Introduction

The One-humped (dromedary) camel (*Camelus dromedarius*) is the most numerous animal in the semi-arid and arid tropical areas of Africa. In the Sudan, the camel population exceeds 3.1 million head (Anon, 2000). In fact about 20% of the world's camels are found in the Sudan. Camels are important source of meat, milk and hair, and are used for transportation and drought power; they are also exported to North Africa and middle east thus contributing a significant proportion to the gross national income.

Trypanosoma evansi (*T. evansi*), the protozoan parasitic cause of camel trypanosomosis (Surra), constitutes one of the major veterinary problems worldwide (Omer *et al*., 2004). Trypanosomosis due to *T. evansi* is a major enzootic disease of the dromedary camel (Gatt-Rutter,1967)

Trypanosomosis, is one of the major and most important diseases in camels in the arid and semiarid zone of the world (Boid, *et al*., 1985). In the Sudan, *T. evansi* is primarily a parasite of camels causing a disease locally known as Guffar. The disease is common in Kordofan and Darfur States in the west, Kassala, Gadaref and Red sea states in the east and to a lesser extent in central Sudan, in the Gezira, Sennar, Blue Nile and Khartoum States (karib, 1961). *T. evansi* is transmitted by the biting flies, particularly, *Tabbanids* and *Stomoxys* (Losos, 1986).

Objectives of the Study:

The general aim of this study is to evaluate the seroprevalence of camels' trypanosomosis (Guffar) in Kassala State, Eastern Sudan, using Card Agglutination Test for *Trypanosoma evansi* CATT/*T. evansi* as well as to compare the prevalence of the disease within male and female camels and the effect of age in the prevalence of this disease in the study area.

Chapter I

Literature review

1.1. Disease and causative agent:

Camel trypanosomosis is of great concern to Sudan. Trypanosomosis was first reported in1904 in cattle arriving to Khartoum from Upper Nile (Karib, 1961).Trypanosomosis is the most important and serious pathogenic protozoal disease of camel caused by *Trypansoma* species. Camel trypanosomosis, also known as surra meaning emaciated (Al-Rawashdeh *et al*., 2000). Mostly, camels suffer from trypanosomosis caused by *T. evansi* that is transmitted biologically by *Tsetse* fly (*Glossina spp.*) further mechanically, non-cyclically, by haematophagus flies such as horse flies (*Tabanus spp.*) and stable flies (*Stomoxys spp.*) in area free of tsetse flies(Anosa, 1983).

The causative agent, *T. evansi*, was discovered by Griffith Evans in 1880 in infected camels and equids in the Dara Ismail Khan District of Punjab (Evans, 1998). Since then, studies have shown that the parasite can infect all species of domesticated livestock, although the principle host varies geographically (Indramhkang, 1998; EL-Sawalhy and Seed, 1999; AL-Rawashdeh *et al.*, 2000). Surra is widespread in different parts of the world and poses a major constraint to camel productivity, in the Sudan the prevalence of Surra caused by *T. evansi* was reported to be 33% (Elamin *et al.*, 1999).

1.2. Clinical manifestation:

Camel trypanosomosis occurs both in chronic and acute forms (Payne *et al*., 1990). The acute form of the disease may last for up to three months and is characterized by irregular fever, reduced appetite and water intake, as the disease progresses hump disappear (FAO, 2000) (Fig. 1), recurrent keratoconjunctivitis and urticarial plaques on the neck and flank, dependent edema under the belly, marked depression, dullness, loss of condition, the hair coat become dull and rough with loss of hair at the tail and often rapid death (Luckins, 1998).

T. evansi has been involved in several reproductive disorders in female and male causes many different manifestations such as abortion, heat repitation, weak newborn,

stillbirth, temporary and permanent anestrus (Batista *et al*., 2008). In male, the disease affects sperm quantity and quality due to testis and epididymis involvement (Mbaya *et al*., 2011).

In chronic cases, camels develop recurrent episodes of fever. Some camels develop edema in their dependent parts of the body, urticarial plaques and petechial hemorrhage in serous membranes. Death finally ensues if untreated, and some may harbor trypanosomes for 2-3 years thus constituting reservoirs of infection to susceptible camels and hosts. Other well documented field reports are death (Tuntasuvan, *et al*. 1997), abortion (Lohr, *et al*. 1986), weight loss, reduced draught power (Luckings, 1998) and nervous signs like circling movement and trembling, unusual aggressiveness, running aimlessly and sudden collapse in severely stressed and over worked 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). The animal stands with its head hanging forward, its eyes turn dull and are closed with considerable amount of tears (Karram *et al*., 1991) inflammation of the nose, eyes, ears and external genitalia (Scheidle, 1982).

