diagnosis:

Many diagnostic techniques including parasitological and serological ones were devised. The techniques differ in their reproducibility, specificity and sensitivity and each can be applied according to the prevailing situation (Abdel Rahman et al, 2001). However, characteristic clinical symptoms of emaciation and anaemia are still used for the provisional diagnosis of the disease (Luckins et al, 1979).

1.10.1. Parasitological diagnosis:

Diagnosis of infection by examination of peripheral blood is satisfactory in animals with acute infections but more difficult in a chronic
disease (Luckins, 1979). Examination of the blood by light microscopy, either by the wet film method or as Gimsa-stained thin or thick smears is quite common. Although examination of wet films is rapid, it is comparatively less sensitive and many sub-patent infections go undetected (Rae et al., 1989).

The microhaematocrit centrifugation technique (MHCT) prepared by Woo (1969) is more sensitive than the wet film or the stained preparation examination and can also indicate the degree of anemia by the percentage of packed cell volume (PCV). However, the method requires centrifugation. To increase the sensitivity of this method the micro-capillary tube is broken and the buffy zone expressed on a microscopic slide. The preparation is then examined in a similar way to dry and wet smears. Wet preparations made of the buffy zone are examined under phase contrast or dark–field illumination microscopes (Murray, Murray and Mc Intyre, 1977). The technique has little application in the field (Kelley and Schillinger, 1983). To conclude no single direct parasitological method alone was totally effective. The Microhaemtocrit centrifuge technique, mouse inoculation of blood and Gimsa–stained smears are proposed as the most effective diagnostic combination (Monzon, 1990). The technique of inoculation of camel blood samples into laboratory rodents is of some diagnostic value as above
(Godfrey and Killick – Kendrick, 1962) but is not used routinely in the field (Boid et al., 1985).

1.10.2. Serological diagnosis:

Recently much attention has been given to the development of specific serological tests for *T. evansi* infections in camels.

1.10.2.1. The indirect fluorescent antibody test (IFAT):

The indirect fluorescent antibody test has been used extensively in the detection of trypanosomal antibodies in animals and humans. Antigens are usually prepared from blood smears which are fixed in acetone and then stored at a low temperature. The IFAT has proved to be both specific and sensitive in detecting trypanosomal antibodies in infected cattle (Wilson, 1969.; Luckins and Mchlitz, 1978) and camels (Luckins et al., 1979).

1.10.2.2. The complement fixation test (CFT):

The complement fixation test was one of the first techniques used to diagnose *T. evansi* infection in camels (Schoening, 1924). However, because the test is difficult to perform and standardization is of paramount importance, it has not been used routinely as a diagnostic assay (Rae. and Luckins, 1984).
1.10.2.3. Enzyme–linked immunosorbent assay (ELISA):

Enzyme-linked immunosorbent assay was a major breakthrough in the diagnosis of animal trypanosomosis. This test is specific and sensitive, and can be used readily for large scale screening of many serum samples (Rae et al, 1989). The ELISA has been used successfully for serodiagnosis of camel trypanosomosis. (Luckins et al, 1979; Boid et al, 1980; Rae et al, 1989).

1.10.2.4. Species–specific monoclonal antibody:

The introduction of this technology has also been a major breakthrough in the diagnosis of trypanosomosis due to T.brucel. No cross reactions occur between other species of trypanosome or other haemoproteozoan parasites (Nantulya et al, 1989).

1.10.2.5. Sandwich ELISA.

T.brucel group specific monoclonal-antibody assay has been used as sandwich ELISA test to diagnose T. evansi infections in different animal species. In camels from an endemic area, the test detected circulating antigens in many T. evansi-parasite positive animals (Nantulya et al, 1989).

The techniques of PCR or ELISA are not only beneficial for diagnosis of the parasite but may also be useful for trypanosomosis control programmes (Chansiri. et al, 2002; Monzon et al, 2003).
1.10.2.6. Capillary agglutination test (CAT):

The test was described by Ross (1971) it is similar to that which has been widely used in the diagnosis of anaplasmosis, and trypanosomosis in man and animals using a particulate antigen prepared from *T. brucei*. The results appeared promising and the test was used by Clarkson *et al* (1973).

1.10.2.7. The Card Agglutination Test (CATT):

A card agglutination test set has been introduced into the market for the diagnosis of Gambian sleeping sickness. After that the test was found suitable for diagnosis of trypanosomosis in camels (Zweygarth, *et al.*, 1984). The test uses the formalin fixed variable antigen types of *T. evansi* that are used in the agglutination test. The test, which is simple to perform, has been used for diagnosis of *T. evansi* (Bajyanasonga *et al.*, 1987), and also used to detect antibodies against *T.evansi* (Hilali *et al*, 2004). The sensitivity of CATT/ *T.evansi* was higher than that of latex agglutination test (SuraTEX), but not significantly so. Both tests had equally high specificity (Ngaira *et al*, 2003). CATT/ *T.evansi* can detect aparsitaemic infection rapidly and it is more sensitive than parasitological methods in revealing the true extent of trypanosomosis in a herd. The test effectively complemented parasitological methods in the detection of *T.evansi* infection in camels.
1.11. Immunity:

Host specific immunity eventually clears the dominant antigenic types of infecting trypanosome; and a new outbreak follows from antigenic types that have apparently been present all along at low frequency. Sequences of dominant antigenic variants tend to follow specific order, although the particular sequence can be influenced by the host immune response and other factors (Gray, 1965; Capbem et al, 1977; Miller & Turner, 1981; Barry & Turner, 1991). Variation in switch rates may also play an important role in the wide variety of pathogenic microorganisms that undergo programmed antigenic variation (Deitsch, et al 1997; Fussenegger, 1997; Nash, 1997; O’Connor et al, 1997; Serkin & Seifert, 1998; Zhang, et al, 1998). The parasite has an ability to change its antigenic surface properties and consequently escape the immune surveillance (Vickerman, 1989). Each parasite cell is covered with a nearly uniform and strongly antigenic glycoprotein coat. The parasites genome contains several hundred alternatives and highly diverse surface antigens, of which only a single one is expressed in any individual. A parasite switches its antigenic expression and entire coat in each cell generation at a rate of one per thousand to one per hundred (Barry & Turner, 1991; Barry, 1997; Turner, 1997). These switches appear to occur in an apparently random way, creating a diverse set
of antigenic variants (Turner & Barry, 1989). In spite of this diversity, the parasitaemia develops as a series of outbreaks, each one dominated by relatively few antigenic types (Barry and Turner, 1991).

