Sudan University of Science and Technology

College of Graduate Studies

Prevalence and Risk factors of *Trypanosoma evansi* infections in Slaughtered Camels (*Camelus dromedarius*) in Tamboul Slaughterhouse, Sudan

نسبة الاصابة وعوامل الخطر لعدوى مثقبية ايفانساي في الابل المذبوحة في مسلخ تمبول بالسودان

A Thesis Submitted to the College of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Preventive Veterinary Medicine (MPVM)

By

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بسم الله الرحمن الرحيم

قال الله تعالى:

(أَفَلَا يَنظُرُونَ إِلَى ٱلْإِبِلِ كَيْفَ خُلِقَتْ)

صدق الله العظيم

الغَاشِيَة الاية (17).

DEDICATION

To my father & my mother

To my brothers & my sisters

To all my friends & colleagues

ACKNOWLEDGMENTS

Firstly, a praise and thanks to Almighty Allah, for giving me the health and strength to complete this study. I would like to express my deepest gratitude and sincere appreciation to my supervisor **Dr. Sara Basher Taha** for her advices, directions and continuous interest.

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ABSTRACT

This study was conducted between November to December 2016 in order to determine the prevalence of camel trypanosomosis (Guffar, or Surra) which caused by haemoprotozan parasite *Trypanosoma evansi* (*T. evansi*) and also to determine the risk factor may associate with the disease in Tamboul Slaughterhouse, East of Gazira state from. Blood samples were collected randomly from 165 camels using heparinized, tubes. These samples were examined for the presence of *T. evansi* using Giemsa-stained smears and Haematocrit Centrifugation Technique (HCT) and the infection rates were 7 out of 165 (4.2%) and 8 out of 165 (4.8%) respectively. These results indicated that the (HCT) was more specificity compared with Giemsa-stained smears to diagnose camel trypanosomosis. Only one risk factor, body condition, was statistical significant (p-value < 0.25) associated with trypanosomosis infection in camel. However, the different in the prevalence rate of camel trypanosomosis based on the sources of animals, breed, sex and age where the higher rate was reported in animal from Butana and West Sudan (5.1% for both), Butana breed and Kassala breed (5.1% for both), female and older animal, respectively.

ملخص الاطروحة

في هذه الدراسة تم مسح لطفيل الدم مثقبية ايفانساي المسبب لمرض التريبانوسوما في الابل (الجفار او سيرا) لتحديد نسبة الاصابه لمرض التريبانوسوما في الابل والعوامل المساعده على حدوث المرض في الابل المذبوحة في مسلخ تمبول بشرق ولاية الجزيره في الفتره من نوفمبر الى ديسمبر 2016. تم جمع 165عينة دم اختيرت عشوائيا عن طريق الوريد الوداجي في انابيب اختبار، تم فحص هذه العينات للكشف عن وجود مثقبية ايفانساي باستخدام المسحه الرقيقه بصبغة جمسا وطريقة التركيز بالطرد المركزي (البوفي كوت) وكان معدل الاصابة كما يلي 7 (4.2%) و8 (4.8%)على التوالي. اثبتت هذه الدراسه ان طريقة التركيز بالطرد المركزي (البوفي كوت) ذات خصوصيه اكثر في تشخيص المثقبيات مقارنة بمسحات الدم. عامل خطر واحد فقط ، حالة الجسم ، كان ذو دلالة احصائية (قيمة $P < 0.25$) المرتبطة بعدوى المثقبيات في الابل. ومع ذلك، هنالك اختلافات في معدل انتشار داء مثقبيات الابل استنادا الى مصادر الحيوانات، السلالة، الجنس والعمر حيث سجلت أعلى نسبة في الحيوانات من البطانة وغرب السودان (5.1% لكليهما)، سلالة البطانة وسلالة كسلا (5.1% لكليهما)، الإناث والحيوانات الأكبر سنا على التوالي.

INTRODUCTION

The importance of camels (*Camelus dromedarius)* is coming from the fact that it is sustainable agricultural resources for millions of people in the arid and semi-arid zones. Camels also provide milk, meat, wool and are used for water traction and the bear of burden. Moreover, the exportation of camels contributes to foreign currency earnings (Abd-Elmajid., 2000).

Sudan is considered as the second largest country in camel population in the world. The Camel population was estimated at three millions. They are distributed in Northern Kordofan and Darfur in the west and the Red sea, Kassala and Butana in the East (Abd-Elmajid., 2000).

Camel Trypanosomosis is a disease caused by *Trypanosoma evansi (T. evansi)*, which was discovered at the first time by Griffith Evans in 1881 in infected camels and equids in the Dara Ismail Khan District of Punjab (Indrakhmang, 1998). Although, the parasite can infect all the species of domesticated livestock and the principle host varies geographically (Al-Rawashdeh *et al.*, 2000).

In Sudan, *T.evansi* is considered as a serious and economical important infection in camels. Moreover, infection with *T. evansi* leads to high mortality and morbidity rate in camel's population. *T. evansi* is transmitted mechanically by biting flies such as *Tabanus spp and Stomoxys spp*, which distributed widely. Although, the diagnosis of Trypanosomosis is largely depends on the direct microscopic examination, some serological tests have been introduced recently. The control of Trypanosomosis can be performed either by using chemotherapy or control of vector (Abd-Elmajid., 2000).

The control of the infectious agents becomes necessary to improve camel health and their productivity. This aim cannot be achieved unless investigations on the epidemiology of the diseases are made.

Objectives:

The main objectives of this study are:

1. To determine the current situation of *Trypanosoma evansi* in Sudanese camel in Tamboul slaughterhouse at Gezira state, Sudan.
2. To investigate the potential risk factors such as (age, sex, breed and body condition), which may associated with *T. evansi* infection in the study area (Tamboul slaughterhouse).

CHAPTER ONE

LITERATURE REVEIWS

1.1. Etiology:

Trypanosoma evansi belongs to the subgenus Trypanozoon and it is the causative agent of camel trypanosomosis. It is hypothesized that *T. evansi* originated from *Trypanosoma brucei* by adaptation to a non cyclical transmission and loss the ability to develop and differentiate in the tsetse fly vector (Luckins, 1998). Camels become infected following contact with biting insect and when these camels moved to non-tsetse areas. Other species of trypanosoma, such as *Trypanosoma congolense, Trypanosoma brucei* and *Trypanosoma vivax* have been also isolated from camels in Sudan, but their effects in camel are insignificant (Mahmoud and Gray, 1980; Elamin *et al.,* 1999).

