

INCIDENCE AND TREATMENT OF CAMEL TRYPANOSOMOSIS (GUFFAR) IN WEST OMDURMAN IN SUDAN

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ABSTRACT: The incidence of camel trypanosomosis (Guffar) caused by *Trypanosoma evansi* (T.evansi) in Omdurman west plain, western Sudan was surveyed using direct smear, Micro Hematocrit Centrifugation Technique (MHCT) and Card Agglutination Test for T.evansi (CATT/T.evansi). In a total of 220 blood and serum samples, 115 (52.2%) were positive by CATT, 72 (32.7%) were positive by the MHCT and 31 (14.1%) were positive by the wet smear preparation. Contingency tables and parcel Chi² - test revealed that CATT/T.evansi was statistically the most sensitive technique for T.evansi followed by the MHCT and lastly the wet smear technique. The percentage packed cell volume (PCV %) differed significantly between the diagnostic techniques used. Thus wet smear technique detected positive camels with the lowest PCV%. Camels infection rate with T.evansi did not differ significantly with sex. Treatment of rats infected with T.evansi isolates from Omdurman west area with quinapyramine pro-salt made by three different manufacturers revealed that Tryquine (Wockharde, India) was the most effective in clearance of parasitaemia within two weeks. Biquin (Star, Pakistan) and quinapyramine (Nicholas primal, India) did not clear the parasitaemia in rats during the same period. The results are discussed in relation to studies leading to control of T.evansi in camels using chemotherapy and chemoprophylaxis.

Keywords: Trypanosomosis, Dromedary camel, Guffar, MHCT, CATT.

ORIGINAL ARTICLE
Received 15 May, 2014
Accepted 30 May, 2014

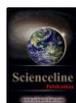
INTRODUCTION

The One-humped (dromedary) camel (*Camelus dromedarius*) is the most numerous animal in the semi-arid and arid tropical areas of Africa. In the Sudan, the camel population exceeds 3.1 million head (Anon, 2000). In fact about 20% of the world camels are found in the Sudan. Here, camels are important source of meat, milk and hair, and are used for transportation and drought power, they are also exported to north Africa and the middle east thus contributing a significant proportion to the gross national product (GNP). The main owners are pastoralists in northern Sudan, and although many are still nomadic, an increasing number are settling as agro-pastoralists into the large agricultural schemes in eastern Sudan, in such schemes they keep camels and small ruminants on the products of, e.g., Durra or sorghum spp. (Abo Sin, 1988).

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

Biologically T. *evansi* is very similar to T. *equiperdum*, the causative agent of dourine (Brun et al., 1998; Claes et al., 2003), and morphologically resembles the slender forms of the tsetse-transmitted species, T. *brucei*, T. *b. gambiense* and T. *b. rhodesiense*. Most of the molecular characterisations indicate that various strains of T. *evansi* isolated from Asia, Africa and South America are very homogeneous and may have a single origin (Ventura et al., 2002), but other works suggest that T. *evansi* could have emerged from T. *brucei* in several instances (Jensen et al., 2008; Lai et al., 2008). Molecular characterisation using random amplified polymorphic DNA techniques and endonuclease fingerprinting showed that isolates of T. *evansi* and T. *equiperdum* formed a closely homogeneous group. The difficulties in differentiating T. *equiperdum* from the other Trypanozoon spp. have been stressed (Claes et al., 2005; Zablotskij et al., 2003), and the existence of T. *equiperdum* was even questioned.

T.evansi belongs to the genus *Trypanosoma* which are haemo flagellate protozoa that belong to the order: Kinetoplastida. They parasitize man, domestic and wild animals causing trypanosomosis, unlike other parts of the world. In the Sudan, T.evansi is primarily a parasite of camels causing a disease locally known as Guffar. The disease is common in Kordofan and Darfur states in the west, Kassala, Gadaref and Red sea states in the east and to a lesser extent in central Sudan, in the Gezira, Sennar, Blue Nile and Khartoum states (Karib, 1961). Guffar is believed to be transmitted mechanically by biting flies, particularly, horseflies (Diptera tabanidae) (Lossos, 1986).



Generally, the disease assumes a chronic course resulting in serious morbidity and moderate mortality. Diagnosis is largely based on demonstration of the causative agent by the standard trypanosome parasitological methods, serological tests have also been developed to detect antibodies (Luckins, et al., 1979; Radostits, et al., 2007).