1.12. Control:

*T. evansi* infection is one of the major constraints for raising camels. Control of vector insects is not feasible now; and despite the encouraging reports of vaccination experiments against *T.evansi* in laboratory rodents (Ryu, 1975; Hertzkorn, 1980, and Bremer, 1982; cited by Schillinger and Rottcher, 1984), the production of a vaccine is still a remote possibility due to the problem of antigenic variation (Gray & Luckins, 1976). The only method available for controlling trypanosomosis infections in camels is therefore chemotherapy and chemoprophylaxis. The efficacy of existing trypanocidal drugs has been reviewed by a number of authors (Finelle, 1973; Borst, 1977; Losos, 1980; and Mahmoud and Gray, 1980).

1.12.1. Chemotherapy:

Treatment of camels infected with *T. evansi* with the current available trypanocides requires greater care. The reasons being their low trypanocidal effect against *T.evansi* on one hand, and their specific toxicity for camels on the other hand. For example Berenil, which is well tolerated in cattle, produces systemic toxicity in some cases ending in death of camels, when
administered in the recommended therapeutic dosage of 3.5mg/kg, (Leach, 1961; Fazil, 1977; Homeida et al, 1981). Four compounds, namely suramin, Berenil, isometamidium and quinapyramine have been used for many years to treat trypanosomosis in camels, cattle, buffalo, horses and pigs.

Treatment of camels was and, has until recently depended upon two drugs: suramin and quinapyramine drugs. However, resistance, poor tolerance and/ or complicated administration highlighted the need for a novel trypanocidal drug (Van Gool et al, 1992). The drug resistance in *T.evansi* might be an underestimated problem with potentially serious implications for the future control of camel trypanosomosis owing to the withdrawal of drugs from the world market. (Mutugi & Boid et al, 1995).

Trypanosomosis is endemic mostly in the developing countries where the resources are very meager. In such countries, purchase of drugs is not usually possible due to economic constraints.

The major problem in the control of camel trypanosomosis is still that of drug resistance. Drug resistance may be caused by under-dosing, using the wrong intervals between chemoprophylactic doses, discontinuing prophylaxis despite continued risk of infection, applying preventive instead of curative doses and injecting the drug inaccurately. Drug resistance of
trypanosomes is now a major problem, but its underlying mechanisms are still not fully understood. (Witola et al, 2004).

1.12.2. Chemotherapy and chemoprophylaxis:

Trypanocides used in treatment and protection are the most common single method employed for the control of camel trypanosomosis, both curative and preventive as in the case of the quinapyramine compounds (Losos, 1986). The difference between cure and prevention depends upon the drug being used and in some cases upon the dosage rate at which it has been administered. Effective application of chemotherapy and chemoprophylaxis in the field depends on several factors which include the strain of trypanosome causing infection, severity of the challenge, species of animal, and lastly the occurrence of resistant strains. (Losos, 1986).

1.12.2.1. Berenil (Diminazene aceturate) (Hoechst, Germany)

This drug qualifies as a wide spectrum trypanocidal drug (Losos, 1986). The drug is notable among anti-trypanosomal drugs in being rapidly excreted from the body, mainly through urine (Kellner et al, 1985) and is therefore not considered to have pronounced prophylactic activity (Fairclough, 1963; Williamson, 1970). Yet, biologically active berenil, which is presumably retained in the tissues, has been shown by others to
have a prophylactic effect that may last for several weeks against natural infections with *T. vivax*, *T. congoense* and *T. brucei* (Van Hove and Cunningham, 1964; Lumsden *et al* 1972; Zahalsky and Weinberg, 1976; Williamson, 1976) and against experimental infection with *T. brucei* in mice (Whitelow and Urguhart, 1985). A curative dose of Berenil against *T. evansi* at 3.5 mg/kg weight could confer protection for a period of 2 days (El-Amin and El Amin, 1992). However, at a dose rate of 10mg/kg the drug was very effective in eliminating *T. evansi* (AL Amin *et al*, 1982) in experimentally infected Asian buffalo calves (Verma *et al*, 1973; Homeida *et al*, 1981).

1.12.2.2. **Antrycide pro-salt (Quinapyramine sulphate and Quinapyramine chloride) Wockhart Ltd, Mumbai, India:**

Antrycide pro-salt is the principal drug in the quinapyramine compounds group, which is curative against *T. evansi* in all species of hosts (Finelle, 1973) and additionally gives camels a prophylactic protection for three to four months (Njogu, 1986). It is administrated subcutaneously as a 10% aqueous solution using cold water at a dose rate of 5.0mg/kg, (Finelle, 1973). The main disadvantage of quinapyramine compounds is cost, as it is more expensive than suramin. The drug was originally supplied for prophylactic use as a mixture of quinapyramine sulphate (3parts) and quinapyramine chloride (2parts) which was made up in water and
administrated at a dose of 7.4mg/kg.s.c. This gave two months protection for camels against *T.evansi* and relapses were treated with suramin (Finelle, 1973).

**1.12.2.3. Ethidum (Homidium bromide) laprovet France:**

Ethidum bromide is used for treatment of bovine trypanosomosis in the Sudan. (Karib *et al.*, 1954), although it had been experimentally used against *T.evansi* in laboratory animals (Abdel Razig *et al.*, 1968).