1.2. Classification of Trypanosoma:

Trypanosomes are classified according to Sousby (1982) as follows:

Kingdom: Protista

Phylum: Protozoa

Subphylum: Sarcomastigophora

Subclass: Mastigophora

Class: Zoomastigophora

Order: kinetoplastida

Suborder: trypansomidae

Family: trypanosomatidae

Genus: trypanosoma

Species: evansi

The principal pathogenic of trypanosomes causing animal Trypanosomosis in the Sudan are *T. congolense, T. vivax* and *T. brucei* which affected cattle, sheep, goats, horses and donkeys, while *T. evansi* affects mainly camels and lesser affects horses (Karib, 1961).

1.3. Morphology and biology:

T. evansi is morphologically identical with subgenus Trypanozoon, and indistinguishable from the other members of this subgenus. *T. evansi* can be distinguished from *T. brucei* by isoenzyme electrophoresis (Gibson *et al.,* 1983).

T. evansi enters the tissue or other body fluids and it may cross the blood -brain barrier (Leese, 1927). It can also enter the joint fluids, thus being less accessible to the chemotherapy. (Jennings *et al*., 1977).

1.4. Distribution:

Trypanosomosis is widespread in different parts of the world and considered as a major constraint for camel production (Elamin *et al.,* 1999). Generally, the geographical distribution of Trypanosomosis is related to the movements of infected animals (Gutierrez *et al.,* 2010). In Africa, the trypanosomosis is present in all countries, where camels are present, from the northern part of Africa through the Middle East to the South-East Asia. The disease exists in Mauritania, Morocco, Algeria, Tunisia, Libya, Egypt, Sudan, Eritrea, and Ethiopia, also in the northern parts of Mali, Burkina Faso, Niger, Nigeria, Chad, Somalia, and Kenya (Hoare, 1972).

Trypanosomosis is continuously present eastwards, in Saudi Arabia, Oman, the United Arab Emirates, Jordan, Israel, Lebanon, Syria, Iraq, Turkey, Iran, Kasakhstan, Afghanistan, Pakistan and Bulgaria (Srivastava *et al.,* 1984; Desquesnes *et al.,* 2003, 2009; Hasan *et al.,* 2006).

Trypanosomosis also exists in India, China, Mongolia, Russia, Nepal, Myanmar, Laos, Vietnam, Cambodia, Thailand, Malaysia, Philippines, and Indonesia (Luckins, 1988; Reid, 2002). The extension of Trypanosomosis toward the West is more recent (Hoare, 1965).

1.5. Hosts:

Although *T. evansi* also has a huge range of domestic and wild hosts worldwide (Lun and Desser, 1995). It is highly pathogenic in Camelids and Equids. *T. evansi* can infect camels, horses, donkeys, dogs, cattle, water buffaloes and elephants. Equines and dogs are very susceptible and usually die after an acute course of the disease (Leese, 1927). Cattle, sheep, goats and antelopes often carry the parasite sub-clinically and acting as asymptomatic reservoirs.

In Africa and the Middle East *T. evansi* is mainly a parasite of camels (*Camelus dromedarius*), and also it is pathogenic in other Camelidae, such as the *Bactrian camel* (*Camelus bactrianus*). Moreover, *T. evansi* is highly pathogenic in Equidae, especially in horses, in asses, in donkeys and also in their crossbreeds (mules). Additionally, *T. evansi* can infect cattle (Dia and Desquesnes, 2007), pigs, sheep, goats, (Reduth *et al.,* 1994), domestic cats (Tarello, 2005) and dogs, which may act as sentinel animals as observed in the surroundings of slaughter houses. To conclude, *T. evansi* in Africa is mainly a parasite of camels, which act as the main host.

In Asia *T. evansi* considered as an economically important disease, which concerns not only in horses and buffaloes, but also in cattle, pigs, and goats (Dargantes *et al*., 2009). Cattle in Asia are more receptive for the infection than in Africa or Latin America, and they can exhibit strong clinical signs (Tuntasuvan and Luckins, 1998). *T. evansi* has been also isolated from elephants in India (Stephen, 1986), and Thailand (Hin *et al.,* 2004).

1.6. Life cycle:

The trypanosoma is replicated by longitudinal binary fission in both host and vector, in which the flagellum and kinetoplast were dividing together (Liu *et al.,* 2005). Adding to that, the cyclical transmission was not observed at any stage of *T. evansi* in the mechanical vectors. Consequently, a procyclic or insect stages (epimastigotes) do not exist in *T. evansi,* which is attributed to lack of maxi circles in the kinetoplast DNA (Ellie *et al*., 1999).

1.7. Transmission:

T. evansi is transmitted in several ways, via biting insects, sucking insects, and vampire bats. The transmission can also be vertical, horizontal and per-oral, with various epidemiological significances, depending on the season, the location, and host species (Desquesnes *et al.*, 2013).

Mechanical Transmission: Mechanical transmission by biting insects is the most important mode of transmission of *T. evansi* in camels, as well as in livestock and other large animals (Gill, 1977). This transmission is a nonspecific process, which can take place when a biting insect initiates a blood meal on an infected host. This process can be interrupted by defensive movements of the host, and then the insect flies

off from the infected host, and landed on another animal to complete its blood meal (Foil *et al.*, 1987).

In biting insects, trypanosomes do not generally survive for a long time. For example, *T. vivax* can survive around 30 min in *Tabanids spp.* and even shorter in *Stomoxys spp*. (Ferenc *et al.*, 1988). An Experimental research shows that the transmission is efficient when the time between two interrupted blood meals, is less than 30 minutes (Sumba *et al.*, 1998; Mihok *et al.*, 1995). Additionally, *T. evansi* cannot survive longer inside Tabanids. Therefore, the probability of delayed transmission by *Tabanids* is very low (Desquesnes *et al.*, 2005).

Thought Mechanical transmission of *T. evansi* is to be essential due to *Tabanids* and *Stomoxys*. However, *Hippobosca equine* and *Hippoboca camelina* were suspected also to transmit *T. evansi* especially in camels and horses (Gill, 1977). Other insects, such as Culicidae, Ceratopogonidae may also have an important role in the transmission of *T. evansi* under special conditions. Furthermore, *T. evansi* has been experimentally transmitted with *Aedes aegypti, Aedes Argenteus,* and *Anopheles fuliginosus* (Gill, 1977).

Sucking flies can also transmit Trypanosomes via simple contamination of wounds, which may be the feeding site of these flies. The transmission of *T. brucei rhodesiense* through *Musca sorbens* has been documented (Lamborn, 1936). A similar observation was also documented for *T. evansi,* which can transmit with *Musca crassirostris* (Gill, 1977).

Other type of Transmission: Besides vector transmissions and the contamination of wounds, iatrogenic transmission caused by the use of non-sterile surgical instruments or needles especially during vaccination and mass treatments (Davila and Silva, 2000).Sexual transmission or transmission from dam to calf/ foal could occur in particular cases,

especially when mucosae are altered or in cases of very close contact (licking) with secretions (mucus, lacrymation, etc.). However, the real impact of horizontal transmission has not been estimated (Sina *et al.*, 1979; Gardiner and Mahmoud, 1990).

