INVESTIGATIONS ON *TRYPANOSOMA EVANSI* AND GASTROINTESTINAL PARASITES IN CAMELS (*CAMELUS DROMEDARIUS*) BROUGHT TO TAMBOUL LIVESTOCK MARKET, SUDAN.

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قال الله تعالى:

(أَفَلَا يَنظُرُونَ إِلَى الْإِبْلِ كَيْفَ خُلِقَتْ) 

الغاشية الآية (17).
Dedication

To my parents & my sisters
To my family with love
To Alsheikh/Al- Buray
I would like to thank Professor Hamid Suliman for his close supervision, encouragement and guidance.

This work was supported and financed by the University of Gazira. In this context I would like to express my deepest thank to Dr. El-Sir A/Elhai Babiker, Dean Faculty of Veterinary Medicine for his support and encouragement throughout this work. I am very indebted to Dr. Badreddin Wasila, Director of Tamboul Camel Research Center (TCRC) and his laboratory staff their assistance. The technical help offered by my colleagues Mr. Hozifa Seddig Faculty of Vet. Med. Miss Abeer Abdalla is highly acknowledged.

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This study surveyed the haemoprotozan parasite *T. evansi* the causative agent of camel trypanosomosis (Guffar) in camels (*Camelus dromedarius*) brought to Tamboul market, East of Gazira by taking 600 blood samples using jugular vein in heparinized and plain tubes. These samples were examined for presence of *T. evansi* using wet and stained smears and Haematocrit Centrifugation Technique (HCT) and revealed an infection rate of (0.83), (2.66%), (3.5%), respectively. These results indicated that the (HCT) was slightly sensitive when compared with blood smears in diagnosing camel trypanosomosis. Out of 600 samples 404 serum prepared and examined serologically using card agglutination trypanosomosis test (CATT) and revealed that 143 (35.1%) serum samples were found to include antibodies against *T. evansi*. This results mean that the CAAT/ *T. evansi* was statistically the most sensitive technique for surveying of *T. evansi*. By using all mentioned methods the rate of *T. evansi* was high during the rainy season (61.1%) and low during the dry season (38.9%).

Out of 600 camels 200 camels examined by using fecal examination for presence gastrointestinal parasites at the same time, using gross fecal examination, floatation, sedimentation and fecal culture and 91% of camels
examined found to be infected at least by one parasite. Gastrointestinal parasites which diagnosed were; *Trichostrongylus* spp., *Strongyloides papilosus*, *Trichuris globulosa*, *Avitellina* spp., *Monezia* spp., and *Fasciola gigantic* and protozoal infection by *Eimeria* spp.

This present study also showed that the mixed infection of gastrointestinal parasites was lower (17.4%) than the rate of single infection (82.6%), also the infection rate was high during rainy season (88%) and low during the dry season (65%).

The concurrent infection of *T. evansi* and gastrointestinal parasites in total of 200 camels examined was seven camels (3.5%).
ملخص الدراسة

في هذه الدراسة تم مسح لطفيل الدم مثقيبات إيفانزاي المسبب لمرض الجفار للإبل التي تباع في سوق تمويل بشرق الجزيرة وذلك باخذ عينات دم من 600 جمل عن طريق الوريد الوداجي في انتابب احتبار، واستخدم في فحص عينات ادم مثقيبات المسحة الرطبة والمصبوبة بطريقة التركيز بالطرد المركزي (الغي كوت) وكان معدل الاصابة كمالي 5%)، 16 (2.66%) و 21(3.5%) على التوالي. أثبتت هذه الدراسة أن طريقة التركيز بالطرد المركزي (الغي كوت) ذات حساسية طفيفة في تشخيص المثقيبات مقارنة بفيلم الدم. من مجموع 600 عينة دم 404 عينة مصل تم فحصها سيولوجياً باستخدام تقنية التلازن على الكرت لطفيل إيفانزاي حيث اوضحت الدراسة 143 عينة اتضح تحوي أجسام مضادة للمثقيبات إيفانزاي 35.1%، هذه النتيجة تدل على أن تقنية التلازن على الكرت احصائياً أكثر حساسية لتشخيص المثقيبات إيفانزاي. وبالاستخدام كل أطراف السابقة كذلك وجد ان معدل الاصابة بهذه المثقيبات ذات اعلى في موسم الامطار مقارنة بمعدل الجفاف بمعدل 61.1% و 38.9% على التوالي.

من مجموع 600 جمل تم أيضاً اخذ 200 عينة برازية واجربت عليها الفحوصات بحثاً عن طفيليات المعدة والامعاء ، حيث اوضحت الدراسة ان 91% من الابل مصابة على الاقل بطفيل واحد . أما الطفيليات التي تم تشخيصها باستخدام الفحص اليدوي للبراز وطريقة المسحة المباشرة ، الطفو و الترسيب وزراعه Trichostrongylus spp., Strongyloides papilosus, Trichuris البرازهي ومن الاصابات Fasciola gigantic, globulosa, Avitellina spp., Monezia spp., Eimeria spp بالاولي.

كذلك أوضحت هذه الدراسة ان معدل الاصابة لأكثر من طفيل واحد على من الاصابة بطفيل واحد بمعدل 82.6% و 17.4% على التوالي. وان معدل الاصابة
هذه الطفيليات أعلى في موسم الأمطار مقارنة مع معدل الإصابة في موسم الجفاف بمعدل 88% و 65% على التوالي.
اما معدل الإصابة المشتركة للمثقبيات ايفانزاي والطفيليات الداخلية للقناة الهضمية للابل فقد اثبتت الفحص وجود عدد سبعة جمال من مجموع 200 عينة (3.5%).
INTRODUCTION

The importance of camels (Camelus dromedarius) stems from the fact that it is sustainable agricultural resources for millions of nomads and pastoralists in the arid and semi-arid zones. Camel population in Sudan exceeds three millions distributed in northern Kordofan and Darfur in the West and Red Sea, Kassala and Butana in the East. Trypanosomosis caused by *T. evansi* and gastrointestinal worms constitute serious and economical important infections in camels in Sudan and elsewhere they cause considerable mortality and high morbidity in camels. *T. evansi* is transmitted mechanically by biting flies such as *Tabanus and Stomoxys spp.* which widely distributed. Diagnosis is largely depends on direct microscopy although some serological tests have been introduced. Due to practical difficulty of vector control, control of trypanosomosis rests inevitably upon chemotherapy and chemoprophylaxis. Similarly, treatment with anthelmentic drugs is main approach for control of worms. Investigation and research on camels since 1905 – 2005 are compiled in the bibliography of camel entitled "The One-humped camel (Camelus dromedarius) in the Sudan" by A/ Majid (2000).

Rationale:

Sudan is the second largest country for camel population in the world. Camels provide milk, meat, and wool and are use for poulphing, water
traction and bear of burden. Camels export contributes to foreign currency earnings. Control of infection agents they becomes necessary for improved camel health and productivity. This can not be attained unless through investigations on the epidemiology of the diseases are made.

Objectives:

1. To determine the prevalence of concurrent *Trypanosoma evansi* and gastrointestinal nematode infections of camel (*Camelus dromedarius*) in Tamboul livestock market.

2. To determine the effect of sex, location and season on the prevalence of *T. evansi* in camels in the study area (Tamboul livestock market).

3. To compare the sensitivity of direct parasitological diagnostic methods and card agglutination trypanosomosis test CATT/*T. evansi* as serological diagnostic technique in detection of camel trypanosomosis in Tamboul livestock market.
CHAPTER ONE

LITERATURE REVIEW

1.1. Historical background:

Camel trypanosomosis is a disease caused by *T. evansi*; which was discovered by Griffith Evans in 1881 in infected camels and equids in the Dara Ismail Khan District of Punjab (Indrakhmang, 1998). The parasite can infect all the species of domesticated livestock, although the principle host varies geographically (Alrawashdeh, *et al*., 2000).