**1.12.2.4. Cymelarsan (Melarsan oxide) Rhone Merieux, France:**

Cymelarsan is an injectable trivalent arsenical. It is a derivative of melarsanoxide, the cysteamine derivative, and is a white powder highly soluble in water. Preliminary work carried out, mainly using mice, indicated that cymelarsan is very active against trypanosomes of *T. brucei* group, the animal pathogens *T. brucei* and *T. evansi* and the human pathogens *T. gambiense* and *T. rhodesiense*. (Raynaud *et al.*, 1989). No activity was demonstrated against the important cattle pathogens *T. congoense* and *T. vivax*.

Cymelarsan has been shown not to be mutagenic or embryo-toxic by standard laboratory procedures. It has also been administered to a number of pregnant camels without ill-effects (Raynaud *et al.*, 1989). The drug showed good efficacy against *T. evansi* infection in camels at dose rate of 1.25,
0.625, 0.6, 0.5 and 0.25mg/kg bwt. It eliminated the parasites rapidly and completely. After treatment all animals recovered quickly and no relapse was observed. Animals which received cymelarsan subcutaneously showed localized swelling and oedema around the injection site during the first 36h post treatment (VanGool et al, 1992).

1.13. Trypanotolerance:

Several indigenous West African taurine (Bos taurus breeds), such as the longhorn (N’Dama) cattle are well known to control trypanosome infection. (Berthier et al, 2003). Range trypanotolent cattle are now widely accepted as an important means of exploiting many tsetse infested areas (Ilard, 1985). All workers agreed that N’Dama and Matura breeds of cattle tolerate infection with pathogenic African trypanosomes better than Zebu or European breeds (Nyindo, 1992). Rahman et al, (1993) reported the existence, of a zebu breed, the western baggara cattle in Sudan which survived a natural tsetse challenge and showed a better ability to control parasitaemia compared to other indigenous zebu, e.g. the Butana and Kinana, which succumbed to trypanosomosis. This probably confirms the historical record noted by Archibald in 1927 (cited by Njogu, 1986) which made the first report of trypanotolerance in cattle from an East African zebu in the Sudan. The gene-based ability called trypanotolerance results from
various biological mechanisms under multigenic control (Berther et al, 2003).

1.14. *T. evansi* infection in camels:

The major protozoal disease affecting camels is trypanosomosis caused by infection with *T. evansi*, (Mahmoud and Gray, 1980). Trypanosomosis in camels in the Sudan was first reported by Evans (1908) at the turn of 20th century. *T.evansi* was first reported from the Sudan in donkeys, mules and camels (Balfour, 1904). The disease is now wide spread in camels in the Sudan.
CHAPTER TWO
MATERIALS AND METHODS

2.1. Study Area (Map 1):

The incidence of camel trypanosomosis (Guffar) caused by *T. evansi* was studied in Butana area (also see section 1. 15. 2) over 13 months from May 2003 to June 2004. Butana area is about 12000 km² and limited by the River Blue Nile to the west, River Atbara to the East and the River Nile to the North, to the South the area is bordered by Durra (*Sorghum* spp.) mechanized farming schemes of Gadaref. It is the richest area in camel population (~ 750,000) in eastern Sudan. These camels are owned by Shukria, Rashida, Butahin and other smaller clans of transhumant tribesmen.

Since camel populations are rarely sedentary, camels tend to move in large groups. The area distribution of these groups is often sparse and animals congregate in masses only during the summer settlements. In addition to the normal human imposition on the distribution of camels, the limitations of food and water often necessitate better and more efficacious use of the available resources by a wide dispersal even of camels within a small herd or group. Camels are more abundant around the agricultural schemes along the main permanent rivers of the region (Babiker, 1984).
Butana area has a semi-desert climate in the north and poor savanna in the south with annual rainfall ranging between 100-300mm. Most of the rains occur between July and September. The soil is of the dark clay type in the south and sandy with land dunes in the extreme North. Scattered small hills and rocky areas are found throughout the area.

The vegetation cover of the Butana was described by Harrison (1955). It is classified as semi-desert grassland occurring on dark cracking clays which are an extension northwards into lower rainfall country of the soil which carries low rainfall woodland savanna on clay. There are patches of *A. mellifera*, but these are usually confined to atypical sites. Perennial grasses are almost absent.

In this study samples were collected from three different locations in Butana area. Alsubagh which is located in the center towards the northern parts of Butana plains and it is characterized by sparse grassland while the woody vegetation is found only in shallow seasonal water courses (wadis). About 74 samples were collected from this area. The second area was Gabt Alfeil (AlRwashda forest); this is characterized by an extensive woody cover comprising *A. mellifera* (kitir), *Capparis deciduas* (Tundub), and grasses. The area lies close to River Atbara and there are a lot of water pools during the rains. The total number of samples collected from this area was 73
samples. The third area was Almagataa which is located in the southern part of Butana plains. In this area there is no vegetation cover because it is utilized in mechanized sorghum and sesame farming. The area is used as livestock route during the annual migration of animals from North to South and vice versa.
1 cm=10,000,000
Map 1. Map of Sudan showing study area. (Source: Agab and Abbas, 1999).
2.2. Experimental animals:
2.2.1. Camels:

About 220 randomly selected adult camels (36 males and 184 females) were examined for infection with *T. evansi*. These included 74 animals at Elsabagh, 73 at Ghabat elfiel and 73 at El mgataa.

2.2.2. Rats:

Clean adult albino rats aged 8-10 weeks and weighing on average 140g were obtained from the Department of preventive Medicine, University of Khartoum, Faculty of Veterinary Medicine. They were kept on a locally made rodent pellets and had free access to water and feed throughout this study.

2.3. Diagnosis of *T.evansi*

2.3.1. Direct parasitological methods

2.3.1.1. Wet blood smears preparation:

Wet blood films were prepared by aseptic puncture of peripheral ear veins of camels using sterile needle. A drop of blood was then taken on to a clean glass slide, and covered with cover slip before examination for *T. evansi* under compound light microscope at X400 magnification.
2.3.1.2. Dry blood smears preparation:

For identification of the causative trypanosome (e.g. *T. evansi*), simultaneous dry blood films were taken from the ear vein. These were stained by 10% Gimsa stain and observed under a compound light microscope using oil immersion lens (X1000) (plate 3,4).