Transplacental infections have been described in *T. equiperdum* and *T. brucei* (Sina *et al.*, 1979; Gardiner and Mahmoud, 1990). Adding to that vertical transmission of *T. evansi* has also been demonstrated as shown in a review on transplacental transmission of trypanosomes (Ogwu and Nuru, 1981).

Trypanozoon, especially *T. evansi*,may be transmitted by pre-oral contamination. This mechanism could occur quite easily when the oral mucosae is damaged, especially when the carnivores eating fresh meat, blood, offal, or bones from animals infected with *Trypanosoma* (Bhaskararao *et al.*, 1995). Transmission by the vampire bat is a new biological system that has been established in Latin America (Hoare, 1972).

1.8. Epidemiology:

Trypanosomosis is often referred as African trypanosomosis. However, there are certain types of trypanosomes cause infection outside this continent. *T. evansi*, the causative agent of Surra, occurs not only in Africa, but also in Central and South America, Middle East, and in Asia.

Although Surra has a wide host spectrum, the main host species varies with the geographical region. In East Africa, camels are the most important host, whilst in Central and South America the horse is principally affected (Dia *et al.*, 1997). In Asia, a wider range of hosts is involved, including camel, cattle, buffalo, horses and pigs (Pacholek *et al.*, 2001). In contrast, there is a little evidence to suggest that domesticated livestock other than camels and horses in Africa and South America respectively, are

infected with *T. evansi* (El-Sawalhy and Seed, 1999).Generally, horse, donkeys, mules, camel, dogs, cats and Asian elephant are more susceptible than sheep, goat, bovines and pigs. Rats and mice are highly susceptible as experimental hosts (Reid and Husein, 2001).

The ability of *T. evansi* to be transmitted by bloodsucking insects allows *T.evansi* to extend its geographical range to the north of Sahara desert, Asia, Pakistan, India, the USSR, China, Sumatra, Java, the Philippines, Mauritius, Madagascar, and South and Central America. Moreover, through camel's exportation the disease was introduced into Australia, North America and South-West Africa. Introduction of the parasite into new areas is generally characterized by a high incidence rate where the mortality rate ranging between 30 to 100% (Elamin *et al.,* 1999).

1.9. Course of infection:

Not all animals get the infection with Trypanosomes showing clinical signs, some may recover without any signs. In camel, the period between initial infection with trypanosoma and the onset of the clinical signs is extremely variable, but generally ranges between 5 and 60 days. However, longer periods such as 3 months have been reported (Aha, 2005). Furthermore the interval between the infection and detection of parasites in the blood stream is usually less than 14 days (Aha, 2005).

A number of factors that can increase the severity of the disease such as initial infective dose, stress, pregnancy, lactation, infection with another parasite,especially helminthes, malnutrition and travelling for long distances (Getahun and Demeke, 1998). Surra affects camels of all ages with a higher incidence rate in young camels shortly after weaning (Evans *et al.,* 1995). Additionally, the severity of the disease depends also on the number of vectors that an animal experiences in a given time.

In camels the disease is manifested by elevation of body temperature, which is directly associated with parasitaemia. Infected animals show progressive anaemia, marked depression, dullness, loss of condition, and often rapid death. Anaemia was observed to be a major clinical finding in camel trypanosomosis (Rami *et al.*, 2003). Some camels develop oedema , urticaria plaques and petechial haemorrhages in serous membranes. Death finally ensues if untreated (Tuntasuvan *et al.*, 1997). Other signs are also reports are such as abortion (Lohr *et al.*, 1986), reduced draught power (Luckins, 1998) and nervous signs like circling movement and trembling, unusual aggressiveness, running aimless and sudden collapse in severely stressed and overworked animals (Manuel, 1998). At post mortem, necrotic foci in the liver and spleen as well as generalized lymphoid tissue hyperplasia are common in camels suffering from surra (Rottcher *et al.*, 1987).

1.10. Immunity:

An increase in gamma-globulin (IgM) during both acute and chronic *T.evansi* infections in camels has been reported (Boid *et al.*, 1981). However,these antibodies are not protective, as the majority of these antibodies are auto antibodies. (Anosa, 1988). Increasing in Leucocytosis, neutrophilia and eosnophilia have been reported in infection with *T. evansi* in camels (Anosa, 1988). Generally the eosinophilia is a feature of parasitic infection and is associated with immediate-type hypersensitivity reactions.

In the acute phase of the disease, lymph nodes and spleen are remarkably reactive. This may account for the generalized lymphoid tissue hyperplasia, which is a characteristic sign for *T. evansi* infections, while in the late stage of the immune system become depleted of lymphoid cells (Losos, 1980).

Circulating and tissue-mediated immune complexes have been demonstrated in laboratory animals infected with *Trypanosoma* (FAO, 1979). These immune complexes are likely to have wide varying pathological effects, including anemia, tissue damage,vascular dilatation and increased permeability (FAO, 1979).

The host immune response to a variety of antigens has been found depressed in animals infected with *Trypanosoma* under experimental conditions (Baltz *et al.,* 1981; Anene *et al.,* 1989; Enwezor and Ekejindu, 1998). Several hypotheses have been put forward to explain trypanosome-induced immunosuppression, and the most favored appeared to be the action of Trypanosome enzymes, such as phospholipases (Tizard *et al.,* 1978), neuraminidases (Esievo, 1983) and proteases (Lonsdale-Eccles and Grab, 1986) which all have been implicated in membrane fluidity and cellular damage.

1.11. Diagnosis:

Trypanosomosis is diagnosed by demonstrating the parasite. The diagnosis is basically divided into clinical, parasitological, serological and molecular methods (Luckins, 1992).

1.11.1. Parasitological diagnosis:

Parasitological methods include microscopic examination of blood, parasite concentration techniques and animal inoculation. The easiest and most frequently used of the three techniques is a direct microscopic examination of blood. In the wet blood smear, the trypanosomes are seen between blood cells (FAO, 2000). Haematocrit centrifugation is one of the concentration techniques that has been used to detect trypanosomosis (Kihurani, 1995).

1.11.1.1. Wet blood films:

A small drop of blood is placed onto a clean glass slide and covered with a cover-slip to spread the blood as a monolayer of cells. The slide is examined using light microscopy (x200) to detect any motile trypanosomes. Although, this technique is simple, inexpensive and gives immediate results, it is not enough to identify the species of the trypanosome properly. Final confirmation of the species is made by the examination of the stained smears. Although, the sensitivity of this method is generally low, the examiner's experience and the level of parasitemia can improve the sensitivity (OIE, 2013).