1.3. Diagnosis of the disease:

Trypanosomosis is diagnosed by demonstrating the parasite. However, because dromedaries are usually far away from laboratory facilities, a tentative diagnosis can be reached without microscopy, by taking into account the owner's observations and clinical examination of camels in the field. The chronic form is most common in camels and may present an association with secondary infections due to immunosuppression caused by *T. evansi* infection, and this complicates clinical diagnosis (Luckins, 1992). The parasites can be detected in blood 13 to 16 days after an infective fly has had a meal. Parasitological diagnosis is mainly carried out by the direct microscopic examination of wet or stained blood films. (Yadvendra *et al*., 1998).Direct parasitological methods include; wet blood smears, dry blood smears, micro hematocrit centrifugation technique (MHCT).

Also serological methods can be used to diagnose trypanosomosis such as Indirect Fluorescent Antibody Test (IFAT), this technique has been used extensively in the

detection of trypanosomal antibodies in animals and human. Antigens are usually prepared from blood smear which are fixed in acetone and then stored at a low temperature. The IFAT has proved to be both specific and sensitive in detecting trypanosomal antibodies in infected cattle and Camel (Wilson, 1969; Lukins and Mehlitz, 1978).

Suratex diagnose the animals with sub-patent infection which couldn't be detect by parasitological method (Olaho *et al.*, 1996). Suratex has been developed for detecting circulating trypanosomal antigen in the blood of infected animal. Serum, plasma or whole blood is mixed with Suratex reagent on a test card which is then rocked manually, in positive reaction, agglutination develops in 2 min. Suratex have a specificity of 99% using sera from horses, camels and cattle, from non-endemic areas. Its sensitivity is also high (93-97%). The test also diagnoses latent infections which can't be detected by parasitological techniques (Nantulya, 1994).

Enzyme-Linked Immunosorbent Assay (ELISA), was a major breakthrough in the diagnosis of animal Trypanosomosis. It is specific and sensitive and it can be used readily for large scale screening of many serum samples (Mahmoud and Elmalik, 1978). ELISA has been used successfully for serodiagnosis of camel trypanosomosis (Rae *et al.*, 1989).

Card Agglutination Test (CATT/*T. evansi*) is a direct card agglutination test for detection of anti-trypanosomes antibodies in serum or plasma of infected animals. It is recommended by OIE (OIE, 2012). The antigen consists of cloned bloodstream form trypanosomes of RoTate 1.2 VSG a predominant variable antigen type (VAT) of *T. evansi* (Magnus, 1988).

1.4. Treatment, prevention and control:

Treatment with trypanocidal drugs is the usual method of control of *T. evansi* and quinapyramine has been used in camels, and only recently melarsomine (cymelarsan) was introduced for the treatment of surra in camels because of the spread of drug resistance (Luckins, 1998; Bourdichon, 1998). Another drug, Trypan, which is a formulation containing diminazene-di-aceturate (diamidino-phenyl-triazene-diaceturate-tetrahydrate), phenazone and procaine hydrochloride is effective against *T. evansi* infections (Bourdichon, 1998).

Trypan can be used for curative and preventive treatment. On the whole, control of surra requires treatment of infected animals with effective drugs and reducing blood sucking flies by regular insecticide treatment.

Figure 1. A photograph showing the disappearance of the hump in a camel confirmed as *T. evansi* positive in this study.

Chapter II

Materials and Methods

2.1. Study area:

The incidence of camels trypanosomosis (Guffar) caused by *T. evansi* was studied in Wd-Ahilio and Sitate River located in Kassala State, eastern Sudan, (Fig. 2 and 3) in February 2016

In these areas camels tend to move in large groups sand congregate in masses only during the summer settlements.

2.2. **Animals and Sampling:**

A total of 58 camel blood samples collected from Wd-Alhilio (30 samples) and Sitate River (28 samples), were examined for *T. evansi*. Approximately 5 ml of blood samples were drawn aseptically from the jugular vein in EDTA-free vacutainers previously labeled with date, location, age (1-4 years, 5-8 years and 9-12 years), sex (male and female). Samples were centrifuged at 3000 rpm for 6 minutes. Sera were then collected in 1.5 ml tubes and then kept on ice until being transported to the laboratory, kept at -20℃ until being tested with CATT/*T. evansi*. .