2.3.1.3. Microhaemtocrit centrifugation technique (MHCT)

During blood collection for serum (see section ...below), two heparinized micro-capillary tubes were filled to the marked level with fresh blood from each vacutainer of each animal. The ends of the tubes were then sealed with crystaceal and the tubes placed in a microhaematocrit centrifuge. They were then centrifuged at 1500 rpm for 5 minutes. Thereafter each tube was placed on a McMaster slide and examined for motile trypanosomes at X100 magnification.

2.3.1.4. Buffy coat examination using wet preparation:

To increase the sensitivity of the MHCT, a wet blood film was made of the buffy coat/plasma junction, after breaking the micro-capillary tube. The preparation was then examined for motile trypanosomes under a compound light microscope at X 400 magnification.
2.3.1.5. Packed cell volume (PCV):

The per cent packed cell volume (PCV %) was determined by the PCV reader. Each rotated capillary tube was placed on the groove and the scale was moved to the point where the length of the packed cells ended.

2.3.2. Serological method:

2.3.2.1. Card Agglutination Test for Trypanosomes (CATT):

CATT is a direct card agglutination test for detection of anti-trypanosome antibodies in serum or plasma of infected animals. The antigen consists of cloned bloodstream form trypanosomes of RoTat 1.2; a predominant variable antigen type (VAT) of *T.evansi*. The antigen was obtained from the Tropical Medicine Institute, Antwerp-Belgium, (Magnus, 1988). The organisms have been fixed, stained and freeze-dried in order to obtain maximal stability. They are agglutinated by antibodies directed against the RoTat 1.2 variable antigen epitopes and also by antibodies against invariable surface antigen components.

For CATT-*T. evansi* test, blood was collected and serum prepared as follows:

After cleaning with 70%, ethanol, blood was taken from the jugular vein of each male or female using a sterile plain glass vacutainer, with a tube-holder and two-way needle (Henke-Sass-Germany). Ten ml of blood
were then withdrawn in the vacutainer and each vacutainer tube labelled with, date, sex and animal number. The tubes were then placed on a rack and kept in shade for at least one hour to allow for clotting of blood. Thereafter the tubes were introduced in an ice box and transferred to the laboratory at the Camel Research Center, Khartoum to be kept overnight at 4°C. In the morning the blood samples were centrifuged at 1500 rpm for 10 minutes to separate serum. Each serum sample was collected in Eppendorf tubes using sterile Pasteur pipette. Each of the latter tubes was labeled with, date, location, sex and animal number stored at -20°C before use.

2.3.2.2. Test Procedure:

Reagents and accessory materials were obtained from the Institute of Tropical Medicine (Antwerp, Belgium). A complete test kit for 250 screening tests contains the following: 6 vials CATT-antigen, 1 vial positive control, 1 vial negative control, and 1 vial CATT-buffer (plate 1). The reagents for the test are mixed as follows:

A 2.5ml of CATT buffer was added to a vial of freeze dried CATT antigen using sterile syringe. The vial was then shaken for a few seconds so as to obtain a homogeneous suspension. 0.5ml of CATT buffer was added to the vials of positive and negative controls using respectively sterile syringe. On a test area of the card, 25µl of the non diluted serum was added the well
containing the homogenized CATT antigen (approximately 45µl).

After tilting the card gently, agglutination was observed and the degree of agglutination was determined as follows:

1- Very strong agglutination (+++).
2- Strong agglutination (++)
3-Moderate agglutination (+).
4- Weak agglutination (±).
5- Absence of agglutination (-).

2.4. Chemotherapy:

2.4.1. *Trypanosoma evansi* isolates:

About 5 ml of blood were taken from the jugular vein of a few infected animals with typical patent parasitaemia. 0.5 ml of the infected blood was then inoculated in each rat inter-peritoneally to establish *T. evansi* infection. Usually the rodents became parasitaemic on Day 3 of inoculation. The isolates were kept alive by serial passage of the first peak of parasitaemia in more uninfected rats until treated with the trypanocide under test.
2.4.2. Infection of rats destined for chemotherapy:
Rats were divided randomly into 4 groups each consisting of three rodents. All individuals in each group were inoculated with 0.5 ml of infected blood containing approximately $10^6$ trypanosomes to propagate patent infections.

2.4.3. Monitoring parasitaemia in infected rats:
Parasitaemia was monitored by daily microscopic examination of wet blood films obtained from clipped tails. The degree of parasitaemia in each rat was estimated using the method of Herbert & Lumsden (1976). When the parasites were not seen in 5, 10 and 20 microscopic fields, parasitaemia was recorded as less than antilog 5.4 organisms/ml (Herbert & Lumsden, 1976). After the parasitaemia had been estimated in each individual rat, the mean level of parasitaemia (parasite/mlg blood) was obtained for each group.

2.4.4. Treatment:
The groups of rats were treated with three different commercial compounds of quinapyramine pro-salt, and these compounds were tryquine (quinapyramine sulphate and quinapyramine chloride) wockharde (India), Biquin (quinapyramine sulphate and quinapyramine chloride) Star (Pakistan) and Quinapyramine injection (quinapyramine sulphate) Nicholas Primal (India). All drugs were administrated at the rate of 4mg/kg when
parasitaemia was $10^6$/ml or above at Day 6. The drugs were administered subcutaneously to rats (Wahba, 1999).
Plate. 1: Reagents of card agglutination test of *T. evansi*.
Plate. 2: Degrees of agglutination of the card test.
2.5. Statistical analysis:

All data on prevalence of *T. evansi* were subjected to Chi-square test using Stata Software (Stata, 2000) loaded in a desk-top personal computer to elicit significant differences in infection rates using direct parasitological and serological methods as related to sex, season and location. To determine the level of agreement among the diagnostic tests, the results of analyses were subjected to cross tabulation and kappa coefficient analysis (Stata, 2000). Kappa statistics is used as a measure of agreement between two tests. It is based on the difference between the observed probability of agreement and the probability of agreement expected by chance and standardized by maximum possible agreement beyond chance. A kappa of 1 indicates perfect agreement and no agreement beyond chance gives a kappa of zero. A kappa of at least 0.4-0.5 indicates a moderate level of agreement.
Plate 3. Field in blood smear stained with Gimsa showing high parasitaemia with *T.evansi*. Blood obtained from rat. (400x magnification)
Plate 4. Slide stained with Gimsa without cover slip showing low parasitamia with *T.evansi*. (400x magnification)
CHAPTER THREE

RESULTS

3.1. Reliability of CATT-\textit{T. evansi}:

\textit{T. evansi} infections were diagnosed by examination of some 220 camels using wet preparations, MHCT (buffy coat) and CATT-\textit{T. evansi} techniques. Table 1 shows that 31 (14.1\%), 72 (32.7\%) and 115 (52.2\%) camels were infected by \textit{T. evansi} by the wet preparation, buffy coat and CATT, respectively. However, 105 (47.7\%) camels were found uninfected irrespective of the diagnostic technique used (Table 1). The contingency table \textit{Chi}^2-test showed that infection rates differed significantly with the diagnostic technique used (Chi$^2 = 72.51$, d.f. = 3, P < 0.0000007). Serial paired comparisons of the same data revealed that the infection rates also differed with the method of diagnosis, the highest difference being recorded between the wet smear and the buffy coat (Chi$^2 = 20.28$, d.f. = 1, P < 0.00001, buffy coat/ CATT, Chi$^2 = 16.41$, d.f. = 1, P < 0.0001). Thus in terms of sensitivity the wet preparation seemed the least effective, The MHCT buffy coat was intermediate between the two techniques while the CATT was the most effective.

Furthermore, when suspected blood samples from camels were tested by CATT using undiluted serum, agglutination was observed. The degree of
agglutination varied from light to intense agglutination. Intense and clear agglutination (+++) were shown by 7 (6.1%), less intense (++) by 26 (22.6%), moderate 72 (62.6%) and light agglutination was shown by 10 (8.7%) and no reaction was seen in 105 (47.80%) (Table 2).

3.2. Per cent packed cell volume (% PCV):

Since all 220 camels were screened for T. evansi using the MHCT together with the wet smear and CATT methods, data on % PCV were, thus, available for each individual animal, regardless of the diagnostic method. These PCV data were then divided into four groups according to the diagnostic technique and whether the animal was infected or uninfected (Table 3). The PCV groups were < 20%, 20-25%, 26-30% and > 30%. Chi² statistics of the grouped data in Table 3 showed that there was a highly significant difference between the diagnostic techniques in the percentages of animal with PCV 20-25% (Chi² = 43.92, d.f. 3, P < 0.0000007), > 30% (Chi² = 58.76, d.f. = 2, P < 0.0000007) but not with animals having a PCV in the range 26-30% (Chi² = 4.68 d.f. = 3, P = 0.199). Data on animals with PCV < 20% had not been included because they were unsuitable for Chi² statistics (no more than 2 animals in a cell). Regards animals in PCV group 20-25%, subsequent paired comparisons showed that there were significant differences between the numbers of infected animals detected with wet
smear and buffy coat ($\chi^2 = 0.0004$, d.f. = 1, $P = 0.98$), buffy coat/CATT
($\chi^2 = 4.96$, d.f. = 1, $P < 0.02$) and CATT/uninfected animals ($\chi^2 = 31.46$, d.f. = 1, $P < 0.0000007$). In other groups i.e. 26-30% and >30% PCV, similar comparisons showed a significant difference between CATT sero positives and the uninfected camels only ($\chi^2 = 58.76$, d.f. = 1, $P = 0.0000$).
Table 1: The number and percentage of animals infected with *T.evansi* using three diagnostic techniques.

<table>
<thead>
<tr>
<th>Test</th>
<th>Total number examined</th>
<th>Number infected</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet smear</td>
<td>220</td>
<td>31</td>
<td>14.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X²=20.28</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Buffy coat</td>
<td>220</td>
<td>72</td>
<td>32.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X²=16.41</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>CATT</td>
<td>220</td>
<td>115</td>
<td>52.2%</td>
</tr>
<tr>
<td>Chi² Probability</td>
<td></td>
<td>X²=72.51</td>
<td>P&lt;0.0000007</td>
</tr>
</tbody>
</table>
Table 2: The degree of intensity of agglutination of positive samples in CATT test (N = 220 camels).

<table>
<thead>
<tr>
<th>Degree of agglutination</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of positive samples</td>
<td>10</td>
<td>72</td>
<td>26</td>
</tr>
<tr>
<td>Percentage</td>
<td>8.7%</td>
<td>62.6%</td>
<td>22.6%</td>
</tr>
</tbody>
</table>
Table 3: Range of PCV % of infected and uninfected camels according to the diagnostic technique used.

<table>
<thead>
<tr>
<th>PCV</th>
<th>Test</th>
<th>&lt;20%</th>
<th>20-25%</th>
<th>26-30%</th>
<th>&gt;30</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Wet smear</td>
<td>2 (6.5%)</td>
<td>17 (54.8%)</td>
<td>10 (32.3%)</td>
<td>2 (6.5%)</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Buffy coat</td>
<td>2 (2.8%)</td>
<td>41 (57%)</td>
<td>22 (30.6%)</td>
<td>7 (9.7%)</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>CATT</td>
<td>2 (1.7%)</td>
<td>45 (39.1%)</td>
<td>51 (44.4%)</td>
<td>17 (14.8%)</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>NO. uninfected</td>
<td>2 (1.7%)</td>
<td>10 (10.9%)</td>
<td>31 (33.7%)</td>
<td>48 (52.2%)</td>
<td>92</td>
</tr>
</tbody>
</table>