1.2. Classification of trypanosomes:

*Trypanosomes* are classified according to Soulsby (1982) as follows:

- **Kingdom:** Protista
- **Phylum:** Protozoa
- **Sub phylum:** Sarcomastigophora
- **Sub class:** Mastigophora
- **Class:** Zoomastigophora
- **Order:** Kinetoplastida
- **Sub order:** Trypanosomidae
- **Family:** Trypanosomatidae
- **Genus:** Trypanosoma
- **Species:** evansi
The principal pathogenic trypanosomes causing animal trypanosomosis in the Sudan are *T. congolense*, *T. vivax* and *T. brucei* which affected cattle, sheep, goats, horses and donkeys, with the *T. evansi* affecting mainly camels and rarely horses (Karib, 1961).

1.3. Morphology:

The morphology of *T. evansi* was described by Soulsby (1986) *T. evansi* is belongs to the brucei group of trypanosomes and it is morphologically identical to the long form of the pleomorphic *T. brucei*, being 15-34um in length (means 24 um). They are characterized by the possession of the kinetoplast, which small and typically occupies a sub terminal or marginal position in the body at the tapering posterior end. Both kinetoplast and nucleus stain red, by using Romanwesky stains. The organism bears a single flagellum. The trypomastigote (blood stream form of mammalian trypanosomes) are basically lanceolate in shape, some forms lack kinetoplast which was thought to develop spontaneously specially after drug treatment. Hoare (1972) postulated that *T. evansi* envolved from *T. brucei* by adaptation to non-cyclic mode of transmission involving of polymorphism. He suggested that camels coming contact with the tsetse flies at their northern most limit of distribution become infected with *T. brucei*, and when the camels returned to tsetse-free area, infection was spread by insects.
1.4. Distribution:

Trypanosomosis is widespread in different parts of the world and affecting all species of domesticated livestock and posses a major constraint to productivity (Elamin et al., 1999). In Africa the disease occurs in many countries and has been given different local names. It occurs in Somalia, Kenya, Sudan, Mauritania, Ethiopia, Egypt, Libya, Mali and Algeria. (Hoare, 1972). In India the disease breaks out in the rainy season, when the insects are multiplying, and it is commonest in riverain and marshy tract or in the region of the forest lands (Edmond and Walker, 2001).

1.5. Hosts:

Trypanosomosis affects equine, cattle, camels, and dogs in varying degrees of virulence. In cattle and camels the disease runs a chronic course (Edmond and Walker, 2001; El-rawashideh, et al., 2000).

Natural infection with T. evansi described in goats produce acute, sub acute and chronic or sub clinical forms (Gutierrez, et al., 2006). In Africa, beyond the northern most limits of the tsetse fly belt, and in part of East Africa, camels are the most important host (Dia, et al., 1997). Whilst in Central and South America the horse is principally affected. In Asia, a much wider range of hosts involved, including the Bactrian camel and dromedaries, cattle, buffalo, horses and pigs (Al-rawashdeh, et al., 2000; Pacholik, et al., 2001).
1.6. Transmission:

*T. evansi* lacks gene necessary for mitochondrial development and therefore unable to undergo growth and differentiation in the insect vector. Nevertheless, this has not precluded transmission by insects. It is speculated that the widespread of occurrence of *T. evansi* is largely due to its being spread mechanically by the bites of haematophagous flies and bats (Losos, 1980).

Mechanical transmission occurs in cases of interrupted feeding, where a fly began feeding on infected animal and completed it in another animal. So it passes infection from an infected animal to non-infected susceptible animals through contamination. The most important mechanical vectors are flies of genus *Tabanus, Haematopoia, Lyperosia, Stomoxys* and *chrysops*. (Mohiuddin, 2007). This mode of transmission is sufficiently effective in maintaining *T. vivax* and *T. evansi* outside tsetse infected area. Wells (1972) reviewed the importance of mechanical transmission in nagana and related it to the presence of *T. vivax* in countries outside Africa where tsetse flies are present. Raymond (1990) was able to prove the role of *Tabanus importules* in *T. vivax* transmission in French Guyana. Mihoks, *et al.* (1995) studied the ability of African *stomoxyniae* to transmit trypanosomes; they concluded five species of the family were capable of transmitting the disease mechanically. Transmission by other means include infection of carnivores with
T. evansi and T. brucei by ingesting meat or organs of infected animals and transmission of T. evansi in Liam America by the bites of vampire bats, but these ways were considered to be of less importance (Ulenberg, 1998).

1.7. Epidemiology:

Trypanosomosis, caused by T. evansi, a blood protozoan, is one of the most important diseases of camels (Luckins, 1992) resulting in high morbidity in countries where the dromedary inhabits. It is the most widespread pathogenic trypanosome in the world. It is mechanically transmitted by haemotophagus biting flies and therefore distributed widely outside the tsetse belts. More than 20 different species of Tabanus have been known experimentally to transmit T. evansi mechanically (Luckins, 1998). The definite correlation between the seasonal outbreaks of T. evansi infections and the increase in numbers of Tabanus during the rainy season is reported (Mahmoud and Gray, 1980). These findings may explain the sporadic occurrence of the disease during the dry season and outbreaks during the rainy. The disease causes significant morbidity and mortality in camels in arid and semi arid regions in the world. The disease is the most important single cause of economic losses in camel rearing areas, causing morbidity of up to 30% and mortality around 3% (Njiru, et al., 2002). It was currently added to the list of notifiable diseases of OIE (Womak, et al., 2006). Camels managed under nomadic
pastoralism have higher risk of being exposed to *T. evansi* infection than camels under extensive system management (Najira, 2003).

The trypanosomosis is insect-born disease of cattle, buffalo, goat, sheep, pig, horse, donkey, camel and dog. This disease is widely distributed in tropical and subtropical countries, (Upadhayay, 2005). Camel trypanosomosis in Sudan exists in arid and semi-arid area north of latitudes 12°N; mainly in provinces of Northern Kordofan, Darfur, Kassala, Blue Nile and Nubian mountains. Animals such as donkeys and cattle are frequently mixed with camels in the pasture although their contribution to the epidemiology of camel trypanosomosis has not yet been evaluated (Ismail, *et al.*, 2007). Natural and experimental camel trypanosomosis have been described in different parts of the world (Alrawashdeh, *et al.*, 2000; Pacholik, *et al.*, 2001; Njiru, 2002). These epidemics considered the major constraint to camel productivity (Elamin *et al.*, 1999).

Tsetse flies in Sudan are active in a total area of 300,000 km² of fertile land. African animal trypanosomosis has been described as a major obstacle to sustainable livestock production and food security, and an important factor of underdevelopment in sub-Saharan Africa. (Swallow, 2000). The belt of animal trypanosomosis in Sudan was described by Rahman (2005) as a horse shoe around the Nile swamps with three gap areas:
(a) The valley of Bahr Al gazal.
(b) The arid country east of Kapoeta.
(c) The Sobat area where the tsetse fringe passes through Ethiopia territory.

1.8. Immunity:

Trypanosomes have ability to evade the host's immunity by changing their surface protein coat (Clarkson, 1976). There are pronounced immune changes occurring in African trypanosomosis. An increase in gamma-globulin (IgM) during both acute and chronic trypanosomosis in camels, although this is not protective, as the majority of the antibodies are auto antibodies. Leucocytes, neutrophilia and eosinophilia have been reported in *T. evansi* infection of camels. These changes occur as a result of an increase in the activity of the mononuclear phagocytic system. The eosinophilia observed is a feature of parasitic infections and is associated with immediate-type hypersensitivity reactions. The cells are expected to accumulate in tissue in response to tissue injury. In the acute phase of the disease, lymph nodes and spleen are remarkably reactive, with plasma cells predominating. This may account for the generalized lymphoid tissue hyperplasia characteristic of *T. evansi* infections, while in the late stages the immune system becomes depleted of lymphoid cells (Felicia and Anthony, 2005).
1.9. Diagnosis:

Definitive diagnosis of a current infection with *T. evansi* relies on the demonstration of the parasite in the blood or tissue fluids of infected animals (Nantulya, 1990).