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

The Objectives of this study is to determine the prevalence of T.evansi infection in camels in Omdurman west, to compare the sensitivity of direct parasitological diagnostic methods versus the most recent serological diagnostic techniques and to document the effect of sex, and location on the prevalence of T.evansi in camels in the study area.

MATERIAL AND METHODS

Description of the study Area and Population:

The incidence of camels trypanosomosis (Guffar) caused by T.evansi was studied in Omdurman west area from February to November in 2013. Location Omdurman at the intersection of latitude 15 degrees 41 minutes north, and longitude 32 degrees 37 minutes east, on the west bank of the Nile opposite the coupler with a tributary of the white Nile, and of both Khartoum north, which are connected by the bridge of the white Nile and bridge Shambat. And likely to Omdurman was the village located to the south of the site with the city today that they were not connected to them at the beginning of its inception.

Since camel populations are rarely sedentary, camels tend to move in large groups. The area distribution of these groups is often sparse and animals congregate in masses only during the summer settlements. In addition to the normal human imposition on the distribution of camels, the limitation of food and water often necessitate better and more efficacious use of the available resources by a wide dispersal even of camels within a small herd or group, camels are more abundant around the agricultural schemes along the main permanent rivers of the region (Babiker, 1984).

In this study samples were collected from three different locations in Omdurman west. Alsufera which is located in the center towards the northern parts of Omdurman west plains and is characterized by sparse grassland while the woody vegetation is found only in shallow seasonal water courses (wadis). About 74 samples were collected from this area. The second area was Omdurman west; this is characterized by an extensive woody cover comprising *A. merillifera* (kitir), *capparis deciduas*, and grasses. The total number of samples collected from this area was 73 samples. The third area was Almweleh which is located in the southern part of Omdurman west plains. In this area there is no vegetation cover because it is utilized in mechanized sorghum and sesame farming. The area is used as livestock route during the annual migration of animals from north to south and vice versa .

Experimental animals

Camels: About 220 randomly selected adult camels (36 males and 184 females) were examined for infection with T.evansi. These included 74 animals at Alsufera, 73 at Omdurman west and 73 at Almweleh.

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

Diagnosis of T.evansi:

Direct Parasitological methods:

Wet blood smears preparation: Wet blood films were prepared by aseptic puncture of peripheral ear veins of camels using sterile needle. A drop of blood was then taken on to a clean glass slide, and covered with cover slip before examination for T.evansi under compound light microscope at X400 magnification (Nantulya, 1990).

Dry blood smears preparation: For identification of the causative trypanosome (e.g. T.evansi), simultaneous dry blood films were taken from the ear vein. These were stained by 19% Gimsa stain and observed under a compound light microscope using oil immersion lens (X1000) (plate 3,4) (Hilali et al., 2004).

Micro Hematocrit Centrifugation Technique (MHCT): During blood collection for serum, tow heparinized micro-capillary tubes were filled to the marked level with fresh blood from each vacutainer of each animal. The ends of the tubes were then sealed with crystalline wax and the tubes placed in a microhaematocrit centrifuge. They were then centrifuged at 1500 rpm for 5 minutes. There after each tube was placed on a McMaster slide and examined for motile trypanosomes at X100 magnification (Kayang et al., 1997).



Buffy Coat examination using wet preparation: To increase the sensitivity of the MHCT, a wet blood film was made of the buffy coat/plasma junction, after breaking the micro-capillary tube. The preparation was then examined for motile trypanosomes under a compound light microscope at X 400 magnification (Jamonneau, 2000).

Packed cell volume (PCV): The percent packed cell volume (PCV %) was determined by the PCV reader. Each rotated capillary tube was placed on the groove and the scale was moved to the point where the length of the packed cells ended (Luckins, 1999).

Serological Method

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

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

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

A 2.5 ml of CATT buffer was added to a vial of freeze dried CATT antigen using sterile syringe. The vial was then shaken for a few seconds so as to obtain a homogeneous suspension. 0.5ml of CATT buffer was added to the vials of positive and negative controls using respectively sterile syringe. On a test area of the card, 25 u 1 of the non-diluted serum was added the well containing the homogenized CATT antigen (approximately 45µ1). After tilting the card gently, agglutination was observed and the degree of agglutination was determined as follows:-

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

Chemotherapy:

Trypanosoma Evansi Isolates: About 5ml of blood were taken from the jugular vein of a few infected animals with typical patent parasitaemia. 0.5ml of the infected blood was then inoculated in each rat inter-peritoneally to T.evansi infection. Usually the rodents became parasitaemic on day 3 of inoculation. The isolates were kept alive by serial passage of the first peak of parasitaemia in more uninfected rats until treated with the trypanocide under test (Luckins, et al., 1979).