2.3. Card Agglutination Test for *Trypanosoma evansi* (CATT/*T. evansi*):

CATT/*T. evansi* is a direct card agglutination test for detection of anti-trypanosomes antibodies in serum or plasma of infected animals. Reagents and accessory materials were obtained from the Institute of Tropical Medicine (Antwerp, Belgium). A complete test kit for 250 screening tests contains the following: 6 vials CATT/T. evansi- antigen, 1 vial positive control, 1vial negative control, and 1 vial CATT/*T. evansi*-buffer (Fig. 4). The reagents for the test were mixed as follows: (A) 2.5 ml of CATT/*T. evansi*-buffer (phosphate buffer saline) was added to a vial of freeze dried CATT/*T. evansi* antigen using sterile syringe. The vial was then shaken for a few seconds so as to obtain a homogeneous suspension. (B) 0.5ml of CATT/*T. evansi* buffer was added to the vials of positive and negative controls, respectively, using sterile syringe. On a test area of the card, 25 u1 of the diluted serum (1:4 serum

: PBS) was added, after being vortexed (Fig 5), to the provided plastic agglutination card containing the homogenized CATT/*T. evansi* antigen (approximately 45µ1/well) and mixed using provided plastic rods. After being rotated using provided CATT/*T. evansi* rotator (Fig. 5) at 70 rpm for 5 min, agglutination was observed and the degree of agglutination was determined as follows: Very strong agglutination (+++), Strong agglutination (++), Moderate agglutination (+), Weak agglutination (±), Absence of agglutination (-)

