

**Sudan University of Science and Technology**

**College of Graduate Studies**

**Seroprevalence of *Trypanosoma evansi* infection in dairy cattle in Khartoum State, Sudan**

مسح مصلي لنسبة الاصابة بطفيل التريبانوسوما ايفانساي وسط ابقار الحليب بولاية الخرطوم، السودان

By

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

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**June 2019**

## **DEDICATION**

*This work is dedicated to my great mother, father, and friends  
with great love, respect and gratitude.*

.

## ACKNOWLEDGEMENTS

Firstly, praise be to Almighty ALLAH (most, gracious most merciful) who gave me the health and strength to complete this work. I would be always indebted to Dr. Ehab Mossaad, my supervisor for planning my study, designing my research work which I was most interested, critical appraisal and correction of the manuscript and compilation of thesis work and above all for supplying my research materials. More importantly, he taught me how to work hard and to come out of the comfort zone in order to achieve greater things in the life. I gained more confidence under his supervision and got lot of knowledge in the research field. I consider myself fortunate enough being his student.

It is my great pleasure to express deepest of gratitude to Dr. Mohei Eldeen Ahmed of the Ministry of Animal resources and Fisheries for helping me in the sampling.

I am also pleased to thank Dr. Elshafee Ibrahim of the Central Veterinary Research Laboratory for helping me with the statistical analysis.

I extend my grateful acknowledgements to Dr. Rawan Satty for helping me with the CATT/*T. evansi*.

I would also like to extend my grateful and thankful acknowledgements to Dr. Fahad Elgazali of Central Veterinary Research Laboratory for helping me in writing my thesis.

Finally I would like to appreciate the financial support received by my supervisor from the Ministry of Higher Education and Scientific Research, Republic of Sudan (Grant No. SRI-VS-2015-71933).

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## **Abstract**

*Trypanosoma evansi* parasite has a wide range of distribution throughout tropical and subtropical regions of the world. In this study we have investigated the prevalence of the disease in dairy cattle in Khartoum State, Sudan using the card agglutination test (CATT/*T. evansi*) which is recommended by OIE. Within 304 blood samples, antibodies against *T. evansi* were detected in 91 (29.9%). Antibodies against *T. evansi* were detected in 31 (31%) animals in Omdurman, 29 (28.4%) animals in Khartoum and 31 (30.4%) animals in East Nile localities. With regard to sex, antibodies were detected in 13 (21%) male's samples and in 78 (32.2%) female's samples. While within different age groups, antibodies against *T. evansi* were detected in 36 (25.7%) of animals < 1year old, in 10 (30.3%) of animals between 1-3 years old and in 45 (34.4%) of animals >3 years. In all risk factors assessed, there was no significant statistical difference recorded. The finding that bovine trypanosomosis caused by *T. evansi* is highly prevalent in the country, suggests the need for stringent control policies and the establishment of measures to help prevent the spread of the parasites within cattle and cattle to other susceptible animals like camels. To the best of our knowledge, this is the first report on cattle trypanosomosis caused by *T. evansi* infection in Khartoum State, Sudan.

## ملخص البحث

طفيل التريبانوسوما ايفانساي من الطفيليات واسعة الانتشار في المناطق المدارية وشبه المدارية.

اجريت هذه الدراسة لتقصي نسبة انتشار الاصابة بالطفيل ومعامل الخطر وسط ابقار الحليب في ولاية الخرطوم، السودان بإستعمال اختبار الكات الموصى به من قبل منظمة الأوبئة العالمية.

من بين ثلاثمائة واربعة عينة دم جمعت من الابقار بتقب الوريد الوداجي وجد ان 91 (29.9%) من الابقار مصابة بالطفيل. منها 31 (31%) في محلية امدرمان، و 29 (28.4%) بمحلية الخرطوم بينما 31 (30.4%) في محلية شرق النيل. كما وجد أن 13 (21%) من الذكور و78 (32.2%) من الإناث مصابة بالطفيل. وعند تقسيم الأبقار حسب فئاتها العمرية وجد أن 36 (25.7%) من الأبقار أصغر من عمر السنه و 10 (30.4%) بين سنة وثلاث سنوات و 45 (34.4%) أكبر من ثلاث سنوات وجدت مصابة بالطفيل. بين كل عوامل الخطر لم يسجل اي فرق إحصائي مؤثر للإصابة بالطفيل.

نتائج هذا البحث تشير إلى ان أبقار الحليب في منطقة الخرطوم وجدت مصابة بنسب مؤثرة مما يتطلب إجراءات إحترازية من السلطات البيطرية في الولاية لمنع مزيد من الإنتشار بين الأبقار ومن الأبقار إلى الحيوانات الأخرى مثل الإبل.

الجدير بالذكر فإن هذه أول دراسة لتقصي معدل إنتشار المرض وسط أبقار الحليب في ولاية الخرطوم.