1.11.1.2. Stained Thick smears:

Smears are made by placing a drop of blood on a clean microscope slide. By using the corner of another slide, the blood was spreading on an area of approximately 2 cm. The thickness of the smear should be such as the figures on a wristwatch dial can just be read through it. The film is dried by rapidly waving in the air, and without fixation the smear is stained for 30 minutes using 4% diluted Giemsa (diluted in phosphate buffered saline, pH 7.2). The stained smear washed with buffered water and examined at ×1000 magnification. This method is simple and relatively inexpensive, but the result is delayed because of the staining process. Trypanosomes are easy to recognize depend on the general morphology (OIE, 2013).

1.11.1.3. Stained Thin smears:

A small drop of blood is placed on the clean microscopic slide around 20 mm from the edge and spread an angle of 30° using another microscopic slide to obtain a thin smear. The film is air-dried briefly and then fixed using methyl alcohol for 2 minutes and then allowed to dry. The

smears are stained using 4% diluted Giemsa for 30 minutes. The stain washed off using distilled water. The stained thin film was viewed under a light microscope using X100 oil immersion objective lens. This technique permits morphological detail and identification of the trypanosome species. Usually, both a thin and thick smear is made from the same blood sample. Thick smears contain more blood than thin smears, thus it has a higher diagnostic sensitivity. On the other side, thin smears allow to identify the species of Trypanosome (OIE, 2013).

1.11.1.4. Haematocrit centrifugation technique (HCT):

The procedure was done as described by Woo (1970). Briefly, each capillary tube was filled with approximately 70 µl of EDTA fresher blood and centrifuged at 12,000 g for 5 min. The capillary tube was then gently snapped at the junction between the lower Buffy-coat and RBC layer using a diamond pen. Plasma and Buffy-coat layer was evacuated onto a clean slide, covered by 18x18 mm cover slip and examined under microscope using x100 or x400 objective lens. This technique can detect around 50–200 trypanosomes/ml of blood (Desquesnes and Tresse, 1996).

1.11.1.5. Animal inoculation:

Laboratory animals may be used to reveal sub-clinical infections in domesticated animals and also when highly sensitive detection is required. This method was able to detect as few as 1.25 *T. evansi*/ml blood (Reid *et al.*, 2001). *T. evansi* has a broad spectrum of infectivity for small rodents, rats and mice. Rodent inoculation is not 100% sensitive, but further improvement in its efficacy can be obtained by the use of Buffy coat technique (Monzon *et al.*, 1990).

1.11.2. Serological diagnosis:

Generally, serological tests are useful to apply for prevalence or incidence studies, seasonal or inter-annual variations and for vector control (Desquesnes *et al.*, 2011). Different methods have been used to detect either specific humeral antibodies or trypanosomal antigens, such as direct or indirect agglutination tests, complement fixation test (CFT), IFAT (Desquesnes, 1997; Uilenberg, 1998), ELISA and CATT (Desquesnes *et al.*, 2011).

1.11.2.1. Indirect fluorescent antibody test (IFAT):

Although the technique does not applied to large-scale surveys, it is still useful to screen a small number of samples. The IFAT- *T. evansi* sero-conversion can be used after 60–90 days from the infection (Jacquiet *et al.*, 1993). Compared with the CATT, IFAT is more sensitive, probably because it can detect the low parasitaemic animals (Dia *et al.*, 1997).

1.11.2.2. Enzyme-linked immunosorbent assay (ELISA):

The principle of this technique is that specific antibodies against trypanosomes can be detected by using enzyme-linked anti-immunoglobulins and solid-phase polystyrene plates coated with soluble antigen (Warburg and Christian, 1942).

Soluble antigens from whole lysate of *T. evansi* is able to detect the immunoglobulins present in blood of the host infected with *T. evansi*. This method can also detect the infections with *T. vivax, T. congolense* and *T. cruzi.* The technique is fast and allowing between 500–1000 samples to be tested daily (Laha and Sasmal, 2008).

1.11.2.3. Card agglutination tests (CATT):

The CATT- *T. evansi* is a quick and easy test which can be performed under field conditions for serological diagnosis of Surra in camels (Luckins, 1988). Demonstration of trypanosomal antigens in the blood of the infected animal would be synonymous with a parasitological diagnosis (Voller and Desavigny, 1981).

1.11.2.4. Complement fixation test (CFT):

The complement fixation test (**CFT**) was one of the first techniques used in the diagnosis of *T. evansi* in camels. The test was also used successfully for the detection of *T. equiperdum* in horses. However, cross reaction with sera of horses infected with other trypanosomes may be occurring. Comparing with ELISA, CFT is less sensitive in the diagnosis of the Dourine disease in equines (Gillbert, 1998; Wassal *et al.,* 1991).

1.11.3. Molecular diagnosis:

Molecular techniques are suitable for detecting parasites in the mammalian host and in the insect vector and currently are the main research tools. The PCR is based on the use of enzyme DNA polymerase that will amplify the sequences of DNA bases, until sufficient DNA is produced to be detectable (Desquesnes *et al.,* 2001). A PCR technique is most suitable for the diagnosis of *T. evansi* infection in camels. This technique has 90 % sensitivity as compared with other parasitological and serological tests. This finding suggests that PCR is a useful diagnostic tool for detecting *T. evansi* infected in the very early stages where the microscopic examination is confusing (Muhammad *et al.,* 2010).

1.12. Control:

1.12.1. Chemical Control of Parasites:

T. evansi can be element using various types of trypanocidal drugs at lethal concentration for the parasite. However, treatment might fail in the case of drug resistance. Trypanocides can be divided into two categories. Therapeutic drugs which are used for treatment and have a short-term effect. Although these drugs can kill the parasites, they do not eliminate the parasite 100% of all cases.

The prophylactic drugs which are used as chemoprophylaxis. They not only kill parasites, but also prevent any new infection, due to the remanence of the sustainable in the blood of animals (Dia and Desquesnes, 2004).

1.12.1.1. Therapeutic and Chemoprophylactic Drugs:

Therapeutic drugs aim to eliminate parasites from a sick animal. Although, Diminazene Aceturate (DA) is the most widely therapeutic drug uses against Surra. Other drugs can be used also such as Isometamidium Chloride (IMC), Melarsominedihydrochloride (cymelarsan), Suramin, and Quinapyramine (Dia and Desquesnes, 2004).

1.12.1.2. Strategies for the Use of Trypanocides:

It is important to determine a strategy and the objectives of a treatment, whichever trypanocide is used. Mild doses of treatment such as 3.5 mg/kg body weight (bw) of (DA) might be sufficient to kill the majority of parasites, ensure clinical improvement, and induce the release of a large amount of parasite antigens to enhance the host's immune response. Animals that are treated in this way, but remain infected, can develop an adapted immune response and leads to the status of subclinical infection or healthy carrying. It is important to emphasize that low dose of

treatments potentially enhance the development of drug resistance (Peregrine and Mamman, 1993; Desquesnes, 2004).