1.9.1. Parasitological methods:

Heparinized blood samples can be collected in clean tubes from camels and examined in the laboratory for presence of blood parasites using standard parasitological methods:

1.9.1.1. Blood films:

Trypanosomes are actively motile, and when present can usually be detected in fresh blood by their movement (Edmond and walker, 2001). The wet smear method is useful, simple and cheap; it is limited by low sensitivity and can't identify the species of trypanosomes. It is also useful in drug trials (Kendrick, 1968).

The blood films are useful in identification of trypanosomes species but less efficient (Kendrick, 1968).

Thick blood method is more sensitive than both wet and thin smear in detection of trypanosomes; it is limited by difficulty in recognizing the parasite species (Kendrick, 1968).

1.9.2. Haematocrit centrifugation technique (HCT):

This method is very sensitive in detecting parasites during the first period of infection. (Wernery, 2001). It is able to detect trypanosomes
infection earlier than other parasitological methods. It has more efficiency in surveys of trypanosomes that are non infective to laboratory animals like *T. vivax* and some strains of *T. congolense*. The method detects *microfilaria* in the blood. The technique is proved to be sensitive in *T. evansi* infections; however it is less sensitive than mice inoculation technique (Monzon, 1990). The technique is used for determining the assessment of anemia and parasitaemia estimation (Kendrick, 1968).

**1.9.1.3. Laboratory animals inoculation:**

Laboratory animals may be used to reveal sub clinical infections in domesticated animals. *Trypanosoma* spp. has a broad spectrum of infectivity for small rodents. 2 ml of infected blood inoculated into rabbit or mouse intraperitoneal (I/P), will demonstrate the trypanosomes in the blood after a few days (Mohiuddin, 2007).

**1.9.2. Serological methods:**

In these methods serum is used to detect the circulating antibodies against the trypanosomes or antigens as a diagnostic tool for trypanosomosis in animals with low parasitaemia. There are many serological and molecular tests used to improve the diagnosis. The serological tests based mainly on detection of circulating antibodies against the trypanosomes.
1.9.2.1. The indirect fluorescent antibody test (IFAT):

The test was performed by Pla tt and Adams (1976) in South American cattle infected with *T. vivax*. The test proved to have no false positive reactions and no cross reactions with other trypanosomes or blood parasites. IFAT also proved to be reliable as ser-epidemiological screening tool for cattle trypanosomosis in Zambia (Kakoma, *et al.*, 1985). This test has been used extensively in the diagnosis of trypanosomosis by detecting trypanosomal antibodies in animals and human. It proved to be both specific and sensitive in detection of trypanosomal antibodies in infected cattle (Luckins, *et al.*, 1979).

1.9.2.2. Complement fixation test (CFT):

The complement fixation test (CFT) was one of the first techniques used to diagnosis of *T. evansi* in camels. The test was used successfully for detection of *T. equiperdum* presence in horses. Cross reactions with sera of horses infected with other trypanosomes may be occurred. However, C.F.T is less sensitive than ELISA in the diagnosis of the Dourine disease in equines (Gillbert, 1998; Wassal, *et al.*, 1991).

1.9.2.3. Enzyme – Linked immunosorbent assay (ELISA):

The enzyme linked immunosorbent assay (ELISA) was developed by Engvall and Pelarmann (1971). The test proved to be as sensitive in
detecting *T. evansi* in rabbits. The Ab-ELISA is not species-specific because of strong cross-reactions between the pathogenic trypanosome species. Technique of ELISA has a beneficial diagnosis of *T. evansi* and it is useful for epidemiological studies and trypanosomosis control programme (Chansiri, *et al.*, 2002; Monozon, *et al.*, 2003). Elamin, *et al.* (1990) were used Ag ELIZA and buffy coat technique (BCT) in diagnosing of camel trypanosomosis of camel in Mid-eastern Sudan. They found that the camel trypanosomosis due to *T. evansi* is endemic among pastural camels with prevalence of 5.4% based on BCT and 31.1% based on Ag-ELIZA.

1.9.2.4. The Card Agglutination Trypanosomosis Test (CATT):

It is well known that certain predominant variable antigen type (VATs) are expressed in common by different strains of salivarian trypanosomes from different areas. On this basis a field technique (CATT) was developed in the laboratory of serology of Institute of Tropical Medicine (Antwerp) for *T. evansi* infection diagnosis in camels (Nantulya, 1999). It is has been introduced for diagnosis of Ghambian sleeping sickness. The test uses the formalin fixed available antigen types of *T. evansi* that are used in the agglutination test and used for detection of antibodies against *T. evansi* and its sensitivity is high, simple to perform (Desquesnes, *et al.*, 2001; Nagira, *et al.*, 2003; Hilal, *et al.*, 2004). Magnus *et al.* (2002) used CATT with stained
trypanosomes successfully for the serological diagnosis of *T. brucei gambiense*. CATT, latex and Ab-ELISA have been used successfully to detect *T. evansi* infection. These three tests were able to detect antibodies or antigens in the serum (Luckins, 1999). Latex test seems to be more ideal for testing small numbers of animals under field conditions infected with *T. brucei, T. congoelense* and *T. vivax* (Kayang, *et al.*, 1997). The CAAT have also been used successfully in diagnosis of human African trypanosomosis (HAT) (Jamonneau, 2000). It is considered as the test of choice for mass screening of the disease under field conditions. Slight modification of the classical card agglutination test was done by Magnus *et al.* (2002) as the former test may give false negative test result. The modification was done by adding EDTA as anticomplementry factor. The test proved to be highly sensitive but also less specific than the classical test. CATT/T. evansi, which is a commercially produced kit, is widely used in the field. However, it is unable to distinguish past or treated from current infections because it detects antibodies that may persist for a long time following treatment (Nagira`, *et al.*, 2003).

**1.9.3. Molecular techniques:**

These are new laboratory procedures. They include restriction enzymes, synthesis of DNA, DNA probing and polymerase chain reaction (PCR). They had considered input into trypanosomes identification,
characterization and diagnosis. DNA technology is the recent development in diagnosis of animal trypanosomosis and having a major impact in many areas of Veterinary Parasitology (Ulenberg, 1998). PCR is highly sensitive in permitting enzymatic amplification of gene fragments of nucleic acids derived limited of parasite material (Gasser, 1999). Aradaib and A/Majid (2006) used a nested polymerase chain reaction (nPCR)-based assay in diagnosing infection of *T. evansi* in naturally infected Sudanese camels and experimentally infected mice. Four oligonucleotides primers (TE1, TE2, TE3 and TE4), selected from nuclear repetitive gene of *T. evansi*. They found that nPCR –base assay is sensitive, specific and rapid of all *T. evansi* strains.

The use of DNA probes and PCR-based assays is technically complex, but highly sensitive. PCR-based assays were more sensitive than DNA probes assays (Weiss, 1995). Combination of DNA probes and PCR revealed trypanosomes in buffy coat samples. The DNA probes proved to be sensitive in detection of *T. evansi* and several species of African trypanosomes (Majiwa, *et al.*, 1994; Gibson, 2002). PCR seems to be one of the most accurate test for the detection of *T. evansi* parasitaemia in camels. PCR has been used successfully in detecting infection with *T. evansi* in buffaloes (Holland, *et al.*, 2001), horses (Clausen *et al.*, 2003) and in camels (Masiga and Nyang’ao, 2001).
Loop-mediated isothermal amplification (LAMP) is a new DNA amplification method that is performed under isothermal condition. This method relies on auto-cycling strand displacement DNA synthesis that is performed by a Bst DNA polymerase with high strand displacement activity (Notomi, et al., 2000). Loop-mediated isothermal amplification (LAMP) was developed for the specific detection of both animal and human trypanosomosis. LAMP has been successfully applied in detection of various pathogens including African trypanosomes (Thekisoe, et al., 2005). LAMP’s rapid (amplification in 1h) and a simple technique (requires only water path/heat block); it amplifies DNA at a contrast temperature, can produce large amount of DNA that can be visualized by the naked eye as white turbidity indicating positive amplification (Mori, et al., 2001) and can amplify trypanosome DNA from blood blotted on filter papers (Kuboki, et al., 2003). All these advantages indicates that LAMP has the potential to be used as an alternative molecular diagnostic method especially at the under resourced laboratories and the field for diagnosis of trypanosomosis especially in countries that lack sufficient resources needed for application of diagnostic technique. The LAMP reaction runs at a constant temperature, usually around 65 °C and uses four primers that recognized six distinct regions of the target DNA combined with the Bst DNA polymerase large fragment which has strand displacement activity.
and is heat inactivated at 80°C (Notomi, et al., 2000). The reaction can be accelerated by inclusion of additional loop primers (Nagamine, et al., 2002). The resulting amplification products are a complex mixture of stem-loop DNAs with inverted repeats and cauliflower-like structures (Notomi, et al., 2000). And induce a white precipitate of magnesium pyrophosphate proportional to the mount of amplified DNA and visible to the naked eye (Mori, et al., 2004).