Chi²

<table>
<thead>
<tr>
<th>Test</th>
<th>&lt;20%</th>
<th>20-25%</th>
<th>26-30%</th>
<th>&gt;30</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet smear</td>
<td>_</td>
<td>X²=0.0004</td>
<td>X²=0.04</td>
<td>X²=1.36</td>
<td></td>
</tr>
<tr>
<td>Buffy coat</td>
<td>_</td>
<td>P&lt;0.02</td>
<td>P&lt;0.09</td>
<td>P&lt;0.000007</td>
<td></td>
</tr>
<tr>
<td>CATT</td>
<td>_</td>
<td>X²=19.50</td>
<td>X²=1.99</td>
<td>X²=58.76</td>
<td></td>
</tr>
<tr>
<td>NO. uninfected</td>
<td>_</td>
<td>X²=43.92</td>
<td>X²=4.68</td>
<td>X²=62.02</td>
<td></td>
</tr>
</tbody>
</table>

Probability

<table>
<thead>
<tr>
<th>Test</th>
<th>&lt;20%</th>
<th>20-25%</th>
<th>26-30%</th>
<th>&gt;30</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet smear</td>
<td>_</td>
<td>P=0.98</td>
<td>P=0.95</td>
<td>P&lt;0.25</td>
<td></td>
</tr>
<tr>
<td>Buffy coat</td>
<td>_</td>
<td>P&lt;0.02</td>
<td>P&lt;0.09</td>
<td>P&lt;0.000007</td>
<td></td>
</tr>
<tr>
<td>CATT</td>
<td>_</td>
<td>P&lt;0.000007</td>
<td>P&lt;0.16</td>
<td>P&lt;0.000000</td>
<td></td>
</tr>
<tr>
<td>NO. uninfected</td>
<td>_</td>
<td>P&lt;0.00000007</td>
<td>P&lt;0.20</td>
<td>P&lt;0.000000</td>
<td></td>
</tr>
</tbody>
</table>
3.3. Effects of sex, location and season on infection of camels with T. evansi:

With regards to infection in male and female camels, the females showed the highest infection (53.8%) by CATT test. The lowest infection (2.8%) was recorded in males using wet smear (Table, 4). In spite of the latter discrepancies, there was however no significant differences in infection rates between sexes regardless of the diagnostic technique used (paired comparisons, Chi² = 0.72 -1.06, d.f. = 1, P = 0.30- 0.40).

The effect of season on trypanosome infection rate in camels (pooled male + female) is shown in (Table 5). The highest infection rates were obtained during the rainy season using the wet smear and the buffy coat techniques. However infection rates varied significantly between seasons irrespective of the diagnostic technique used (Chi² = 23.30- 49.90, d.f. = 2, P < 0.00001- 0.00000001). Moreover, with any method used, the infection rate was higher during the rainy season probably highlighting the importance of biting flies in mechanical transmission of the disease during this season. It is a well known fact that tabanids and biting muscids become abundant during the rainy season.

The infection rates of T. evansi of camels (male + female) in the selected three locations are presented in (Table 6). With any one diagnostic method the disease prevalence was higher in Ghabat El fiel followed by
Elsobagh and lastly Magataa. Despite the latter statement there were no significant differences in infection rates between locations, except during the wet season ($\chi^2 = 7.65$, d.f. = 2, $P < 0.02$). This significant discrepancy in the incidence of the disease was, nonetheless, largely or wholly attributed to the difference between Ghabat El fiel and Elmagtaa during the rains ($\chi^2 = 5.72$, d.f. = 1, $P < 0.02$).
Table 4: Prevalence (%) of *T. evansi* in male and female camels using three diagnostic techniques in Butana area, eastern Sudan.

<table>
<thead>
<tr>
<th>Test</th>
<th>Wet smear</th>
<th>Buffy coat</th>
<th>CATT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>female</strong></td>
<td>30 (16.3%)</td>
<td>65 (35.3%)</td>
<td>99 (53.8%)</td>
</tr>
<tr>
<td>(N=184)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>1 (2.8%)</td>
<td>7 (19.4%)</td>
<td>16 (44.4%)</td>
</tr>
<tr>
<td>(N=36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chi²</strong></td>
<td>-</td>
<td>X²=1.06</td>
<td>X²=0.72</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td>P=0.3</td>
<td>P=0.39</td>
</tr>
</tbody>
</table>
Table 5: Prevalence of camel’s trypanosomosis in various seasons.

<table>
<thead>
<tr>
<th>Test</th>
<th>Wet smear No.=31</th>
<th>Buffy coat No.=72</th>
<th>CATT No.=115</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry cool season</td>
<td>6(19.4%)</td>
<td>11(15.3%)</td>
<td>23(20%)</td>
</tr>
<tr>
<td>Hot dry season</td>
<td>5(16.1%)</td>
<td>14(19.4%)</td>
<td>35(30.4%)</td>
</tr>
<tr>
<td>Rainy season</td>
<td>20(64.5%)</td>
<td>47(64.3%)</td>
<td>57(49.6%)</td>
</tr>
<tr>
<td>Chi²</td>
<td>X²=26.81</td>
<td>X²=49.9</td>
<td>X²=23.3</td>
</tr>
<tr>
<td>Probability</td>
<td>P=1.61</td>
<td>P=0.0000007</td>
<td>P=0.0000008</td>
</tr>
</tbody>
</table>


Table 6: Prevalence of infected camels according to location in Butana area.