In bovine, a higher dose of drug should be used such as DA (at concentration 7mg/kg bw), IMC (at concentration 1mg/kg bw) or melarsominedihydrochloride (at concentration 0.5–0.75mg/kg bw) (Peregrine and Mamman, 1993; Payne *et al.*,1994; Desquesnes, 2004; Dia and Desquesnes, 2004; Desquesnes *et al.*, 2011). In dogs, DA (at concentration 3.5mg/kg) could be enough to treat the infected animal (Peregrine and Mamman, 1993; Desquesnes, 2004).

T. evansi in horses and dogs can be treated by using DA (at concentration 7mg/kg bw) or melarsomine dihydrochloride (at concentration 0.5mg/kg bw), or quinapyramine sulfate (at concentration 8mg/kg). However, in the case of an invasion of the nervous system, none of these drugs have yet been proven to be efficient (Finelle, 1973; Peregrine and Mamman, 1993; Payne *et al.*, 1994; Desquesnes, 2004; Desquesnes *et al.*, 2011).

1.12.2. Prevention and Control:

Preventing infection is an important part of disease control, especially for highly susceptible species or in non-endemic area.

1.12.2.1. Vector Control:

The control of mechanical vectors is not easy because of the diversity of *Tabanid* species and their high mobility. In addition, the larval stages of *Tabanids* are generally spread over a wide area and various landscapes (Foil and Hogsette, 1994).

The control of Tabanid using insecticide sprays was proven to be efficient in small closed deforested areas (Raymond and Favre, 1991). Even in this case, *Tabanid* infestation was reappeared 2-3 years after the

end of the control program (Desquesnes, 2004). When the control of *Tabanid* is carried out in an open area, it is not sustainable because *Tabanids* move to the surrounding areas to fill the ecological gap created by the control program. (Foil and Hogsette, 1994).

Stomoxys species differ from *Tabanids* in that they are developing within the livestock area or within the farm and they are closely related to the farming systems (Foil and Hogsette, 1994). The control of these vectors can be attempted using traps and/or impregnated screens or using insecticides on livestock (Laveissiere and Grebaut, 1990; Mihok, 2002).

1.12.2.2. Other Methods for Prevention:

In situations where it is difficult to control the biting insect populations, it may be easier to control transmission. *Tabanids* are naturally persistent feeders and they do not leave the animal to bite another if the latter one is more than 50 meters away (Foil *et al.*, 1985). Therefore, 200m is considered to be a safe distance to control the mechanical transmission by biting insects (Foil, 1983; Foil *et al.*, 1985; Barros and Foil, 2007).

However, separating bovine from equine is highly recommended to prevent the transmission of *T. evansi* from a reservoir such as buffalo or cattle to the highly sensitive host such as camel and horses. Thus, it is advisable to housing the cattle and camel/ horses in completely different areas that are separated at least by several kilometers (Da Silva *et al.*, 2007; Vergne *et al.*, 2011).

As mention before carnivores may be infected when they eat the bones, flesh, or blood of an infected animal that has only just died. Rodents, which are omnivorous, may become infected like carnivores and get the infection via oral as demonstrated in a trial (Da Silva *et al.*, 2007; Vergne *et al.*, 2011). To control this type of transmission, the dead animal

carcasses should be eliminated as soon as possible and the stray dogs, around the slaughterhouses, as well as around the farms should be control (Gutierrez *et al.*, 2010).

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study Area:

This study was conducted in Tamboul Slaughterhouse, which lies at the east part of Algezira state around 150 km south Khartoum. The district is located at latitude 14° 52′ N and longitude 33° 31′ E.

2.2. Study type:

The study was a cross-sectional study to estimate the prevalence of camel trypanosomosis, and the risk factors associated with the disease.

2.3. Sampling Method and Sample Size Determination:

By using systematic random sampling methods the samples were selected. The size of the sample was determined by using the formula of Thrusfield (2007) and 95% confidence interval as following:

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

n = sample size

P_{exp}= expected prevalence

1.96 = the value of Z at 95% confidence interval

d = desired accuracy level at 95% confidence interval

In a previous study performed in Tamboul livestock market (Eldaw, 2009) they found that, the prevalence of camel Trypanosomosis (*T. evansi*), was 3.5%. Referring to that, the size of the sample was calculated as following:-

$$n = \frac{\mathbf{1.96^2 . (0.035) .(0.965)}}{\mathbf{(0.0025)}} = \mathbf{52}$$ animal samples.

To increase the precision of the study, the sample size was multiplied by three. So the size of the sample became 165 samples.

2.4. Collection of blood samples:

Whole blood samples from 165 camels were collected from the jugular vein into a heparinized vacationer tube (5ml). The tubes were labeled with a number and kept in ice box and then were transported immediately to the laboratory of Al Butana University. At the same time the information regarding sex, age, breed, body condition……. etc was recorded in an especial form (questioner).

2.5. Diagnosis of Trypanosomosis:

2.5.1. Thin blood smears:

The Thin blood smears were performed as described by (Murray *et al.*, 1977). A small drop of blood was placed onto a clean microscope slide around 20 mm from the edge and a then spread with a glass spread an angle of 30° to obtain a thin smear The film was air-dried briefly and then fixed using absolute methyl alcohol for 2-3 minutes and then allowed to dry. The smears were stained using 4% diluted Giemsa for 30 minutes. The stain washed off using distilled water and also air dry. After air-drying the slides were examined under oil immersion objective lens (100x) to detect and identify the *Trypanosoma* species based on the morphological characters.

2.5.2. Microhaematocrit centrifugation technique (MHCT):

A heparinized capillary tube (75 × 1.5 mm) was filled with blood until two thirds its size and then was sealed from one side using crest seal. The tube was centrifuged for 4 minutes at 12,000 rpm/minute using microhaematocrit centrifuge. After centrifugation, the capillary tube was placed on a microscopic slide and the interphase between the Buffy coat layer and the plasma was examined under a microscope using 10X objective lens (Wernery *et al*., 2001). After examination the capillary tube was cut at 1mm below the Buffy coat to include the upper layers of red blood cells. The contents of the capillary tube were gently spread onto a microscopic slide, and then covered with a coverslip. The slides were examined under a microscope for the presence of the motile trypanosome using 10x or 40x objective lenses (Murray *et al*., 1977).

2.6. Questionnaire:

Data regarding the characteristics of individual camels, including age, sex, breed, body condition, and sources of animals, were obtained by asking the owner.