1.10. Control:

Absolute control of animals trypanosomosis cannot be achieved with the available current methods, which are inadequate to prevent enormous socio-economic losses caused by this disease. These methods include animal treatment with trypanocides, using livestock that are more resistant to disease, reducing the proximity of livestock to reservoir hosts and controlling the population of vectors by spraying of insecticides or by trapping (Desquesnes and Davila, 2002). Since control of the vector flies is not feasible at present, control of the disease is basically achieved through chemotherapy and chemoprophylaxis (Haroun, et al., 2003).

Chemotherapy depends on few trypanocides. Their effectiveness is diminished by the increased occurrence of chemo-resistance (Soulsby, 1982). The trypancidal drugs include suramin (Antrypol or Neganol) which is introduced as a chemotherapeutic and a chemoprophylactic drug against *T. evansi* (Thomas and Jeemy, 1981). It was
shown to be effective in the treatment of naturally occurring *T. evansi*; Dimiazene (Berenil) is another drug but as it rapidly excreted from the body. It was previously believed that the drug doesn’t promote the development of resistant trypanosomes even if sub-therapeutic doses were used (Leach and Robert, 1981); Quinapyramine was introduced in the early fifties after a series of field trials in Africa (Davey, 1957). Quinapyramine dimethosulphate (Antrycide) is active against *T. brucei, T. conglense, T. vivax* and *T. evansi*. It used for curative treatment of all domestic animals at a recommended dose subcutaneously only (Hassan, 2003) and lastly Cymerlarson is a new compound for a group that has not previously been used against animal trypanosomosis. It is very effective against the *T. brucei* group specially *T. evansi* infection (Raynaud, *et al.*, 1985).

1.11. Drug resistance:

Resistance to drugs used for the treatment of trypanosomosis was reported in various high transmission areas and for most established drugs, but mainly for melarsoprol (Basel and Wirz, 2006). Resistance is likely to occur when only a few drugs are available to treat the disease over a long period of time and when a sufficient plasma level of drug cannot be reached (under-dosing) due to compliance problems of failure in treatment schedules. This favors selection of less susceptible trypanosomes. Reports on occurring relapses often do not distinguish
whether these relapses were due to drug resistance trypanosomes or if they were due to animal related problems.

2. Brief history of gastrointestinal parasites of camels in Sudan:

Helminthes infections in the Sudan were the main cause of death of camels during autumn (57.9%) (Agab and Abbas, 1992). Steward (1950) reported *Haemonchus longistips*, *Tichostrangylus probolurus*, and species of *Strongloides*, *Stilesia hepatica*, hydatid cysts, *Onchocerca fasciata* and *Deipetalonema evansi* in camels in the Sudan. Malik (1959) examined 17 camels from Central Sudan and he found that 70.5%, 70.5%, 52%, 23.5%, 23.5%, 11.7%, 11.7%, 5.9%, and 5.9% of camels examined were infected with *Avittelina woodlandi*, *Trichuris globulosa*, *Echinococcus granulosus* cyst, *Haemonchus longistipes*, *Impalaila* spp. *Oesphagastomum venulosus*, *Schistosama bovis*, *Moneizia expansa* and *Nematodirus* spp., respectively.

Arzoun, *et al.* (1984) observed that *Haemonchus longistipes* was the only helmith parasite found in naturally infected camels with prevalence rate of 89% during the rainy season and 64% during the dry season. Elgezuli, *et al.* (1978) found that all of the 12 camels examined, were infected with *Haemonchus* spp.

Kheir, *et al.* (1982) carried out a preliminary survey of haemonchosis of camels in Eastern Sudan during December 1980, January and February 1981. They examined 203 animals at slaughter houses in different districts and the incidence of infection during these
months was found to be 77.7% 79% and 86% respectively. Fecal samples of 356 camels were taken and examined microscopically; the incidence of infection of these camels was 54.8%, 63.2% and 74.5% for the same previous months, *Trichuris globulosa, Strongyloides lapiatopapitosa* and *Schistosoma bovis* were encountered during the fecal examination. Saad, *et al.* (1983) observed that 64(45%) of 141 heads of camels slaughtered at Tamboul were infected with hydatid cysts. Magzoub, *et al.* (1983) conducted a study on the prevalence of internal parasites in camels with the emphasis on the epidemiology of infection in the Sudan. Fecal examination carried out by these authors, revealed the presence of *Strongyle, Trichostrongyle, Trichuris, Strongyloides* together with the *Monezia* eggs and *Eimeria* spp. Oocyst. The fecal cultures showed the presence of 4 genera. These comprised *Haemonchus, Trichostrongylus, Oesophgastomum* and *Impalaia*.

Gaffar (1984) examined 120 camels at El-Gadarif slaughter house by means of post-mortem examination. They reported that the genus *Haemonchus* was prevalent among 67% of the camels examined *Strongyloides* in 15.8% and *Trichuris* 5%. Saad (1985) investigated that the high prevalence of hydatid cyst in Sudan was found in camels (43.9%), cattle (3.89%) and sheep (12.2%).

Hamza (1987) carried out a various sero-epidemiological aspects of hydatid disease caused by *Echinococcus granulosus* in some selected
area in the Sudan. His study showed that the high infection rates were obtained in camels from Butane plains are (61.5), in Khartoum (41%) and 55% in the Sudanese camels used for exported to Egypt. The average prevalence rate in camels was found to be (56.4%).

Magzoub, et al. (1988) studied the degree of pasture infestation with *Trichostrongylus* larvae of camels in Eastern Sudan. Samples of vegetation were examined monthly throughout a period of one year. The study revealed the presence of *Trichostrongylus* spp. and *Haemonchus* spp. Larvae. High numbers of *Trichostrongylus* spp. Larvae were observed during the rainy reason and started to decline towards winter, till they disappeared from herbage during the following summer. On the other hand, *Haemonchus* spp. Larvae was detected only during the rainy season.

Burger, et al. (1988) examined 429 camels in Butane area (central Sudan) by means of fecal and autopsy examination. They encountered 7 species of gastrointestinal nematodes. These were *Haemonchus longistipes*, *Trichostrongylus* probolurus, *Trichostrongylus* spp. *Cooperia pectinata*, *Impalaia tuberculata*, *Oesophagostomum columbiaum* and *Trichuris globulosa*. The most prevalence species was *Trichostrongylus* spp. Positive correlation between rainfall and egg counts was established. The highest incidence of infection however was detected in the rainy season.
Saad and Magzoub (1989) carried out survey studies of hydatidosis in camels and cattle in the Sudan. They stated that out of 1169 cattle and 191 camels examined, 45(3.84%) and 93(48.69%) respectively were found to harbour hydatid cysts. Awad, et al. (1990) reported the occurrence of aortic onchocercosis due to *onchoerca armillata* in 45 (41%) out of 109 Sudanese camels. El-Hussein, et al. (1991) examined 74 camels in Eldamer province, northern state of the Sudan. Their study showed that 37% of camels harboured hydatid cyst.

Fadl, et al. (1992) investigated the gastrointestinal nematodes of camels in Butana collected faecal samples female dromedaries of 3-12 years of age at Tamboul market over a 12-month period. Their study indicated that fecal egg count increased during the raining season (May-October with a peak in July), with the increase being mainly due to *Haemonchus* and *Impalaia* egg. There was a high prevalence of *Trichostrongylus spp.* throughout the dry and raining season.