## **Introduction**

*Trypanosoma evansi*, the agent of surra, is a salivarian trypanosome, originating in Africa. It is thought to derive from *Trypanosoma brucei* by deletion of the maxicircle kinetoplastic DNA (genetic material required for cyclical development in tsetse flies). It is mostly mechanically transmitted by tabanids and stomoxes, initially to camels, in sub-Saharan area (Desquesnes et al., 2013). It causes chronic wasting disease in a wide range of animals including cattle and buffalos (Sengupta et al., 2019). In Sudan, the parasite is known to affect camels (Mossaad et al., 2017a; Elamin et al., 1998; Babeker and Hassab Elrasoul, 2014; Salim et al., 2011; Ali et al., 2011), equines (Salim et al., 2014) and recently in dogs in Khartoum (Mossaad et al., 2017). In Sudan, cattle, sheep and goats undergo protracted infection in which they may play the role of a reservoir host (Malik and Mahmoud, 1978). Serological diagnostic methods are still commonly applied in large-scale epidemiological surveillance (Verloo et al., 2000). The recombinant variable surface glycoprotein RoTat1.2 showed no differences to the native antigen in serological diagnostic tests for *T. evansi* infection in dromedary camels (Lejon et al., 2005). The card agglutination test for *T. evansi* - CATT/*T. evansi* which is recommended by OIE (OIE, 2012) is a test utilizing the recombinant antigen RoTat1.2, and it is widely applied in the serodiagnosis of trypanosomosis caused by *T. evansi* in different animal species in Sudan including camels (Mossaad et al.,

2019) and dogs (Mossaad et al., 2017). In Khartoum State, Sudan, the dairy industry is very important production sector that provide milk to millions of people. However, no data describing the prevalence of the disease in cattle is available in Sudan.

**General objectives:**

In general the objective of this study is to investigate the prevalence of *T. evansi* infection in dairy cattle Khartoum State, Sudan.

**Specific Objective:**

1. To detect antibodies against *T. evansi* infection using the CATT/*T. evansi*
2. To assess some risk factors that may affect the prevalence of the diseases including area, sex and age of the animals.

# Chapter 1

## Literature review

### 1.1. *Trypanosoma evansi*

Trypanosomes found in mammals, including humans, are blood and sometimes tissue parasites of the order Kinetoplastida, family Trypanosomatidae, genus *Trypanosoma*. In the subgenus *Trypanozoon*, mechanical transmission of *T. evansi* usually takes place through contamination by sucking flies such as tabanids and stomoxes (Gingrich et al., 1983; Mihok et al., 1995) and tsetse as mechanical vectors (Roberts et al., 1989). In the particular case, *T. evansi* due to a loss of genetic material (maxicircle kinetoplastic DNA), the parasite can no longer undergo its cycle in tsetse flies, thus it is mainly mechanically transmitted by biting insects. For this reason, *T. evansi* spread outside the tsetse belt in Africa.

### 1.2. Origin, history, and geographical distribution

*T. evansi*, is the first pathogenic mammalian trypanosome to be described in the world, in 1880, by Griffith Evans, in the blood of Indian equines and dromedaries (Desquesnes et al., 2013). Its principal host is originally the camel but it is also present in horses and other equidae as well as in a wide range of other hosts. In Africa *T. evansi* is present in all countries where camels are bred, north of a line extending from Senegal (15° North) to Kenya (equator), above the tsetse belt. It is

found not only in Mauritania, Morocco, Algeria, Tunisia, Libya, Egypt, Sudan, Eritrea, and Ethiopia, but also in the northern parts of Mali, Burkina Faso, Niger, Nigeria, Chad, Somalia and Kenya (Desquesnes et al., 2013). Currently, its geographical distribution is continuous from the northern part of Africa through the Middle East to South East Asia. *T. evansi* is continuously present eastwards, in the Arabian peninsula, including Saudi Arabia, Oman, the United Arab Emirates, Jordan, Israel, Lebanon, Syria, Iraq, and Turkey, and even with one occasional record in Bulgaria; it is present from Iran to Kazakhstan as well as in Afghanistan and Pakistan (Desquesnes and Dia , 2003; Desquesnes et al., 2009; Hasan et al., 2009; Srivastava et al., 1984). *T. evansi* is present in India, China, Mongolia, Russia, Bhutan, Nepal, Myanmar, Laos, Vietnam, Cambodia, Thailand, Malaysia, the Philippines, and Indonesia (Luckins, 1988; Reid, 2002). It's so far absent from Australia (Luckins ,1988). The extension of *T. evansi* toward the West is more recent. It was introduced into Latin America in the fifteenth century on the Island of Marajo (Amazon estuary) in 1827 Paraguay, 1847 in Pantanal, 1850 in Brazil, and 1860 in Mato Grosso, Brazil. It is present in Central America up to Mexico (Desquesnes et al., 2013). *T. evansi* recently arrived in the Canary Islands (Spain) (Desquesnes et al., 2013). The geographical distribution of surra in the world is represented in (Fig. 1)