Infection of rats destined for chemotherapy: Rats were divided randomly into 4 groups each consisting of three rodents. All individuals in each group were inoculated with 0.5ml of infected blood containing approximately 106 trypanosomes to propagate infections (Hoare, 1972).

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

Treatment: The groups of rats were treated with three different commercial compounds of quinapyramine pro-salt, and these compounds were tryquine (quinapyramine sulphate and quinapyramine chloride) wockarde (India), Biquin (quinapyramine sulphate and quinapyramine chloride) star (pakistan) and Quinaoyramine injection (quinapyramine sulphate) Nicholas Primal (India). All drugs were administrated at the rate of 4mg/kg when parasitaemia was 106/ml or above at day 6. The drugs were administered subcutaneously to rats (Wahaba, 1999).

Statistical analysis:

All data on prevalence of T.evansi were subjected to Chi-square test using stata software, loaded in a desk-top personal computer to elicit significant differences in infection rats using direct parasitological and serological methods as related to sex, and location. To determine the level of agreement among the diagnostic tests, the results of analyses were subjected to cross tabulation and kappa coefficient analysis (Saad and Magzoub, 1989). Kappa statistics used as a measure of agreement between two tests. it is based on the difference between the observed probability of agreement and the probability of agreement expected by chance and standardized by



maximum possible agreement beyond chance. A kappa of 1 indicates perfect agreement and beyond chance gives a kappa of zero. A kappa of at least 0.4-0.5 indicates a moderate level of agreement.

RESULTS

Reliability of CATT-T.evansi:

T.evansi infections were diagnosed by examination of some 220 camels using wet preparations, Buffy Coat and CATT-T.evansi techniques. Table (1) shows that 31 (14.1%), 72 (32.7%) and 115 (52.2%) camels were infected by T.evansi by the wet preparation, Buffy Coat and CATT, respectively. However, 105 (47.7%) camels were found uninfected irrespective of the diagnostic technique used (Table 1). The contingency table Chi²-test showed that infection rates differed significantly with the diagnostic technique used (Chi² = 72.51, df = 3, p = 0.0000007). Serial paired comparisons of the same data revealed that the infection rates also differed with the method of diagnosis, the highest difference being recorded between the wet smear and the Buffy Coat (Chi² = 20.28, df = 1, p = 0.00001, buffy coat/ CATT, Chi² = 16.41, df = 1, p = 0.0001). Thus in terms of sensitivity the wet preparation seemed the least effective, The Buffy Coat was intermediate between the two techniques while the CATT was the most effective.

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

Table 1 - The number and percentage of animals infected with T.evansi using three diagnostic techniques

Test		Total number	Number of Infected	Examined	Percentage
Wet smear		220	31		14.1%
Buffy coat	X ² =20.28 P≤0.00001	220	72		32.7%
CATT	X ² =16.41 P≤0001	220	115		52.2%
Chi ² Probablity			X ² =72.51 P≤0.0000007		

X²= Chi², P= Probablity and CATT= Card Agglutination Test for Trypanosomes.

Table 2 - The degree of intensity of agglutination of positive samples in CATT test (N = 220 camels)

Degree of agglutination	±	+	++	+++
Number of Positive Samples	10	72	26	7
Percentage	8.7%	62.2%	22.6%	6.1%

±: Weak agglutination. +: Moderate agglutination; ++: Strong agglutination. +++: Very strong agglutination.