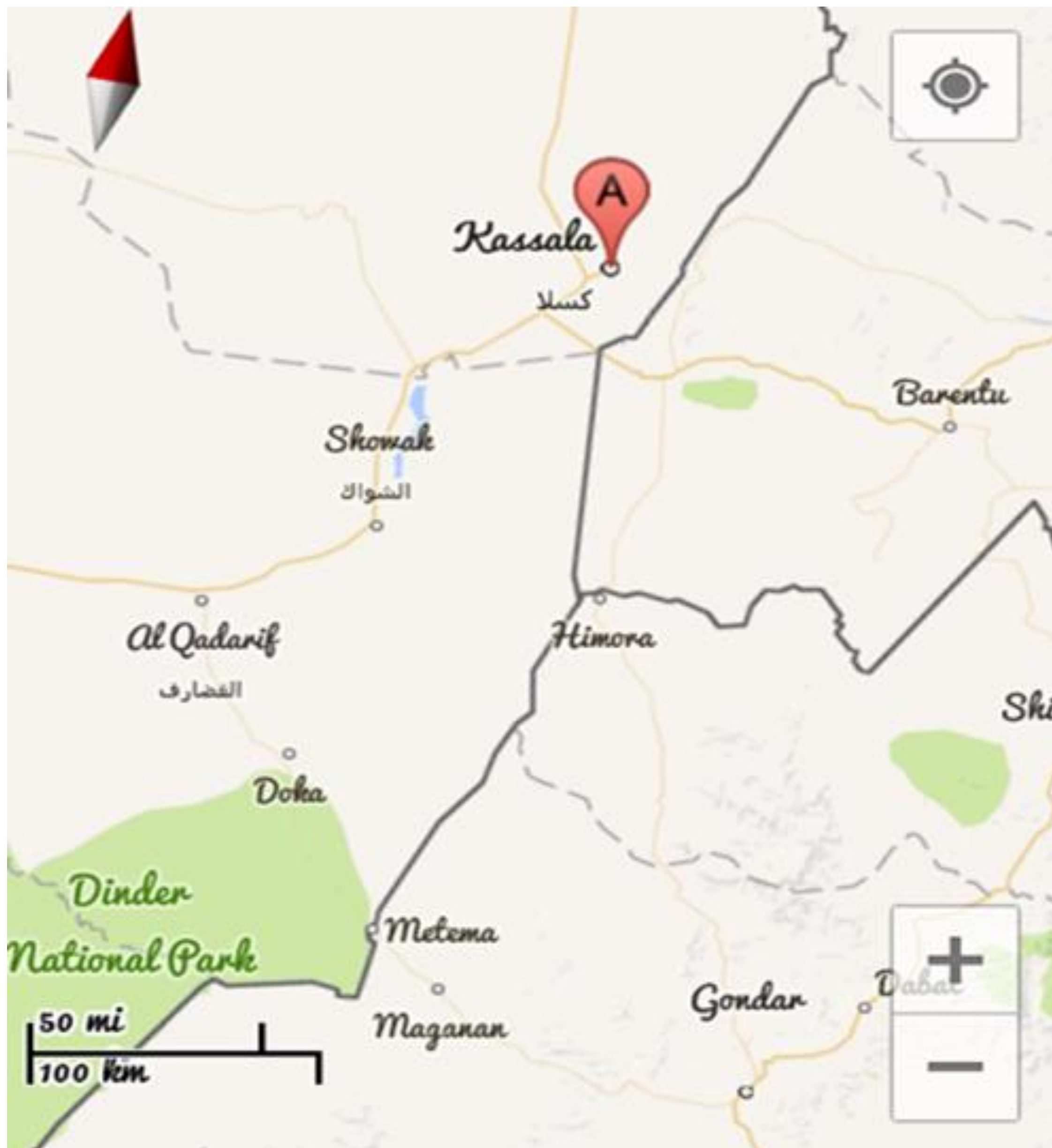

Figure (2): Map showing the study area.