Siddig and El-Hussein (1997) examined post-mortemly 41 carcasses of camels slaughtered at Atbara abattoir, beside that they collected fecal sample from 33 alive animal kept for slaughter. Their study stated that 12 of 33 faecal samples (36.4%) were positive for helminth parasite, *Monezia expansa* was the commonest parasite (23 camels) followed by *Haemonchus longistipes* (16 camels), *Strongyloides papillosus* (9), *Avitellina* spp. (7) and *Stilesia* spp.(3). On the other hand,
the result should that 34.3% harboured single infection, 51.4% double infection and 14.3% triple infection.
CHAPTER TWO
MATERIALS AND METHODS

2.1. Area of Study (Map 1):

This study was conducted in Tamboul town in state of Gazira Region. It lies 150 Km south of Khartoum. Tamboul is a large and it is an important camel market in Sudan.

2.2. Experimental Animals:

Six hundred randomly selected camels (*Camelus dromedarius*) from Tamboul livestock market 165 males (27.5%) and 435 females (72.5%) were examined for infection with *T. evansi* using the conventional parasitological methods. They include 174 camels from Kassala, 97 from Gedarif, 254 from west and middle of Butana and 75 from western Sudan. Two hundred camels examined for gastrointestinal parasites at same time using fecal sample examination methods. These camels were brought to Tamboul market for slaughter.
Fig (1): Map of the study area (Butana area – Taboul area)

Source: Government of Gezira State
2.3. Collection of blood samples:

Blood was collected from jugular from each camels into heparinized vacationer tube (7ml) and plain (non-heparinized tubes). The tubes were labelled with (sex, age, location etc) and were kept in ice box. They were then transferred to Tamboul Camel Research Center laboratory for testing. Heparinized blood was utilized for microscopic examination and non-heparinized blood was allowed to clot for serum preparation. Collected sera were kept in deep freezer (– 22 ° C) for the card agglutination test (CATT).

2.4. Diagnosis of Trypanosomosis:

2.4.1. Wet blood smears:

A drop of heparinized blood was placed on a clean slide, covered with a cover slip (18x18mm) and examined under X40 objective (Kendrick, 1968).

2.4.2. Stained thin blood films:

It was made by placing a drop of blood on a clean slide, spread out, dried, fixed with methanol and stained with 10% Giemsa for 30 minutes. After staining, the slide was washed gently under tap water, allowed to dry and examined under X100 oil-immersion objective lens.
2.4.3. **Thick blood film:**

It was made by placing a drop of blood on a clean slide and spread out into area of approximately 2cm diameter, it was then dried, fixed, stained with 10% Giemsa stain and examined as blood film.

2.4.4. **Hematocrit centrifugation technique (HCT):**

A heparinized capillary tube (75 x 1.5 mm), was filled to \( \frac{2}{3} \) it size sealed from side using crestseal, and centrifuged in a microhaematocrit centrifuge (Haematokrit 210, GmbH & Co, Germany) for 4 minutes at 12,000 rpm/minute, After centrifugation the capillary tube was placed on slide and the interphase between the buffy coat layer and the plasma was examined under a microscope using X10 objective lens. The capillary tube was rotated from time to time during the examination to ensure that all sides of the tube have been examined (Wernery, 2001).

2.4.5. **Buffy coat technique (BCT):**

This technique was made according to Murray (1977). After examination the capillary tube, the tube was cut 1mm below the buffy coat to include the upper most layers of red blood cells and 3mm above to include the plasma. The contents of the capillary tube were gently expressed onto a slide, mixed and covered with a cover slip (18x18mm). The preparation was then examined using ordinary microscope using X10 and X40 objective lens.
2.5. Card Agglutination Test (CATT):

The procedure:

Reagents and accessory materials were obtained from Belgium Institute of Tropical Medicine, Antwerp, Belgium. It comprise a complete test kit for 250 screening tests, containing the following: 26 plastic test cards, 3 stirring rods, 1 syringe (2.5 ml), 8 droppers, 5 vials CATT-antigen, 1 vial positive control, 1 vial negative control and 1 vial CATT-buffer (30ml). The reagents were mixed as follows: 2.5 ml buffer was added to a vial of freeze dried antigen using sterile syringe. The vial was then shaken for a few seconds so as to obtain a homogenous suspension. Similarly, 0.5 ml buffer was added to each of the vials of the negative and positive controls using sterile syringe. On a test area of the card, 25 µl of non diluted serum was added to well containing homogenized CATT antigen by special dropper approximately 40-45 µl mixed and spreaded out the to about 1mm from the edge of test area by using stirring rod. The test card was rotated on a flat bed orbital rotator for 5 minutes at 70 rpm, after which agglutination was observed and the degree of the agglutination (Fig 3) was determined as follows:

Very strong agglutination (++++)
Strong agglutination (++)
Moderate agglutination (+)
Weak agglutination (±)
Absence of agglutination

(−)

2.6. Diagnosis of gastrointestinal internal parasites:

2.6.1. Fecal sampling:

Two hundred fecal samples from individual males and females camels, intended for parasitological examination were collected directly from rectum of randomly selected camels during defecation. The fresh feces were placed in clean plastic bags, closed, labeled, kept in refrigerator (4°C) until they processed in the laboratory of Tamboul Camels Research Center (TCRC).

2.6.2. Fecal samples examination:

The fecal samples collected from individual camels were examined macroscopically and microscopically.

2.6.2.1. Macroscopic examination:

A preliminary or an oriented diagnosis was made to check the presence of adult worms or segments of tapeworms in fecal samples (Charles and Robinson, 2006).

2.6.2.2. Microscopic examination:

2.6.2.2.1. Direct smear method:

This method was made by making a thin smear on a slide by taking a small amount of feces, place it on a clean slide, mix it with a
drop of water, cover it with cover slip (22X22mm) and examined under 10X, 40 X objective lens (Charles and Robinson, 2006).

2.6.2.2.2. Floatation Method:

This method was made by Willis technique (Soulsby, 1986). About 1.0 ml of mixed specimen was diluted with a concentrated salt solution (Sodium chloride for nematode eggs) in a tube, which was filled to top with the salt solution. A cover glass was placed on top of the mouth of the tube that it comes in contact with the solution. After 30 minutes, the cover glass was quickly removed, put on a clean side and examined under a low power microscope.

2.6.2.2.3. Sedimentation Method:

For high recovery of trematode eggs, the following sedimentation technique was performed:

Five to ten grams of feces were mixed with sufficient volume of normal saline. After through disintegration, the suspension was passed through a tea strainer into a beaker and then into a conical flask. To the filtrate more normal saline was added until it filled the flask. The suspension was allowed to sediment and clarifies the fecal mass. Most of supernatant materials were decanted carefully and the flask containing the sediment was refilled with normal saline. The process was repeated several times until the supernatant become clear. After discharging the supernatant fluid, and by using a pipette, few drops of the sediment were
transferred to a clean glass slide, covered with cover slip (22x22mm) and examined under microscope by using 10X, 40X objective lens (Soulsby, 1986).

### 2.6.2.2.4. Fecal culture:

Fecal culture was used to differentiate Strongylid and Trichostrongylidae eggs as the third stage larvae (L3). The technique applied in the study was according to the method of Charles and Robinson, (2006) which was carried out as follows: Twenty grams of feces were broken up sometimes a comparatively large quantity of feces was used to secure sufficient larvae from feces registering low egg counts. Fairly fine sawdust was added to the wet samples so as to reduce the moisture and was mixed well. The mixture was wrapped in a piece of gauze and then suspended in a clear-glass, wide mouthed, screw-capped jar containing a small amount of water to provide the media with moisture. The culture was kept at room temperature for about 10-12 days. Then the culture jars were completely filled with water covering the suspended feces. They were left to stay overnight, allowing the larvae to migrate down the wrapped feces and settle on the bottom of the jar. On the next day the suspension in the jar was decanted and a small amount was left for 1-2 hours for the larvae to settle on the bottom. The suspension on the top was decanted and the sediment in all test tubes was pooled in one test tube, labeled and store at 4 °C until
examined. One hundred larvae from each individual culture were identified to the generic level according to Soulsby (1986).