2.7. Data analysis:

The overall prevalence was calculated by dividing the number of positive results by the total number of animals examined in this study. The whole data (laboratory results, risk factors such as age, breed ….etc.) were entered into Microsoft excel sheets (Microsoft office excel 2007) and then transferred to SPSS version (16.0) to analyse.

First the data were analyzed by simple descriptive statistic using relative frequencies, cumulative frequencies and cross tabulation. A univariate analysis was used to estimate the strength and statistical

significance of the association between risk factors and the disease. Chi - square (x^2) test was used to determine the significant (p-value <0.25) of each factors. All potential risk factors which shown a significant association with the disease in the univariate analysis were entered for further analysis using multivariate analysis and logistic Regression. The factor shown p-value < 0.05 by using logistic Regression was considered statically significant association with disease.

The comparison between two tests (Buffy coat and Giemsa's stain) including the sensitivity and specificity was performed using Roc curve.

CHAPTER THREE

RESULTS

3.1. Overall prevalence:

From a total of 165 animals 8 were positive (4.8%). The overall prevalence of camel Trypanosomosis in Tamboul slaughterhouse, Sudan was 4.8% **(Table 1).**

Table (1): The overall prevalence of camel trypanosomosis in Tamboul Slaughterhouse:

Results	Frequencies	Cumulative frequency%
Positive	8	4.8
Negative	157	95.2
Total	165	100.0

3.2. Examination of blood samples:

Infection with *T. evansi* was diagnosed by examination of 165 camels using two different techniques, Giemsa's stain **(Fig 1)** and MHCT (Buffy coat techniques) **(Fig 2).** The numbers of positive samples were detected in this study was 7 and 8 by using Giemsa's stain and Buffy coat techniques, respectively **(Table 2 and 3)**.

Table (2): The number and Prevalence of infected camels with *Trypanosoma evansi* using Giemsa's stain inTamboul Slaughterhouse:

Results using Giemsa's stain techniques	Number	Prevalence %
Positive	7	4.2
Negative	158	95.8
Total	165	100%

Table (3): The number and Prevalence of infected camels with *Trypanosoma evansi* using Buffy coat inTamboul Slaughterhouse:

Results using Buffy coat techniques	Number	Prevalence %
Positive	8	4.8
Negative	157	95.2
Total	165	100%

3.3. Distribution of the camels according to the potential risk factor:

As shown in **Table 4,** the breed of camels was classified into 3 breed Darfur (78 camels), Butana (59 camels) and Kassala (28 camels) were examined respectively. The source of camels was divided into 3 groups; west of Sudan (78 camels), Butana (59 camels) and east of Sudan (28 camels) respectively.

The body condition was classified into 3 groups; fat (35 camels), normal (113 camels) and poor (17 camels) group. According to the sex, 20 males and 145 females. Regarding age, the animals were divided into 2 groups; young (1-5 years), and old (>5 years) group. From each group, 45 and 120 camels were examined respectively **(Table 4 and 5).**

Table (4): Distribution of camels examined for Trypanosomosis according to the potential risk factor inTamboul Slaughterhouse:

Risk factor	Number tested
Breed	
Darfur	78
Butana	59
Kassala	28
Total	165
Source of animal	
West of Sudan	78
Butana	59
East of Sudan	28
Total	165
Body condition	
Fat	35
Normal	113
Poor	17
Total	165
Sex	
Male	20
Female	145
Total	165
Age	
Young	45
Old	120
Total	165

Table (5): The frequency, percent and cumulative percent of the camels examined for Trypanosomosis according to the potential risk factor inTamboul Slaughterhouse:

Risk factor	Frequency	Percent	Cumulative Percent
Breed			
Butana	59	35.8	35.8
Darfur	78	47.3	83.0
Kassala	28	17.0	100.0
Total	165	100.0	
Source of animal			
Butana	59	35.8	35.8
East of Sudan	28	17.0	52.7
West of Sudan	78	47.3	100.0
Total	165	100.0	
Body condition			
Fat	35	21.2	21.2
Normal	113	68.5	89.7
Poor	17	10.3	100.0
Total	165	100.0	
Sex			
Female	145	87.9	87.9
Male	20	12.1	100.0
Total	165	100.0	
Age			
Old	120	72.7	72.7
Young	45	27.3	100.0
Total	165	100.0	

3.4. The distribution of the positive camels examined for Trypanosomosis according to the potential risk factor:

Four out of 78 (5.1%), 3 out of 59 (0.51%) and 1 out of 28 (3.6%) of camels were infected with *T. evansi* from Darfur, Butana and Kassala respectively **(Table 6 and 7)**.

Referring to the sources of animals **(Table 6 and 7)**, the positive samples were 4 out of 78 (0.51%) from West of Sudan, 3out of 59 (0.51%) from Butana and 1 out of 28 (3.6%) from East of Sudan. All positive camels were females (8 out of 145 / 5.5%) and there is no infection was detected in male (0 out of 20 / 0%) **(Table 6 and 7)**.

One out of 45 (2.2%) and 7 out of 120 (5.8%) of camels were infected with *T. evansi* in young and old group, respectively **(Table 6 and 7)**.

Based on the body condition, the number of positive samples was 8 (7.1%) in the group of normal body condition. No infection was detected in fat group or poor body condition group **(Table 6 and 7).**

Table (6): Distribution of positive camels examined for Trypanosomosis according potential risk factor inTamboul Slaughterhouse:

Risk factor	Number of tested	Number of positive
Breed		
Darfur	78	4
Butana	59	3
Kassala	28	1
Total	165	8
Source of animal		
West of Sudan	78	4

Butana	59	3
East of Sudan	28	1
Total	165	8
Sex		
Male	20	0
Female	145	8
Total	165	8
Age		
Young	45	1
Old	120	7
Total	165	8
Body condition		
Fat	35	0
Normal	113	8
Poor	17	0
Total	165	8

Table (7): Cross tabulation of trypanosomosis in165 camels examined according to the potential risk factor in Tmboul slaughterhouse:

Risk factor	No. tested	No. positive	Percent%
Age			
Old	120	7	5.8
Young	45	1	2.2
Total	165	8	
Sex			

Female	145	8	5.5
Male	20	0	__
Total	165	8	
Breed			
Butana	59	3	5.1
Darfur	78	4	5.1
Kassala	28	1	3.6
Total	165	8	
Body condition			
Fat	35	0	__
Normal	113	8	7.1
Poor	17	0	__
Total	165	8	
Source of animal			
Butana	59	3	5.1
East of Sudan	28	1	3.6
West of Sudan	78	4	5.1
Total	165	8	

3.5. Univariate analysis:

The chi- square test showed that only one factor (body condition) out of five risk factors was statistical significant (p-value .144) associated with trypanosomosis in camels **(Table 8).**

Table (8): Univariate analysis of the risk factor associated with trypanosomosis infection in camels slaughtered at Tamboul slaughterhouse, Sudan:

Risk factor	No. tested	No. Positive	d.f	Chi	p-value*
Age			**1**	**.925**	**.336**
Old	120	7			
Young	45	1			
Total	165	8			
Sex			**1**	**1.160**	**.282**
Female	145	8			
Male	20	0			
Total	165	8			
Breed			**2**	**.119**	**.942**
Butana	59	3			
Darfur	78	4			
Kassala	28	1			
Total	165	8			
Body condition			**2**	**3.869**	**.144**
Fat	35	0			
Normal	113	8			
Poor	17	0			
Total	165	8			
Source of animal			**2**	**.119**	**.942**
Butana	59	3			

East of Sudan	28	1			
West of Sudan	78	4			
Total	165	8			

***** Significant association (p-value< 0.25)

3.6. Multivariate analysis:

Multivariate analysis using logistic Regression showed that there was no significant association (p-value< 0.05) between the risk factors and camel trypanosomosis at Tamboul slaughterhouse.

3.7. Rock curve Test:

Table (9): Cross tabulation of stained smear and Buffy coat technique of camel Trypanosomosis at Tmboul slaughterhouse in Sudan:

		Buffy coat		
		+ve	**-ve**	**Total**
Stained Smear	**+ve**	**7**	**0**	**7**
	-ve	**1**	**157**	**158**
	Total	**8**	**157**	**165**

- Value under the curve **(0.938)** its excellent test.
- **Sensitivity = 0.875**
- **Specificity = 1**

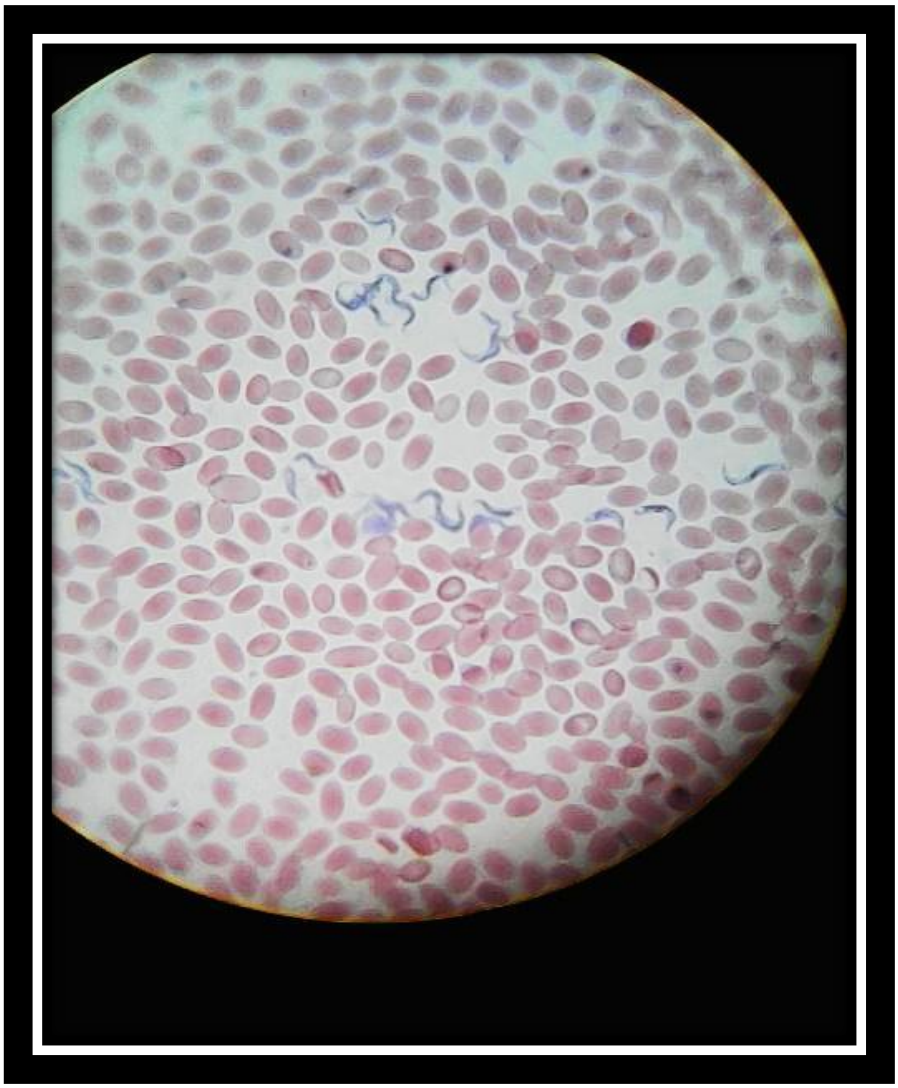

Figure (1): Photomicrograph of camel blood sample infected with *Trypanosoma evansi* using 100× lens under microscope and Giemsa's stain in Tmboul slaughterhouse, Sudan.