Percent Packed cell volume (PCV%):

Since all 220 camels were screened for T.evansi using the Buffy Coat together with the wet smear and CATT methods, data on PCV% were, thus, available for each individual animal, regardless of the diagnostic method. These PCV data were then divided into four groups according to the diagnostic technique and whether the animal was infected or uninfected Table 3. The PCV groups were <20%, 20-25%, 26-30% and >30%. Chi² statistics of the grouped data in Table 3 showed that there was a highly significant difference between the diagnostic techniques in the percentages of animal with PCV 20-25% (Chi² = 43.92, df = 3, p < 0.0000007), >30% (Chi² = 58.76, df = 2, p < 0.0000007) but not with animals having a PCV in the range 26-30% (Chi²= 4.68 d.f = 3, p = 0.199). Data on animal with PCV < 20% had not been included because they were unsuitable for Chi² statistics (no more than 2 animals in a cell). Regards animals in PCV group 20-25%, subsequent paired comparisons showed that there were significant differences between the numbers of infected animals detected with wet smear and Buffy Coat (Chi² = 0.0004, df = 1, P = 0.98). Buffy Coat/CATT (Chi² = 4.96, df = 1, P<0.02) and CATT/uninfected animals (Chi²=31.46, df=1, P < 0.0000007). In other groups i.e. 26-30% and >30% PCV, similar comparisons showed a significant difference between CATT servo positive and the uninfected camels only (Chi² =58,76, df= 1,P=0.0000).

Effects of sex and location on infection of camels with T.evansi:

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

The infection rates of T.evansi of camels (male + female) in the selected three locations are presented in Table 5. With any one diagnostic method the disease prevalence was higher in Almweleh followed by Alsufera and lastly Omdurman west. Despite the latter statement there were no significant differences in infection rates between locations.



Comparison between parasitological and serological finding using Kappa statistics:

The level of agreement between Buffy Coat and Direct Smear was found to be high (81.36%) with kappa coefficient value of 0.504 (Table 6). On the other hand, the level of agreement between Direct Smear and CATT was relatively low (58.18%) with low kappa coefficient value of 0.190 (Table 6). Also the level of agreement between Buffy Coat and CATT found to be high (73.18) with kappa coefficient value of 0.472 (Table 6).

Table 3 - Range of PCV% of infected and uninfected camels according to the diagnostic technique used

PCV Test	<20%	20 - 25%	26 - 30%	> 30	Total
Wet smear	2 (6.5%)	17 (54.8%)	10 (32.3%)	2 (6.5%)	31
Chi² Probablilty	- -	$\chi^2=0.0004$ $p=0.98$ $df=2$	$\chi^2=0.04$ $p=0.95$ $df=3$	$\chi^2=1.36$ $p<0.25$ $df=1$	
Buffy coat	2 (2.8%)	41 (57%)	22 (30.6%)	7 (9.7%)	72
Chi² Probablilty	- -	$\chi^2=4.96$ $p<0.02$ $df=1$	$\chi^2=2.98$ $p<0.09$ $df=2$	$\chi^2=31.46$ $p<0.000007$ $df=3$	
CATT	2 (1.7%)	45 (39.1%)	51 (44.4%)	17 (14.8%)	115
Chi² Probablilty	- -	$\chi^2=19.50$ $p<0.0001$ $df=1$	$\chi^2=1.99$ $p<0.16$ $df=4$	$\chi^2=58.76$ $p<0.000007$ $d.f=2$	
Number of Uninfected	2 (1.7%)	10 (10.9%)	31 (33.7%)	48 (52.2%)	91
Chi² Probablilty	- -	$\chi^2=43.92$ $p<0.00000007$ $df=3$	$\chi^2=4.68$ $p<0.20$ $df=3$	$\chi^2=62.02$ $p<0.0000000$ $df=2$	

$\chi^2=$ Chi², P= Probability, d.f= degree of freedom and CATT= Card Agglutination Test for Trypanosomes.

Table 4 - Prevalence % of T.evansi in male and female camels using three diagnostic techniques in Omdurman west area

Sex	Wet smear	Buffy coat	CATT
Female (N=184)	30 (16.3%)	65 (35.3%)	99 (53.8%)
Male (N=36)	1 (2.8%)	7 (19.4%)	16 (44.4%)
Chi² Probablilty	-	$\chi^2= 1.06$ $P=0.3$ $df= 1$	$\chi^2=0.72$ $P=0.39$ $df= 1$

$\chi^2=$ Chi², P= Probability, D.F= degree of freedom, N= Number of camels and CATT= Card Agglutination Test for Trypanosomes.