2.7. Statistical method:

The statistical programme used in analysis data of this study was Statistic Package for Social Science (SPSS). The data which collected computerized individually in hard disc and analyzed with Chi-square, cross tabulation and correlation.
CHAPTER THREE
RESULTS

3.1. Examination of blood samples Results:

*T. evansi* infections were diagnosed by examination of 600 camels using wet and stained smear and MHCT (buffy coat) techniques. Table (1) showed that 5 (0.83%), 16 (2.66%) and 21 (3.5%) camels were infected by *T. evansi*. Out of 600 samples 404 tested for camel trypanosomosis by card agglutination trypanosomosis test (CATT) using undiluted serum agglutination. This showed that, 143 (35.3%) camels were positive. The observed degree of agglutination 41(++)ve, 56 (+ve), 46 (+ve) correlation to 32.2%, 39.2% and 28.6%, respectively (Table 2). Chi-square analyses showed that infection rates differed significantly with the diagnostic technique used (Chi-square = 339.10, d.f. = 3, P < 0.00000). The comparison between the stained smears and buffy coat showed high difference (Chi-square = 537.71, d.f. = 1, P < 0.00000), (Chi-square = 518.94, d.f. = 1, P < 0.00000), respectively.

3.2. Effect of sex, location and season on infection of camels:

Examination revealed that an infection rate was significantly higher females by all diagnostic methods (Chi-square = 121, d.f.=1, P<0.00000). (Table 3).
Using CATT, the infection rates of *T. evansi* was higher in animals from East and West of Butana 59(41.2%) followed by Kassala 48(33.6%), Gedarif 19(13.3%) and the last western Sudan 17(11.9%), Chi-square (132, d.f=3, P> 0.0000), (Table 4).

The effect of season on infection rate due *T. evansi* in male and female camels is shown in (Table 5), (Chi-square =58, d.f. =3, P>0.0000). The highest infection rates were obtained during the rainy season by using wet and dry smear and MHCT.

3.3. **Mean Packed Cell Volume (PCV%)**:

The mean PCV of non-infected camels was 41% while the mean PCV of infected camels was 27.1%, 28.4%, 28.0% and 36.2% examined with wet and stained smears, buffy coat and CATT, respectively. The analysis of Chi-square indicates clear relationship between camel trypanosomosis and low PCV% (d.f. = 1, P> 0.0000., (Table 6).

Out of 404 samples examined only 16 were positive by stained smears, whole 388 negative. A total of 261 samples were negative by both CATT and stained smears (Table 7).

Table (8) showes that out of 600 samples examined only 21 were found positive by (HCT), and 579 negative, whole 573 negative by both HCTand stained smears.
Table (1): The number and percentage of infected camels with *Trypanosoma evansi* using four diagnostic methods:

<table>
<thead>
<tr>
<th>Method</th>
<th>No. examined</th>
<th>No. infected</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet smear</td>
<td>600</td>
<td>5</td>
<td>0.83%</td>
</tr>
<tr>
<td>Stained smear</td>
<td>600</td>
<td>16</td>
<td>2.66%</td>
</tr>
<tr>
<td>Buffy coat</td>
<td>600</td>
<td>21</td>
<td>3.5%</td>
</tr>
<tr>
<td>CATT</td>
<td>404</td>
<td>143</td>
<td>35.3%</td>
</tr>
</tbody>
</table>

Table (2): The degree of intensity of agglutination of positive samples in Card Agglutination Trypanosonosis Test (N= 404 samples).

<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>46</td>
<td>56</td>
<td>41</td>
</tr>
<tr>
<td>Percentage</td>
<td>32.16%</td>
<td>39.16%</td>
<td>28.7%</td>
</tr>
</tbody>
</table>

Table (3): Prevalence (%) of *Trypanosoma evansi* in male and female camels in Tamboul market by using three diagnostic methods:

<table>
<thead>
<tr>
<th>Sex</th>
<th>CATT</th>
<th>Buffy coat</th>
<th>Stained smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>39(29.3%)</td>
<td>7(33.3%)</td>
<td>4(25%)</td>
</tr>
<tr>
<td>Female</td>
<td>104(72.7%)</td>
<td>14(66.7%)</td>
<td>12(75%)</td>
</tr>
<tr>
<td>Total</td>
<td>143(100%)</td>
<td>21(100%)</td>
<td>16(100%)</td>
</tr>
</tbody>
</table>
Table (4): Prevalence of *Trypanosoma evansi* in camels coming from different localities using CATT test:

<table>
<thead>
<tr>
<th>Test Location</th>
<th>No. of examined</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. E. Butana</td>
<td>143</td>
<td>19</td>
<td>29</td>
<td>11</td>
<td>59(41.2)</td>
</tr>
<tr>
<td>Kassala</td>
<td>143</td>
<td>13</td>
<td>17</td>
<td>18</td>
<td>49(33.6)</td>
</tr>
<tr>
<td>Gedarif</td>
<td>143</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>19(13.3)</td>
</tr>
<tr>
<td>West of Sudan</td>
<td>143</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>17(11.9)</td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>46</td>
<td>56</td>
<td>41</td>
<td>143(100)</td>
</tr>
</tbody>
</table>

Table (5): Prevalence of camel's trypanosomosis in rainy and dry season in Tamboul area using three diagnostic methods.

<table>
<thead>
<tr>
<th>Season</th>
<th>No. of exam.</th>
<th>Stained</th>
<th>B.C</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>206</td>
<td>7(43.75%)</td>
<td>4(19%)</td>
<td>37(22.4%)</td>
<td>169(38.9%)</td>
</tr>
<tr>
<td>Rainy</td>
<td>394</td>
<td>9(56.25%)</td>
<td>17(81%)</td>
<td>128(77.6%)</td>
<td>266(61.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>16(100%)</td>
<td>21(100%)</td>
<td>165(100%)</td>
<td>435(100%)</td>
</tr>
</tbody>
</table>
Table (6): Mean PCV% of camels infected with *Trypanosoma evansi* and uninfected by using four diagnostic methods:

<table>
<thead>
<tr>
<th></th>
<th>No. of infected camels</th>
<th>Non-infected PCV</th>
<th>Infected PCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet smears</td>
<td>5</td>
<td>41%</td>
<td>27.1%</td>
</tr>
<tr>
<td>Stained smears</td>
<td>16</td>
<td>41%</td>
<td>28.4%</td>
</tr>
<tr>
<td>Buffy coat</td>
<td>21</td>
<td>41%</td>
<td>28.0%</td>
</tr>
<tr>
<td>CATT</td>
<td>134</td>
<td>41%</td>
<td>36.2%</td>
</tr>
</tbody>
</table>

Table (7): Cross tabulation between results obtained from Card Agglutination TrypanosomosisT test and stained smear:

<table>
<thead>
<tr>
<th>CATT</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>_</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stained smear</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ve</td>
<td>3</td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>-ve</td>
<td>43</td>
<td>48</td>
<td>36</td>
<td>261</td>
<td>388</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>56</td>
<td>41</td>
<td>261</td>
<td>404</td>
</tr>
</tbody>
</table>

Table (8): Cross tabulation between results obtained from stained smear and buffy coat technique:

<table>
<thead>
<tr>
<th>Stained smear</th>
<th>+ve</th>
<th>-ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffy coat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ve</td>
<td>10</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>-ve</td>
<td>6</td>
<td>573</td>
<td>579</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>583</td>
<td>600</td>
</tr>
</tbody>
</table>
3.4. Fecal examination results:

Out of two hundred fecal samples collected from camels of different breeds and ages, 182 camels (91%) were infected with at least one gastrointestinal parasite.

3.4.1. Gross examination of fecal samples:

Out of 200 camels, 44 camels (22%) were infected with *Avitellina* spp., *Monezia* spp. and *Trichuris* spp. with prevalence rate 8%, 9% and 5%, respectively. (Table 9).