<table>
<thead>
<tr>
<th>Test</th>
<th>Location</th>
<th>Wet smear No.31</th>
<th>Buffy coat No.72</th>
<th>CATT No.115</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GhabtAlfeal</td>
<td>16 (51.6%)</td>
<td>30 (41.7%)</td>
<td>44 (38.3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chi²</td>
<td>X²=5.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability</td>
<td>P&lt;0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magataa</td>
<td>6 (19.4%)</td>
<td>19 (26.4%)</td>
<td>30 (26.1%)</td>
<td></td>
</tr>
<tr>
<td>Chi²</td>
<td>X²=0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probability</td>
<td>P=0.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-Sobag</td>
<td>9 (29%)</td>
<td>23 (32%)</td>
<td>41 (35.6%)</td>
<td></td>
</tr>
<tr>
<td>Chi²</td>
<td>X²=7.65</td>
<td>X²=3.88</td>
<td>X²=4.25</td>
<td></td>
</tr>
<tr>
<td>Probability</td>
<td>P=0.02</td>
<td>P=0.14</td>
<td>P=0.12</td>
<td></td>
</tr>
</tbody>
</table>
3.4. Comparison between parasitological and serological findings using Kappa statistics:

The level of agreement between buffy coat and direct smear was found to be high (81.36%) with kappa coefficient value of 0.504 (Table, 7). On the other hand, the level of agreement between direct smear and CATT was relatively low (58.18%) with low kappa coefficient value of 0.190 (Table, 7). Also the level of agreement between buffy coat and CATT found to be high (73.18) with kappa coefficient value of 0.472 (Table, 7).

The cross tabulation between direct smear and buffy coat examination results revealed that 31 positive *T.evansi* samples were agreed upon by the two tests (Table, 8). Out of 72 +ve sample detected by buffy coat. Also the two tests agreed on 148 negative samples, out of 189 negative samples by direct smear on the other hand the two tests were disagreed on 41 samples, which were positive, by buffy coat but negative using direct smear (Table, 8).

The cross tabulation between direct smear and CATT results showed that the two tests agreed on 27 positive samples out of 31(87.1%) positive samples detected by direct smear and 115 positive samples using CATT (Table,9). Also 101 negative samples were agreed upon by the two tests, out of 189 (53.4%) negative samples detected by direct smear and 105 negative
samples by CATT. On the other hand, the two tests disagreed on 4 samples, which were positive by direct smear but negative by CATT. Even there were 88 samples positive by CATT but negative by direct smear (Table, 9).

The cross tabulation between buffy coat and CATT results showed that 64 positive samples agreed upon by the two tests out of 72 positive samples detected by buffy coat and 115 positive samples using CATT (Table, 10). Also the two tests agreed upon 97 negative samples out of 148 negative samples using Buffy coat and 105 negative samples using CATT. The disagreement between the two tests was reflected in 8 samples, which were positive by buffy coat, but negative, by CATT. Also there were 51 samples positive by CATT but negative by buffy coat (Table, 10).
Table 7: Agreement between different diagnostic techniques using sigma Stata for windows version 2.0 (Kappa coefficient).

<table>
<thead>
<tr>
<th></th>
<th>Agreement</th>
<th>Expected Agreement</th>
<th>Kappa</th>
<th>Z</th>
<th>Pr&gt;Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS x BC</td>
<td>81.36%</td>
<td>62.40%</td>
<td>0.5043</td>
<td>8.61</td>
<td>0.0000</td>
</tr>
<tr>
<td>DS x CATT</td>
<td>58.18%</td>
<td>48.37%</td>
<td>0.1901</td>
<td>4.19</td>
<td>0.0000</td>
</tr>
<tr>
<td>BC x CATT</td>
<td>73.18%</td>
<td>49.21%</td>
<td>0.4719</td>
<td>7.58</td>
<td>0.0000</td>
</tr>
</tbody>
</table>
Table 8: Cross tabulation between results obtained from Buffy Coat and Direct Smear.

<table>
<thead>
<tr>
<th></th>
<th>DS</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>148</td>
<td>41</td>
<td></td>
<td>189</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>31</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>72</td>
<td></td>
<td>220</td>
</tr>
</tbody>
</table>
Table 9: Cross tabulation between results obtained from CATT test and Direct Smear.

<table>
<thead>
<tr>
<th></th>
<th>CATT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS</td>
</tr>
<tr>
<td>Negative</td>
<td>101</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
</tr>
</tbody>
</table>
Table 10: Cross tabulation between results obtained from Buffy Coat test and CATT.

<table>
<thead>
<tr>
<th>BC</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>97</td>
<td>51</td>
<td>148</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
<td>64</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>115</td>
<td>220</td>
</tr>
</tbody>
</table>
3.5. Chemotherapy:

3.5.1. Estimation of parasitaemia:

Following inoculation of rats with *T.evansi*, the infected rats became parasitaemic on Day 2. From Day 2 onwards parasitaemia increased progressively when it reached about antilog 8 on Day 14. Rats showing parasitaemia at log $6.9 \pm 0.50$ on Day 6 were treated with one of quinapyramine pro-salt. Those treated with Quinapyramine (Quinapyramine sulphate Nicholas Primal, India) showed drop in parasitaemia from antilog 7 to 5.4 on Day 8. Therefore the parasitaemia increased to reach antilog 7.9 on Day 14. The parasitaemia in the rats treated with Biquin (Star, Pakistan) fell to antilog less than 5.4 on Day 8 and increased again till it reached antilog 8.5 on Day 14. Tryquin (Wockharde, India) treated rats witnessed a drop of parasitaemia to antilog 5.7 on Day 8 and remained so till the parasitaemia finally disappeared on Day 12.
Fig (4): The effect of treatment with three drugs on *T.evansi*.
CHAPTER FOUR

DISCUSSION

Camel trypanosomosis 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 (Boid et al, 1985). Although significant improvements have been made recently in diagnosis, a high proportion of infections still remain undetected as the chronic, more common form of the disease is often aparasitaemic (Luckins et al, 1979). In the face of these constraints, alternative methods of diagnosis have been developed, most of which are for the detection of antibody response to the antigens of the circulating trypanosomes (Allen et al, 1992).

The results obtained in this study using dry and wet smear preparations showed that, overall, 14.1% (31 out of 220 camels) were infected with T. evansi. This rate is higher than that reported by Dafaalla (1988) who surveyed camel trypanosomosis in Gedarif, Showak, Kassla, and New Halfa. The infection rates reported by Dafaalla (1988) in these localities were comparatively very low ranging between 1.12% and 2.13%. Examination of camels with the MHCT, in the present work showed an
infection rate of 32.7% which was double the rate obtained by the dry and wet smear methods above. This indicates that the MHCT method was at least two times as sensitive as the blood films techniques. El-Amin et al, (1998) working in Butana using the same diagnostic methods as ours reported a rather lower infection rates with *T. evansi* in camels which ranged between 1.8% to 7.3%.