Table 5 - Prevalence of infected camels according to location in Omdurman west area

Location	Wet smear N= 31	Buffy coat N= 72	CATT N= 115
Almweleh	16 (51.6%)	30 (41.7%)	44 (38.3%)
Chi² Probablilty	$\chi^2 = 5.7$ $P\leq 0.02$		
Omdurman west	6 (19.4%)	19 (26.4%)	30 (26.1%)
Chi² Probablilty	$\chi^2=0.35$ $P=0.55$		
Alsufera	9 (29%)	23 (32%)	41 (35.6%)
Chi² Probablilty	$\chi^2=7.65$ $P=0.14$	$\chi^2=3.88$ $P=0.02$	$\chi^2=4.25$ $P=0.12$

$\chi^2=$ Chi², P= Probability, N= Number of camels and CATT= Card Agglutination Test for Trypanosomes.

Table 6 - Agreement between different diagnostic techniques using sigma stata for windows version 2.0 (Kappa coefficient)



Agreement Expected Kappa Z					Pr ≥ Z
Agreement					
DS x BC	81.36%	62.40%	0.5043	8.61	0.0000
DS x CATT	58.18%	48.37%	0.1901	8.61	0.0000
BC x CATT	73.18%	49.21%	0.4719	7.58	0.0000

DS= Direct Smear, BC= Buffy Coat, CATT= Card Agglutination Test for Trypanosomes and Pr = Probability.

Comparison between parasitological and serological finding using the cross tabulation statistics:

The cross tabulation between Direct Smear and Buffy Coat examination results revealed that 31 positive T.evansi samples were agreed upon by the two tests (Table 6). Out of 72 positive sample detected by Buffy coat. Also the two tests agreed on 148 negative samples, out of 189 negative samples by Direct Smear on the other hand the two tests were disagreed on 41 samples, which were positive, by Buffy Coat but negative using Direct Smear (Table 6).

The cross tabulation between Direct Smear and CATT results showed that the two tests agreed on 27 positive samples out of 31 (87.1%) positive samples detected by Direct Smear and 115 positive samples using CATT Table 7. Also 101 negative samples were agreed upon by the two tests, out of 189 (53.3%) negative samples detected by Direct Smear and 105 negative samples by CATT. On the other hand, the two tests disagreed on 4 samples, which were positive by Direct Smear but negative by CATT. Even there were 88 samples positive by CATT but negative by Direct Smear (Table 7).

The cross tabulation between Buffy Coat and CATT results showed that 64 positive samples agreed upon by the two tests out of 72 positive samples detected by Buffy Coat and 115 positive samples using CATT (Table 8). Also the two tests agreed upon 97 negative samples out of 148 negative samples using Buffy Coat and 105 negative samples using CATT. The disagreement between the two tests was reflected in 8 samples, which were positive by Buffy Coat, but negative, by CATT. Also there were 51 samples positive by CATT but negative by Buffy Coat (Table 8).

The cross tabulation between Direct Smear and CATT results showed that 27 positive samples agreed upon by the two tests out of 31 positive samples detected by Direct Smear and 115 positive samples using CATT Table 9. Also the two tests agreed upon 101 negative samples out of 189 negative samples using Direct Smear and 105 negative samples using CATT. The disagreement between the two tests was reflected in 4 samples, which were positive by Direct Smear, but negative, by CATT. Also there were 88 samples positive by CATT but negative by Direct Smear (Table 9).

Table 7 - Cross tabulation between results obtained from Buffy Coat and Direct Smear

Direct Smear	Buffy Coat		
	Negative	Positive	Total
Negative	148	41	189
Positive	0	31	31
Total	148	72	220

Table 8 - Cross tabulation between results obtained from Buffy Coat test and CATT

Direct Smear	Card Agglutination Test for Trypanosomes (CATT)		
	Negative	Positive	Total
Negative	97	51	148
Positive	8	64	72
Total	105	115	220

Table 9 - Cross tabulation between results obtained from CATT test and Direct Smear

Direct Smear	Card Agglutination Test for Trypanosomes (CATT)		
	Negative	Positive	Total
Negative	101	88	189
Positive	4	27	31
Total	105	115	220

Chemotherapy:

Estimation of Antilog Parasitaemia: Following inoculation of rats with T.evansi, the infected rats became parasitaemic on day 2 from day 2 on wards parasitaemia increased progressively when it reached about antilog 8 on Day 14. Rats showing parasitaemia at log 6.9 ± 0.50 on Day 6 were treated with one of quinapyramine pro-salt. Those treated with Quinapyramine (Quinapyramine sulphate Nicholas primale, India) showed drop in parasitaemia from antilog 7 to 5.4 on Day 8. There for the parasitaemia increased to reach antilog 7.9 on Day 14. The parasitaemia in the rats treated with Biquin (Star, Pakistan) fell to antilog less than 5.4 on Day 8 and increased again tell it reached antilog 8.5 on Day 14. Tryquin (Wockarde, India) treated rats witnessed a drop of parasitaemia to antilog 5.7 on Day 8 and remained so till the parasitaemia finally disappeared on Day 12 (Figure 1).