3.4.2. Direct smear:

Using direct smear method 72 camels (36%) out of total 200 camels were found to infected. The prevalence rates were 16%, 5.5%, 5.5%, 5%, 2% and 2% for *Trichostrongylus* spp., *Strongyloides papillosus*, *Strongylus* spp., *Eimeria* spp. *Trichuris* spp and *Fasciola gigantica*, respectively. (Table 10).

3.4.3. Flotation method:

Out of total 200 camels, 182 (91%) were infected with at least of these worms: *Trichostrongylus* spp., *Strongyloides* spp., *Monezia* spp., *Eimeria* spp., *Trichuris* spp., and *Strongylus* spp. with prevalence rate 39%, 13.5%, 17.5%, 9.5%, 6% and 5.5%, respectively. (Table 11).
3.4.4. Sedimentation method:

Out of total 200 camels examined, 52(26%) were infected with one of these parasites: *Fasciola gigantica*, *Paramphistomum* spp. and *Monezia* spp. with prevalence rate 10.5%, 6% and 6.5%, respectively, and 6 camels (3%) were found to be infected by mixed infection with *Fasciola gigantica* and *Monezia* spp. (Table 12).

3.4.5. Fecal culture:

In vitro cultivating of eggs revealed that 100 camels (50%) were infected with one parasite including *Trichostrongylylus* spp., *Oesophagostomum columbianum*, *H. longistipes* and *Nematodirus* spp. with prevalence rate 7%, 5%, 29% and 9%. Also 21(10.5%) camels were found to harbour double infections such as *H. longistipes* and *Oesophagostomum columbianum*, (4%) and *H. longistipes* and *Nematodirus* spp. (6.5%), (Table 13).

3.5. Single and mixed infection of gastrointestinal parasites:

Table (14) showed that the single infection rate lower than mixed infection rate using sedimentation and fecal culture method, 21(17.4%) and 100(50%) respectively.

3.6. The effect of sex on gastrointestinal parasites infection rate:

The infection rate of gastrointestinal parasites was found to be higher in females and low in male using flotation method, 84% and 16% respectively. Table (15).
3.7. Effect of season on gastrointestinal parasites infection:

Using conventional parasitological methods indicated that the infection rate of gastrointestinal nematodes was highly in rainy season (early weeks of autumn) and low in dry season with prevalence rate (88.0%), 65(65%), respectively. Table (16).

3.8. Concurrent infection of *T. evansi* and gastro-intestinal parasites:

Examination of two hundred camels 7(3.5%) camels infected as concurrent mixed infection with *T. evansi* and gastro-intestinal parasites, (Table 17).
Table (9) Camels infected with gastrointestinal parasites using gross examination technique in Tamboul area:

<table>
<thead>
<tr>
<th>parasite</th>
<th>No. of examined</th>
<th>Frequency</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Avitellina</em> spp.</td>
<td>200</td>
<td>16</td>
<td>8%</td>
</tr>
<tr>
<td><em>Monezia</em> spp.</td>
<td>200</td>
<td>18</td>
<td>9%</td>
</tr>
<tr>
<td><em>Trichuris</em> spp.</td>
<td>200</td>
<td>10</td>
<td>5%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>44</td>
<td>22%</td>
</tr>
</tbody>
</table>

Table (10): Camels infected with gastrointestinal parasites using direct smear technique in Tamboul area:

<table>
<thead>
<tr>
<th>parasite</th>
<th>No. of examined</th>
<th>Frequency</th>
<th>Percent%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichostrongylus</em> spp.</td>
<td>200</td>
<td>32</td>
<td>16%</td>
</tr>
<tr>
<td><em>Strongyloides</em> spp.</td>
<td>200</td>
<td>11</td>
<td>5.5%</td>
</tr>
<tr>
<td><em>Strongylus</em> spp.</td>
<td>200</td>
<td>11</td>
<td>5.5%</td>
</tr>
<tr>
<td><em>Eimeria</em> spp.</td>
<td>200</td>
<td>10</td>
<td>5%</td>
</tr>
<tr>
<td><em>Trichuris</em> spp.</td>
<td>200</td>
<td>4</td>
<td>2%</td>
</tr>
<tr>
<td><em>Fasciola</em> spp.</td>
<td>200</td>
<td>4</td>
<td>2%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>72</td>
<td>36%</td>
</tr>
</tbody>
</table>
Table (11): Camels infected with gastrointestinal parasites using floatation method in Tamboul area:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. of examined</th>
<th>frequency</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichostrongylus</em> spp</td>
<td>200</td>
<td>78</td>
<td>39%</td>
</tr>
<tr>
<td><em>Strongyloides</em> spp.</td>
<td>200</td>
<td>27</td>
<td>13.5%</td>
</tr>
<tr>
<td><em>Eimeria</em> spp.</td>
<td>200</td>
<td>35</td>
<td>17.5%</td>
</tr>
<tr>
<td><em>Trichuris</em> spp.</td>
<td>200</td>
<td>12</td>
<td>6%</td>
</tr>
<tr>
<td><em>Monezia</em> spp.</td>
<td>200</td>
<td>19</td>
<td>9.5%</td>
</tr>
<tr>
<td><em>Stongylus</em> spp</td>
<td>200</td>
<td>11</td>
<td>5.5%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>182</td>
<td>91%</td>
</tr>
</tbody>
</table>

Table (12): Camels infected with gastrointestinal parasites using sedimentation method in Tamboul area:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. of examined</th>
<th>frequency</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fasciola</em> spp.</td>
<td>200</td>
<td>21</td>
<td>10.5%</td>
</tr>
<tr>
<td><em>Paramphistomum</em> spp.</td>
<td>200</td>
<td>12</td>
<td>6%</td>
</tr>
<tr>
<td><em>Monezia</em> spp.</td>
<td>200</td>
<td>13</td>
<td>6.5%</td>
</tr>
<tr>
<td><em>Monezia</em> spp + <em>Fasciola</em> spp.</td>
<td>200</td>
<td>6</td>
<td>3%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>52</td>
<td>26%</td>
</tr>
</tbody>
</table>
Table (13): Camels infected with gastrointestinal parasites using fecal culture technique in Tamboul area:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. of exam.</th>
<th>Freq.</th>
<th>Perce%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichostrongylus spp.</td>
<td>200</td>
<td>14</td>
<td>7%</td>
</tr>
<tr>
<td>Oesophgastomum spp.</td>
<td>200</td>
<td>10</td>
<td>5%</td>
</tr>
<tr>
<td>H. longistipes</td>
<td>200</td>
<td>58</td>
<td>29%</td>
</tr>
<tr>
<td>Nematodirus spp.</td>
<td>200</td>
<td>18</td>
<td>9%</td>
</tr>
<tr>
<td>H. longistipes + Oesophgastomum</td>
<td>200</td>
<td>8</td>
<td>4%</td>
</tr>
<tr>
<td>H. longistipes + Nematodirus spp.</td>
<td>200</td>
<td>13</td>
<td>6.5%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>121</td>
<td>60.5%</td>
</tr>
</tbody>
</table>

Table (14): The mixed and single nematode infection using three diagnostic methods in camels in Tamboul area:

<table>
<thead>
<tr>
<th></th>
<th>No. of infected Flotation method</th>
<th>No. of infected Sedimentation method</th>
<th>No. of infected Fecal culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed</td>
<td>0</td>
<td>6(11.5%)</td>
<td>21(17.4%)</td>
</tr>
<tr>
<td>Single</td>
<td>182</td>
<td>46(88.5%)</td>
<td>100(82.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>182</td>
<td>52(100%)</td>
<td>121(100%)</td>
</tr>
</tbody>
</table>
Table (15): The effect of sex on gastrointestinal nematode infection by three diagnostic methods:

<table>
<thead>
<tr>
<th></th>
<th>Flotation method</th>
<th>Sedimentation method</th>
<th>Fecal culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Female</td>
<td>153</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>Total</td>
<td>182</td>
<td>18</td>
<td>52</td>
</tr>
</tbody>
</table>