Comparisons of previous, recent and present *T. evansi* infection rates in camels in the Butana area suggest that the prevalence of camel trypanosomosis due to *T. evansi* in eastern Sudan is increasing. One reason for this increase may be the inadequate treatment. Field veterinarians use an array of trypanocidal drugs against gufār such as Cymelarsan, Ethidium bromide, Isometamidium chloride and Qinopyramine compounds but critical assessment of the efficiency of these drugs in the national laboratories has yet to be fully instituted. The second reason for the inadequacy in treatment is that herds are driven southwards to areas of high savanna with relatively very high challenge of biting flies such as Tabanidae and Stomoxyinae. Herds are always taken southwards during the dry season and in all seasons during drought years in search of water and grazing higher up in the Ethiopian plateau at the Sudanese-Ethiopian borders. The third reason may be drug-resistance of *T. evansi* to the drugs in common use (El
Rayah et al., 1999; Mohamed-Ahmed et al., 1992; Luckins, 1988). Drug resistance may arise as a result of subdosing, high trypanosomosis challenge and the faulty administration of drugs.

Of the diagnostic techniques employed in the present work, the CATT was relatively highly more sensitive and specific for detection of *T. evansi* infection than the wet and dry smears or MHCT. Similar results were also reported by Nantulya (1990). Our results obtained by CATT indicated that 52.2% of the 220 camels were infected which was three times as sensitive as the MHCT. The latter technique was more than two times as sensitive as the stained blood films. As far as we know this is the first record of employment of CATT-*T. evansi* in the diagnosis of camel trypanosomosis in the Sudan. However, a recent study in Egypt conducted on imported Sudanese camels using CATT showed that 28% of the animals were positive for *T. evansi* (Elsaid et al., 1998). The latter authors concluded that the test was both sensitive and reliable for the diagnosis of camels with chronic and latent *T. evansi* infections.

Considering the intensity of agglutination, it appears that four levels are manifested (Table 2 or Fig.2). The most frequent intensities were observed to manifest themselves at the moderate and less intense agglutination levels. This indicates that the titers of antibody in the sera were
not high enough which might be a reflection of the chronicity of the infection.

The Kappa crosses tabulation between direct smear and CATT showed that the sensitivity and specificity were 87% and 53%, respectively. Similarly, cross tabulation between MHCT and CATT showed sensitivity and specificity at 88% and 65%, respectively. These results agree with Magnus (1988) who suggested that CATT test was highly sensitive but was not strictly species- specific. In conclusion these results show that CATT-*T. evansi* was reliable enough to detect aparasitaemic infection rapidly and was more sensitive than parasitological methods in revealing the true extent of trypanosomosis in a herd (Ngaira *et al*, 2003; Delafosse and Doutoum, 2004; Hilali *et al*, 2004).

*T. evansi* infected camels show a low grade anemia, 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 (Onah *et al*, 1996). In this study the PCV of the infected camels was lower than that of the negative or uninfected animals. This low PCV level can be attributed to parasitaemia and subsequently the destruction of erythrocytes by *T. evansi*
haemoflagellates. However, this may not a universal proposition, since Boid et al (1981) reported that *T. evansi* appeared to have little effect on the haematological picture and PCV of infected sheep, though there was a progressive fall in the PCVs of similarly infected goats and camels.

The present results show that there was no significant difference in *T. evansi* infection rates between males and females. Similar results were reported by El-Amin et al, (1998) who showed that there was no significant difference between the infection rates of males and females of camels in several locations in Butana area. However, *T. evansi* infection rate differed significantly between seasons and between locations during the rainy season. Regards the latter, it is well known fact that biting and non-biting haematophagous muscids (Diptera: Muscidae) and horseflies (Diptera: Tabanidae) breed and proliferate during the rains (Abdu El Karim, 2003). These flies, Tabanidae in particular, are considered the main mechanical vectors of *T. evansi*. This may help to explain why infection rates of camels with *T. evansi* were higher during the wet season. It also explains why camels in Ghabat El Fiel area had a higher infection rate with *T. evansi* than El Subagh and El magataa area. Ghabat El Fiel is very close to R. Atbara and has higher number of pools and water bodies, hence higher fly denities, during the rains than the other two locations.
The level of parasitaemia (parasites/ml of blood) in *T. evansi*-infected rats treated with Biquin and quinapyramine was similar to or was not significantly different from that of the infected untreated control rats. This suggests that both drugs were ineffective against the *T. evansi* isolates at the therapeutic dosage used. In contrast the complete clearance of parasitaemia in rats treated with Tryquin indicates the high efficacy of this drug. It therefore seems reasonable to suggest that isolates of *T. evansi* may be more sensitive to some quinapyramine salts than others. This suggestion agrees with Haroun et al (2003) who obtained similar results with tryquin against *T. evansi*. It is also possible that *T.evansi* could have become resistant to the recommended therapeutic dose of both quinapyramine and Biquin (Zweygarth, *et al.*, 1992).
Conclusions and recommendations

1. - The card agglutination test for trypanosomosis (CATT-\textit{T. evansi}) was found to be a good serological test in the field and as a screening test for guffar among camel herds. It is also easy to use in day – to – day diagnosis.

2. - It is suggested that camel trypanosomosis is a serious problem in Butana plains. There is thus a need to continue studies focusing on the control of the disease in the area.

3. - Since treatment of \textit{T. evansi} with Antrycide pro-salt and Biquin did not clear completely the parasitaemia in infected rats we suspect that some isolates had acquired some resistance to both drugs. We therefore recommend investigations into drug resistance of isolates of \textit{T.evansi} from various locations in Butana area.
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