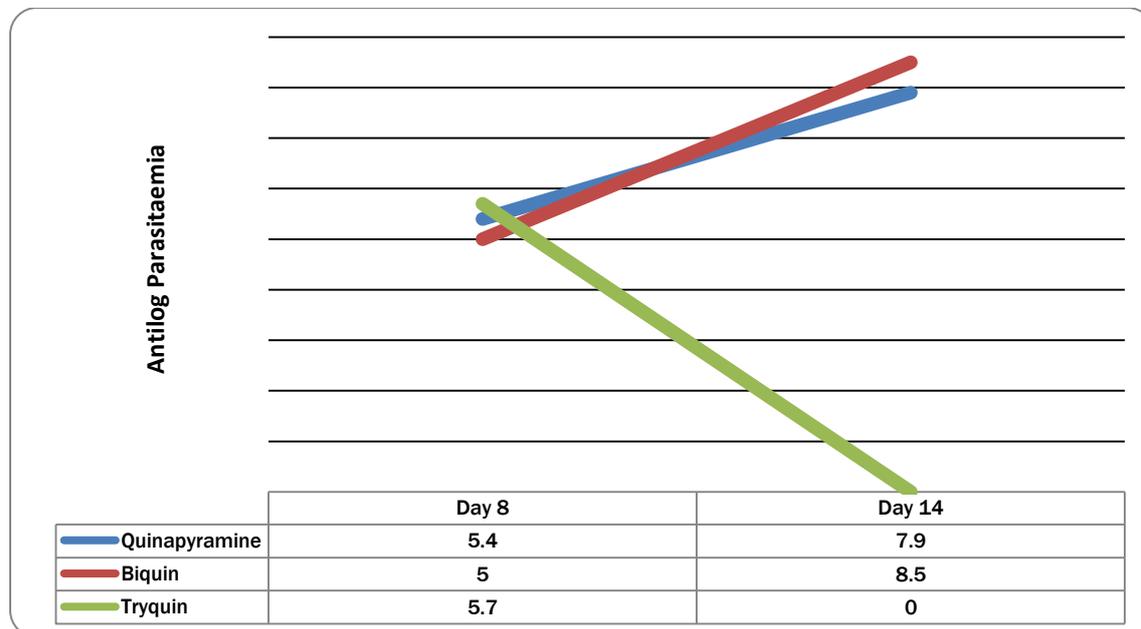


Figure 1 - Estimation of Antilog Parasitaemia of rats with *Trypanosoma Evansi* to three drugs: Quinapyramine sulphate Nicholas primale; India, Biquin (Star, Pakistan) and Tryquin (Wockarde; India)

DISCUSSION

Camel trypanosomosis present special problems with regard to diagnosis. The clinical signs are not pathognomonic and the standard techniques for the detection of trypanosomes are not sufficiently sensitive (Boid et al., 1985; Tran et al., 2009). Although significant improvement have been made recently in diagnosis, a high proportions of infections still remain undetected as the chronic, more common form of the disease is often aparasitamic (Luckins et al., 1979; Brown and Torres, 2008). In the face of these constraints, alternative methods of diagnosis have been developed, most of which are for the detection of antibody response to the antigens of the circulating trypanosomes (Allen et al., 1992; Anonymous, 2008).

The number and percentage of animals infected with *T.evansi* using three diagnostic techniques (wet smear, Buffy Coat and CATT) are shown in table (3.1). The results obtained in this study using wet smear preparations showed that, overall, 14.1% (31 out of 220 camels) were infected with *T.evansi*. This rate is higher than that reported by Dafaalla(1988), similar of that obtained by seroprevalence of the trypanosomosis using card agglutination test for trypanosomiasis (CATT) in Morocco, Spain and France (Atarhouch et al., 2003; Tamarit et al., 2010; Desquesnes et al., 2008). Who surveyed camel trypanosomosis in Gedarif, Showak, Kassla, and New Halfa. The infection rates reported by Dafaalla (1988). In these localities were comparatively very low ranging between 1.12% and 2.13%. Examination of camels with the buffy coat, in the present work showed an infection rate of 32,7% which was double the rate obtained by the wet smear methods above. This indicates that the Buffy Coat method was at least two times as sensitive as the blood films techniques. El-Amin et al., (1998) working in omdurman west using the same diagnostic methods as ours reported a rather lower infection rates with *T.evansi* in camels which ranged between 1.8% to 7.3%.