Table (16): The effect of the season of on gastrointestinal parasites:

<table>
<thead>
<tr>
<th></th>
<th>No of examined camels</th>
<th>No. of infected camels and %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainy season</td>
<td>100</td>
<td>88(88%)</td>
</tr>
<tr>
<td>Dry season</td>
<td>100</td>
<td>65(65%)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100(100%)</td>
</tr>
</tbody>
</table>

Table (17): Concurrent infection of camels with *T. evansi* and gastrointestinal parasites:

<table>
<thead>
<tr>
<th></th>
<th>No. of examined</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. evansi</em></td>
<td>200</td>
<td>7(3.5%)</td>
<td>197(96.5%)</td>
</tr>
<tr>
<td>GIT parasites</td>
<td>200</td>
<td>182(91%)</td>
<td>18(9%)</td>
</tr>
</tbody>
</table>
CHAPTER FOUR
DISCUSSION

Camels are known to harbour many endo and ecto parasites. In Sudan, little information on the occurrence of gastrointestinal helminthes in camels are available. Such information is derived from Annual reports of the Ministry of Animal Resources, Stewerd (1950), Malik (1959), Eisa, et al. (1979), Arzoun, et al. (1984), Fadl, et al. (1992) and Agab and Abbas (1992). Fadl (1987) stated that the sudanese camels are seriously affected by three major debilitating diseases, namely mange, haemonchosis and trypanosomosis, and that gastrointestinal parasites of camels other than haemonchosis, were not fully investigated in the Sudan.

The results obtained in this study using wet and stained smears 0.83% and 2.66%, respectively, were similar to those obtained by Dafalla (1988) who did similar work in Gedarif, Kassala and New Halfa. He also found that the infection rates in these localities range to range between 1.12% and 2.13%. This rate is lower than that reported by Elzakiy (2005) who surveyed camel trypanosomosis using the same methods in three localities in Butana area; Alsubag, Gabt Alfeil and Almagatta, and found high infection rate (14.1%). The high prevalence reported by Elzakiy (2005) may be attributed to increasing numbers of biting flies in those localities. Another possible explanation is that camels encroach into
Ethiopian peninsula and thus become exposed to high challenge of biting flies such as *Tabanids* and *Stomoxinae*.

Examination of camels in Tamboul area with HCT in this study revealed an infection rate of 3.5%. This rate is slightly higher than that obtained by wet and dry smear methods. This indicates that the HCT is slightly more sensitive in diagnosing camel trypanosomosis when compared to the wet and stained methods. The present results are also in agreement with Elamin, *et al.* (1990) who used wet and dry smear and HCT in Butana area and found that HCT was at least two times sensitive in diagnosing camel trypanosomosis in blood films. In addition to its sensitivity HCT can also be used as indicator of anemia. These merits coupled with the fact that it can be applied in the field make this test highly recommended for epidemiological surveys and investigations.

Serological examination of camels by CATT carried in the present study revealed that 143 out 404 (35.1%) camels were infected. This clearly indicates a highly infection rate. Similar results were reported by Elzakiy (2005) in sudanese camels that probably might have used this technique for the first time in the Sudan. That means that the card agglutination trypanosomosis test (CATT) is an effective method in detection of antibodies against camel trypanosomosis due to *T. evansi*. The infection rate was higher during the rainy season and low during the dry season, 61.1% and 38.9%, respectively in both sexes. The high
infection rates of *T. evansi* reported currently in the study area can be attributed to increasing numbers of biting flies especially in the rainy season. Movement of camel herds south of Gedarif in the dry season expose them to a high concentration of *Tabanids* and *Stomoxinae*. In addition, resistance to antitrypanosomal drugs may be attributing factor in increased prevalence. In this and that study, CATT showed positive results in parasitaemic and a parasitaemic camels. In other wards, the test determines circulating antibodies irrespective of camels being cutely and chronicly infected. Other serological were used for detection tests for *T. evansi* infections. Suratex card latex agglutination antigen detection test was compared with other method for diagnosis of *T. evansi* in nine herds in Eastern Province and Rift Valley Province, Kenya. Of the examined camels 9.1%, 23.1% and 46.3% were positive by the HCT, mouse sub-inoculation and suratex respectively. Trypanosomal antigens were detected in 92% and out of 50 HCT positive camels and 88% out of 121 mouse inoculated positive camels (Olaho, *et al.*, 1996).

The present results revealed that the infection rate was high in females than males by all diagnostic methods. Similar results were reported by Najira (2003) who surveyed camel trypanosomosis in Kenya. This may be attributed to pregnancy and lactation stresses.

The PCV of infected camels for *T. evansi* was lower than that of non-infective camels. This result agrees with that reported by Elzakiy (2005)
and Njira (2003) who found significant relationship between infection and low PCV. This may be due to hemolytic factors produced by trypanosomes which lead to destruction of RBCs and anemia.

In this study 91% of the examined camels were found to be harbour gastrointestinal parasites. Fadl (1987) found similar findings indicated with point prevalence rate (PPR). In Sudan, infections may probably originate from grazing dry hay and wet grasses. As known, camels are browsers who used to consume leaves of *Accacia* and other trees. However, due to extensive erosion of forests in many areas in the country, camels become campelled to feed on ground. This makes the expose to eggs of helminth parasites possible. In this study several parasites were encountered. These include; *Trichostrongylus* spp., *Strongyloides papilosus*, *Trichuris globulosa*, *Monezia expansa*, *Strongylus* spp., *Fasciola gigantica* and *Monezia* spp.. Protozoal infections were exemplified by *Eimeria* spp.. The parasites were found in various prevalence rates.

*Trichostrongylus* spp. eggs were detected all throughout the study period in both seasons (rainy and dry) for camels examined in this area with high prevalence rate (91%). However, *Trichuris globulosa* eggs were detected just during the rainy season with low prevalence rate (3%). This may be due to fact that the eggs of this genus needs high humidity and low temperature than the eggs of the genus
Trichostrongylus. Monezia spp. and Strongyloides papilosus eggs were found through the study period with prevalence rate of 6.5% and 17.5%, respectively. Eimeria spp. oocysts were detected during the rainy season with high prevalence rate (17.5%). The low prevalence rate in the dry season may be due to the fact that the oocysts were destroyed during the hot dry season thereby the difficulty to diagnose them. Oocysts are easy to diagnose in rainy season due to suitable temperature and moisture. The results revealed that the rate of single parasitic infection in camels was higher than mixed infection. Similar results obtained by Inas (2003). Multiple infections may be due to stress by first parasitic infection leading to second parasitic infection. The present results are not in agreement with Abu baker, et al. (2002) who found higher mixed infection than the single infection.

In the present results revealed that the infection with gastrointestinal parasites was higher in females than male. These results were in agreement with those reported by Bekel (2002). Who attributed it to pregnancy and lactation stress. However this result disagreed with that of Sena, et al. (2000) who reported that the male camels showed higher prevalence of infection compared to females. As there is no clue that males develop more resistance to some gastro-intestinal parasites than female camels, the high prevalence may thus be due to the large numbers of females in the herds. The current results showed that the
gastrointestinal parasites infection rates were higher in the rainy season than the dry season (88%), (65%). Such high incidence of camel nematodes infection during the rainy season was also reported by Agab and Abbas (1992) and may be attributed to the fact that the environmental conditions for pasture contamination with the nematodes eggs were optimal in this reason.
Conclusions and recommendations

1. Antibody detection test (CATT) are useful for screening purposes and it is not distinguish between past and present infections.

2. The current reliability of antigen detection test is limited, those reasons required specific and sensitive molecular methods for diagnosing of camel trypanosomosis such as PCR and LAMP and considered for researches.

3. Using of blood films in diagnosing camel trypanosomosis is less efficient in revealing parasites compared to (HCT) in the fields.

4. Worms burden in camels were found to be very abundant. This warrants through look into the transmission dynamics and control strategies.
REFERENCES


**Hoare, C. A. (1972).** The Trypanosomes of mammals, Black well scientific publication, Oxford.