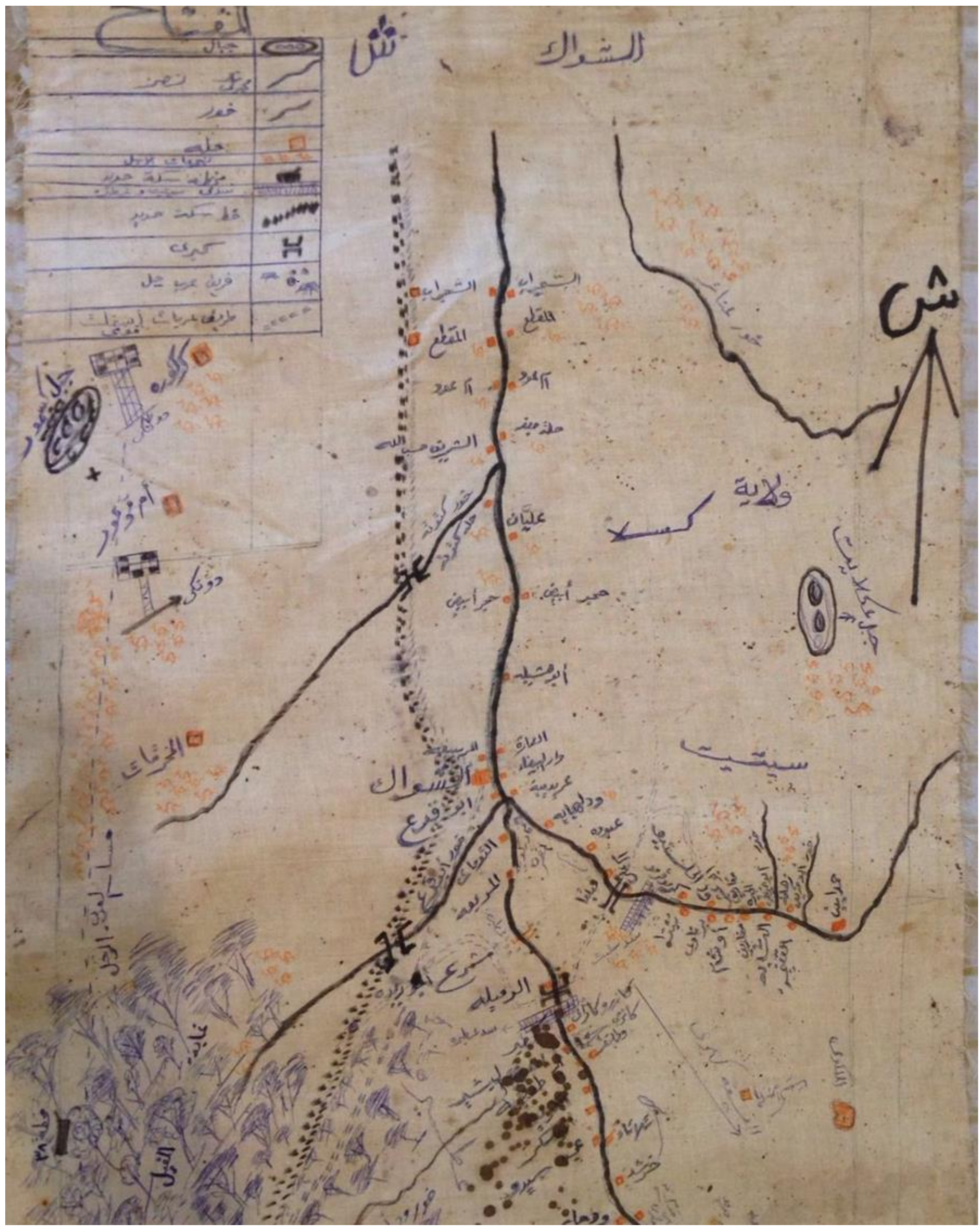

Figure (3): Map showing the study area. Source: Alshowak Camel's Research Center, Khartoum University.

Figure (4): CATT/*T. evansi* kit’s components (Buffer, Antigen, positive control, negative control), respectively from left to right.

Figure (5): CATT/*T.evansi* rotator, card and rod.

Figure (6): Autovotrex for samples mixing

Chapter III

Results and Discussion

3.1. Prevalence of *T. evansi* in camels using CATT/*T. evansi*:

A total of 58 camels at Wd-Alhilio and Sitate River were screened for *T. evansi* infection using (CATT/*T. evansi*). Overall prevalence of *T. evansi* infection was 47(81%) of which 28 (48%) in Wd-Alhilio and 19 (33%) in Sitate River. While 11(19%) showed the negative result.

The intensity of the infection within individuals was recorded with various agglutination levels (Fig.7). The very strong agglutination was recorded in 14 (24%), strong agglutination 7 (12%), moderate agglutination 16 (28%) while weak agglutination was recorded in 10 (17%) (Table. 1 and Fig.8), indicating the high prevalence corresponded with possibly heavy parasite load in the tested animals. It is known that CATT/*T. evansi* can't differentiate between the current (active) and previous infections. However, it has also been observed that the very strong and strong agglutination might indicate that animals most probably are currently infected with *T. evansi* (OIE, 2012).