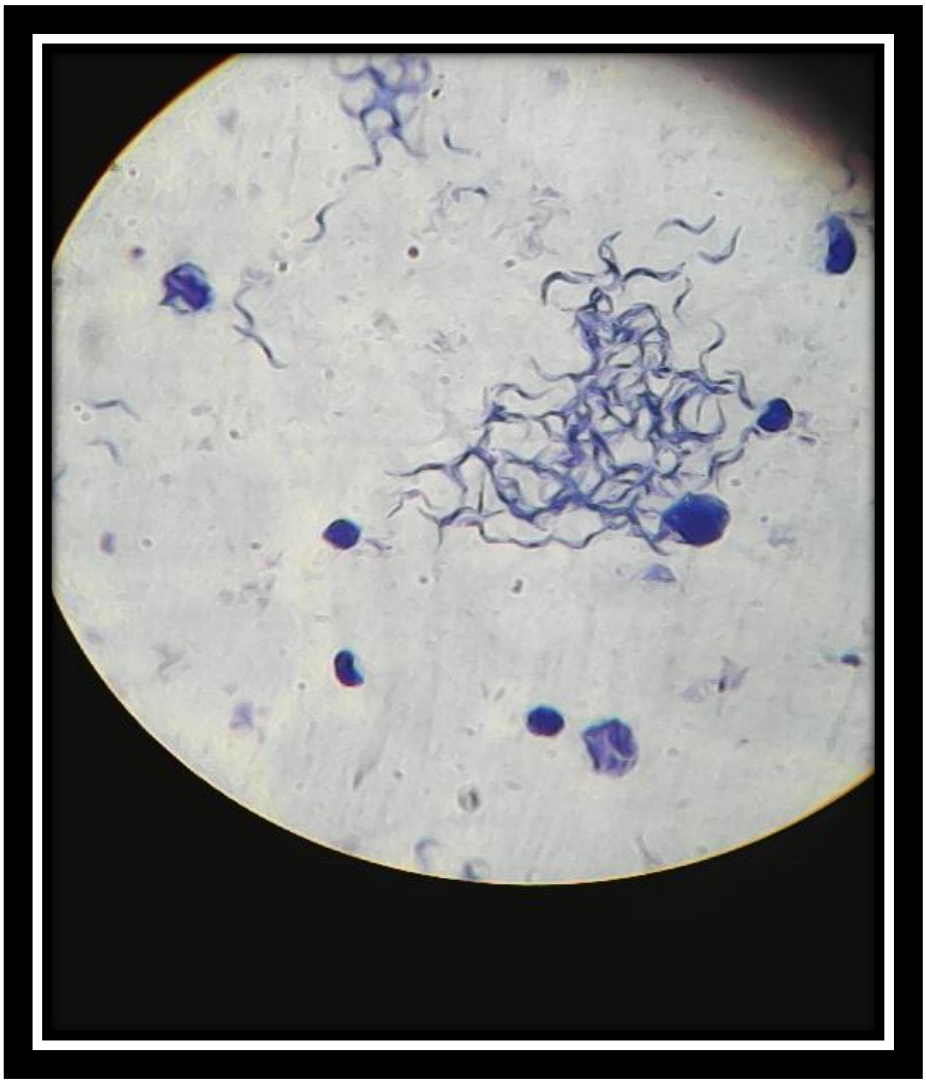

Figure (2): Photomicrograph of camel blood sample infected with *Trypanosoma evansi* using Buffy coat, in Tmboul slaughterhouse, Sudan.

CHAPTER FOUR

DISCUSSION

Camel trypanosomosis is a disease of major economic importance in many countries in Africa, Asia and South America. Although, the devastating effect caused by the disease is not frequently seen nowadays, the disease still occurs with high prevalence rates in many countries. Moreover, *T. evansi* as extracellular parasites are permanently faced with the multiple components of the host‘s immune system ranging from innate to adaptive immune defenses (Luckins, 1998).

In our study the prevalence and risk factor associated with camel trypanosomosis were investigated. The overall prevalence was 4.8% in Tamboul Slaughterhouse, at Algezira state. This result is in agreement with previous reported performed by Khalid (2015) where the prevalence was 5.1% in Butana area at Al Gedarif State, Sudan. On the other hand, our result was higher than that demonstrated by (Ibrahim *et al*., 2011) and co-worker where the prevalence of trypanosomosis was 2.46%. However, the prevalence of disease in the present study is low compared with that reported by Ibrahim and co-worker (6.08%) (Ibrahim *et al*., 2015). This difference may refer to the difference in the number of camels investigated in each study, which may reflected in the prevalence rate of the disease.

In the present study, the univariate analysis showed that only one risk factor (body condition, p-value = 0.144) was statistical significant (p-value < 0.25) association with trypanosomosis infection.

The distribution of Trypanosomosis according to the source of the animals from Butana (5.1%), West of Sudan (5.1%) and East of Sudan (3.6%) respectively. These results were slightly higher comparing with

previous report conducted at Al Gedarif (2.04%) and Kassala (1.12%) (Dafalla, 1988).

Generally, the lower prevalence rate was reported in animals from East of Sudan comparing with Butana and West of Sudan, this finding may be attributed to the lower number of camels examined from this area.

In alignment with previous results, the low prevalence rate was reported in Kassala breed (3.6%) comparing with Butana and Darfur breed (5.1% for both). This result could be due to fewer numbers of camels examined from Kassala breed (28 camel).

The present results revealed that the prevalence rate was higher in females (5.5%) than males (0%). Similar results were reported by Najira (2003) and Eldaw (2009), who investigated camel trypanosomosis in Kenya and Tamboul area in Sudan, respectively. This observation may be attributed to stress induced by pregnancy and lactation (Tayib *et al.*, 2015). Moreover, the majority of animals slaughtered during this study were females.

In this study, the oldest animal (age > 5 years) showed an infection rate (5.8%), which is higher comparing with young animals (age ≤ 5 years) (2.2%). This result is in alignment with Dia *et al*., (1997), Gutierrez *et al*., (2000) and Atarhouch *et al*., (2003), who reported that a tendency for infection was increased in correlation with the increase of age. The higher prevalence of trypanosomosis in old camel might be due to heavy stress through their use for transportation and long-term exposure under poor management. These factors increase the risk of infection in old camel comparing with younger. Furthermore, young animals are often browsed within the vicinity of human dwellings, which in turn reduced the exposure to the vectors. In contrast, the older camels are browsed and watered in the open area, where they have a greater chance of contact with the vectors. Additionally, young animals are also bitten less frequently comparing with

older ones. This phenomenon due to the greater defensive behavior they exhibit, which is making them hard for biting flies to attach (Tayib *et al*., 2015).

Examination of camels in Tamboul Slaughterhouse with MHCT in this study revealed an infection rate (4.8%) which is slightly higher than that obtained by stained methods. This result indicates that the MHCT is more specific in the diagnosing of camel trypanosomosis compared with stained methods.

CONCLUSION AND RECOMMENDATIONS

Camel trypanosomosis is the most important diseases causing the economic losses, particularly in areas where camel contributes in human activity. Moreover, camels provide milk, meat, wool and used for water traction and the bear of burden. Thus, it is very important to focus on the control strategies and launch the most effective campaign to control camel trypanosomosis. In the majority of the camel trypanosomosis the diagnoses only based on the clinical sign. Subsequently the control of the disease using treating depends on these signs, which in turn leads to either mistreatment or increase the risk of drug resistance in future life. Therefore, the following points are recommended:

- The practitioners must focus on the accurate diagnosis and using the most reliable and sensitive technique.
- Effective prevention and control measures should be designed against the parasite and their vectors to minimize the disease.
- Expand government veterinary services to serve the community in the study areas.
- Awareness program for the owner about the disease should be promoted.
- Develop control measures in affected herds to avoid spreading the disease.
- Use more sensitive methods for diagnosis of trypanosomiasis such as (PCR, ELISA), which can give a higher prevalence rate and to improve the diagnostic tool.

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Appendix

Questionnaire:

Investigation of camel trypanosomosis in Tamboul Slautghterhouse Gezira state. Conducted by: The Epidemiology Department of Sudan university of science & technology.

Locality_____date_____

The individual risk factors:

1-Age: (years)

≤ 5 ( )

> 5 ( )

2-Sex:

Males ( )

Females ( )

3-Body condition:

Fat ( )

Normal ( )

Poor ( )

4-Breed:

Darfur ( )

Butana ( )

Kassala ( )

5-Source of animal:

West of Sudan ( )

Butana ( )

East of Sudan ( )