Comparison of previous, recent and present *T.evansi* infection rates in camels in Omdurman west area suggest that the prevalence of camels trypanosomosis due to *T.evansi* in western Omdurman is increasing. One reason for this increase may be the inadequate treatment. Field veterinarians use an array of trypanocidal drugs against guffar such as Cymelarsan, Ethidium bromide, Isometamedium chloride and Qinopyramine compounds but critical assessment of the efficiency of these drugs in the national Laboratories has yet to be fully instituted.

The second reason may be drug-resistance of *T.evansi* to the drugs in common use (Elrayah et al., 1999; Mohamed Ahmed et al., 1992; Luckins, 1988). Drug resistance may arise as a result of sub dosing, high trypanosomosis challenge and the faulty administration of drugs.

Of the diagnostic techniques employed in the present work, The CATT was relatively highly more sensitive and spensitive for detection of *T.evansi* infection than the wet smears or buffy coat. Similar results were also reported by Nantulya (1990). Our results obtained by CATT indicated that 52.2% of the 220 camels were infected which was three times as sensitive as the buffy coat. The later technique was mor than two times as sensitive as the stained blood films. As far we know this is the first record of employment of CATT-*T.evansi* in the diagnosis of camel trypanosomosis in the Sudan.

However, a recent study in Egypt conducted on imported Sudanese camels using CATT showed that 28% of the animals were positive for *T.evansi* (Elsaid et al., 1998). The latter authors concluded that the test was both sensitive and reliable for the diagnosis of camels with chronic and latent *T.evansi* infections.

Considering the intensity of agglutination, it appears that four levels are manifested (Table 2). The most frequent intensities were observed to manifest themselves at the moderate and less intense agglutination levels.

This indicates that the titers of antibody in the sera were not high enough which might be a reflection of the chronicity of the infection.

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

T.evansi infected camels show a low grade anemia, which is the main feature of camel trypanosomosis (Fatihu et al., 2000). Death due to trypanosomosis is usually a result of severe anaemia, and animals that are capable of compensating the reduction in PCV and erythrocytes indices during the course of infection often survive (Onah et al., 1996). In this study the PCV of the infected camels was lower than that of the negative or uninfected animals. This low PCV level can be attributed to parasitaemia and subsequently the destruction of erythrocytes by T.evansi haenogellates. However, this may not be a universal proposition, since Boid et al. (1981) reported that T.evansi appeared to have little effect on the haematological picture and PCV of infected sheep, though there was a progressive fall in the PCVs of similarly infected goats and camels.

The present study shows that there was no significant difference in T.evansi infection rates between males and females. Similar results were reported by El-Amin et al. (1998). Who showed that there was no significant difference between the infection rates of males and females of camels in several locations in Omdurman west area. However, T.evansi infection rate differed significantly between locations.

These flies, Tabanidae in particular, are considered the main mechanical vectors of T.evansi. This may help to explain why infection rates of camels with T.evansi were higher in the wet locations. It also explains why camels in Almweleh area had a higher infection rate with T.evansi than Elsufera and Omdurman west area. Almweleh is very close to higher number of water bodies, therefore, increase the percentage of the presence of the fly.

Conclusions and Recommendations:

- 1- The card agglutination test for trypanosomosis (CATT-T.evansi) was found to be a good serological test in the field and as a screening test for guffar among camel herds. It is also easy to use in day to day diagnosis.
- 2- It is suggested that camel trypanosomosis is a serious problem in Omdurman west. There is thus a need to continue studies focusing on the control of the disease in the area.
- 3- Since treatment of T.evansi with Antrycide pro-salt and Biquin did not clear completely the parasitaemia in infected rats we suspect that some isolates had acquired resistance to both drugs. We therefore recommend investigations into drug resistance of isolates of T.evansi from various locations in Omdurman west area.

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