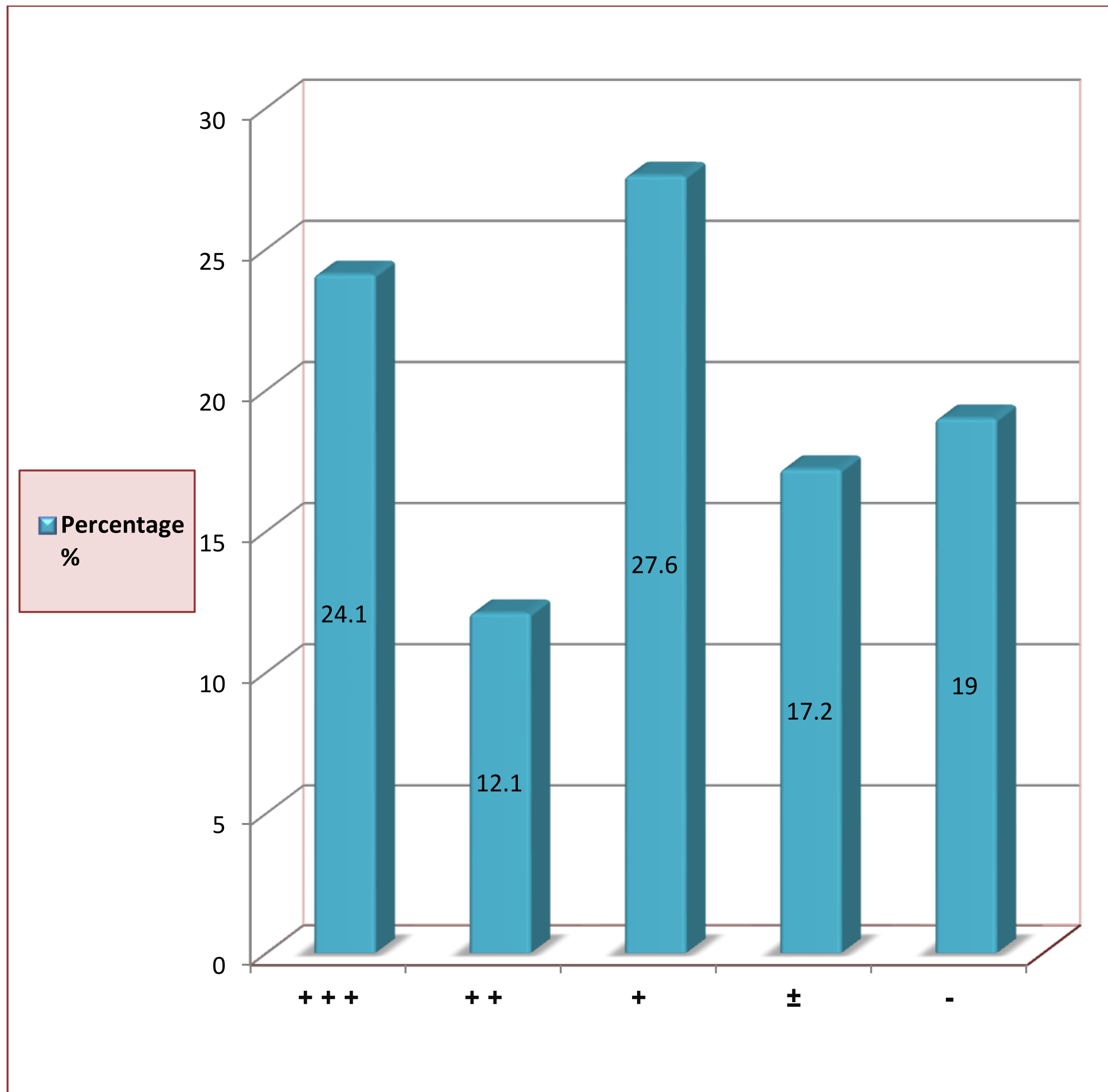

Figure (7): The intensity of the agglutination of studied camel's blood samples using CATT/*T. evansi*.

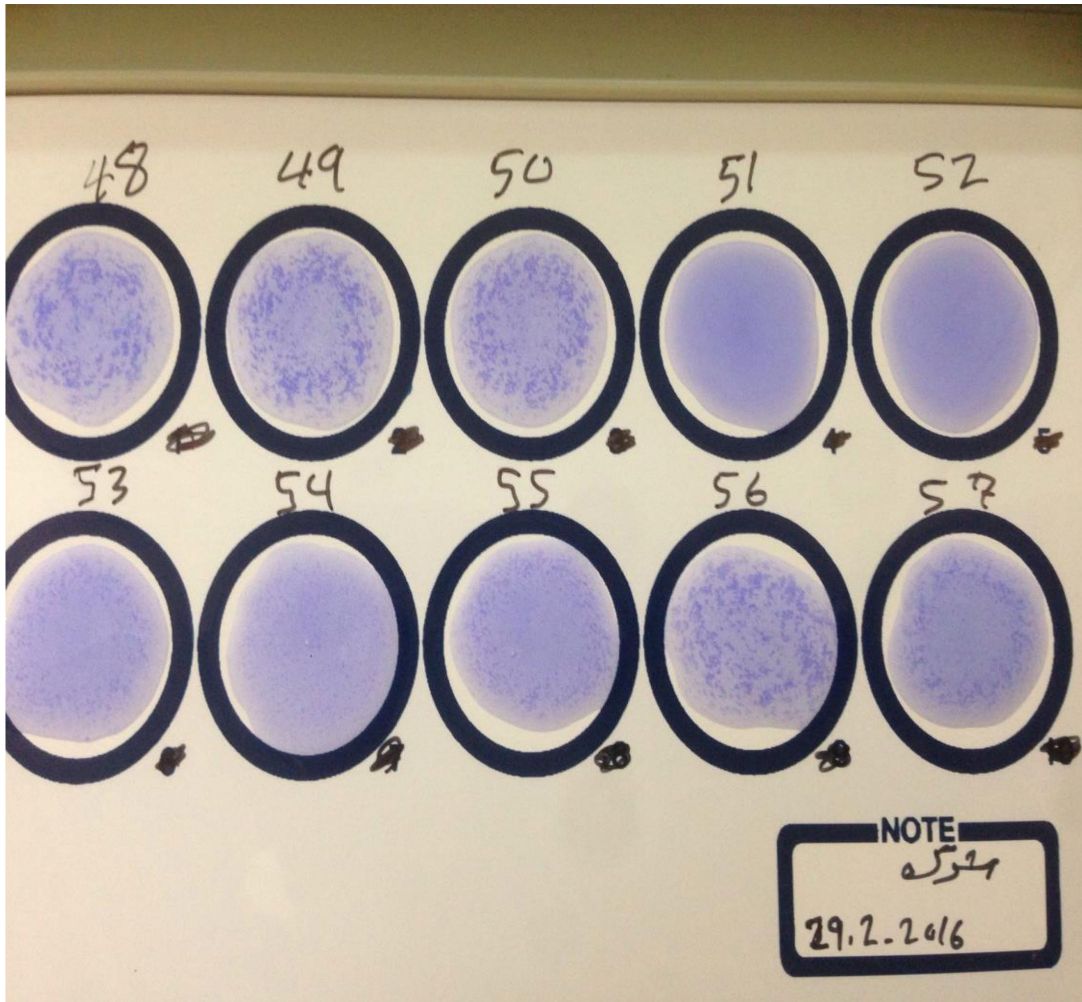

Figure (8): Various agglutination levels. Samples 48, 49 and 50 (very strong agglutination), Sample 56 (strong agglutination), sample 55 and 57 (moderate agglutination) and sample 54 (weak agglutination).

Statistically there was no significant difference ($p<0.05$) in the prevalence of the disease within the two areas. However, the prevalence was higher in Wd-Alhilao than Sitate River.

The prevalence of the disease (81%) is higher than the previous report 31% (Salim *et al*, 2011). This might be due to the spread of drug resistance reported by (Babeker and Hassab Elrasoul, 2014; Elrayah*et al*., 1999). It may also be due to the small sample size in our study (58 samples).

Our study has also reported higher prevalence as compared to the previous study (Babeker and HassabElrasoul in 2014) who reported 52.2% in west Omdurman. The difference in the prevalence between the two studies could be due to the difference in the study areas.

Another study in Egypt conducted by (EL Said *et al*., 1998) in Sudanese camel exported to Egypt using CATT/*T. evansi* showed lower prevalence of 28%. It is known that exported animals are chosen based on their health condition. Therefore, apparently healthy animals are expected to show lower prevalence.

3.2. **Effect of sex on infection of camels with *T. evansi*:**

We investigated the relationship between CATT/*T. evansi*-positive samples and sex. The infection rates of *T. evansi* in camels (males and female) in two locations are presented in table (2 and 3). The prevelance of Guffar disease in female in 44 she camels (75.9%). while in male only 14 (24.1%) in Total of 58 camels in Two study areas.

The present study shows that there was no significant difference ($p<0.05$) between the prevalence of the disease in males and females; similar results were reported by (Elamin *et al*., 1998), who reported that there was no significant difference between the infections rates of males and females of camels in several locations in the Butana plain.

	Male	Female	Total
Positive	7 (24%)	21 (70%)	28 (94%)
Negative	1 (3%)	1 (3%)	2 (6%)
Total	8 (27%)	22 (73%)	30 (100%)

Table (1): The prevalence of *T. evansi* infection within males and females in Wd-Alhilao area.

	Male	Female	Total
Positive	4 (14%)	15 (54%)	19 (68%)
Negative	2 (7%)	7 (25%)	9 (32%)
Total	6 (21%)	22 (79%)	28 (100%)

Table (2): The prevalence of *T. evansi* infection within males and females in Sitate River area

3.3. Effect of age on infection of camels with *T. evansi*:

Animal were sorted into three groups based on their ages (Table. 4). No significant difference was observed ($p<0.05$) in the prevalence of the disease within different age groups. However, it is clear that the more getting old the more positive animals were reported. This could be observed in the (9-12) group.

Age (years)	Positive	Negative	Total
1-4	3 (5%)	1 (2%)	**4 (7%)**
5-8	11 (19%)	5 (9%)	**16 (28%)**
9-12	33 (56%)	5 (9%)	**38 (65%)**
Total	47 (80%)	11 (20%)	**58 (100%)**

Table (3): The prevalence of *T. evansi* infection within different camels' ages groups.

Conclusion

1. The results obtained during this study using CATT/*T. evansi* showed camel trypanosomosis caused by *T. evansi* is endemic with prevalence of 81% in 58 tested camels from Wd-alhilo and Sitate River.

2. No significant difference in the prevalence of the disease between males and females or between age groups.

3. There is a high need to improve control policies and institute measures that help prevent the spread of the parasite.

3. It's being a zoonotic parasite (Van Vinh Chau *et al*., 2016), The endemicity of *T. evansi* in the study area in our study may have economic consequences, and highlights the potential for further human cases.

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