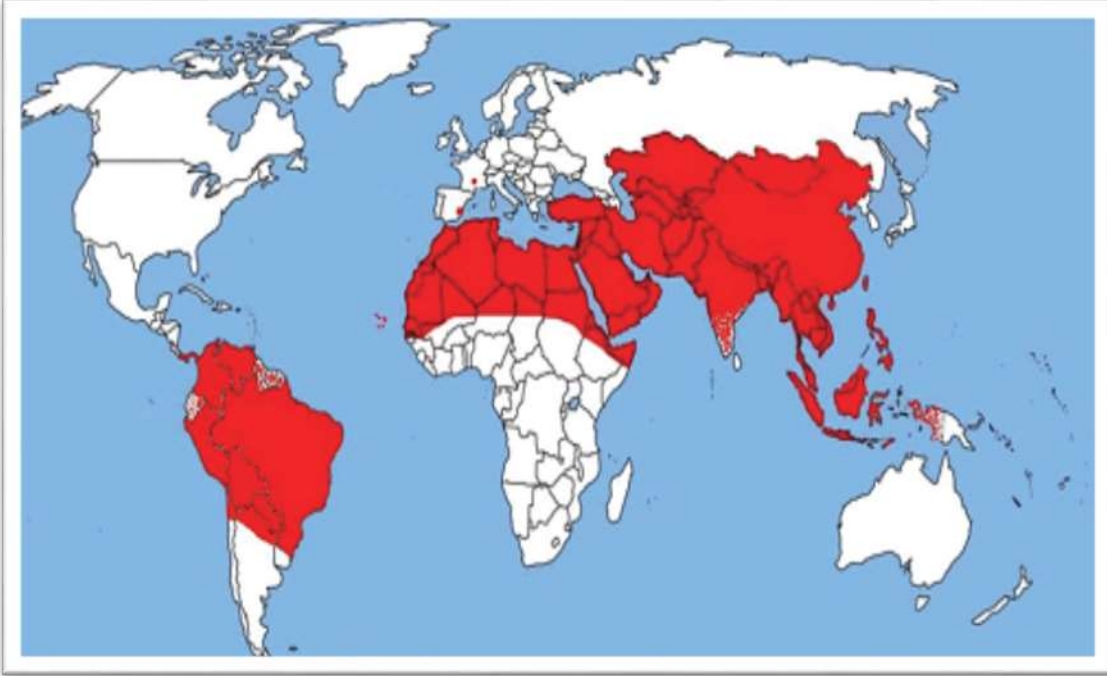


Fig. 1: Geographical distribution of *Trypanosoma evansi* in the world

Historically, *T. evansi* could only be eradicated from areas where it was detected very early and controlled. When introduced into America and Australia, in 1906 and 1907 most probably due to the existence of wild and domestic reservoir, the ability to be transmitted by nonspecific mechanical vectors present all over the world and its ability to diffuse silently via healthy carriers. The evolution of the geographical distribution of *T. evansi* is related to the movements of infected animals. Transmission inside an infected country, especially with healthy carriers such as bovine, carrying the parasite with mild or subclinical signs was recently observed in the Canary Islands, Spain and France (Gutierrez et al., 2009).

### **1.3. Disease synonyms and parasite taxonomy**

*T. evansi* belongs to the genus *Trypanosoma* subgenus *Trypanozoon* (salivarian section) together with (i) *T. brucei brucei*, one of the agents of a disease called Nagana in livestock. Nagana is a complex of diseases due to a number of *Trypanosoma* species including *T. brucei brucei*, *T. vivax* and *T. congolense* which have a great impact on cattle breeding in Africa. (ii) *T. brucei rhodesiense* and *T. brucei gambiense* are responsible for Human African Trypanosomosis (iii) *T. equiperdum*, which is sexually transmitted in equidae and is responsible for a disease called dourine. The word “surra” which describes the infection with *T. evansi*, came from the India and means “rotten” which qualifies the state of the animals after chronic evolution of the disease. In Africa, for example, surra is



found under the Arabic name Debab (El debab in Algeria) which means fly (linked to the vector) (Atarhouch et al., 2003) it is also known as Guifar in Sudan (Desquesnes et al., 2013),

#### **1.4. Morphology of *T. evansi***

*T. evansi* has the characteristics of slender *Trypanozoon* parasites: it is monomorphic thin trypomastegote parasite, with thin posterior extremity, free flagellum, central nucleus, small subterminal kinetoplast and highly visible undulating membrane. It has active movements but producing limited displacements in the microscope field. *T. evansi* is sharing some characteristics with *T. brucei brucei* and more generally with the subgenus *Trypanozoon* (Lai et al., 2008). *T. evansi* and *T. equiperdum* are different from *T. b. brucei* since they suffer from a mutation leading to the homogenization of their kinetoplastic minicircles, for this reason, they are unable to transform into procyclic stage, thus to implement a cycle in tsetse flies. They are consequently locked into the host as a blood stream form (Lai et al., 2008). They are also unable to recombine their DNA since this event occurs during the implementation of the cycle in the tsetse fly (Gibson and Stevens, 1999).

### **1.5. The Host Range of *T. evansi***

*T. evansi* has the widest host range amongst salivarian trypanosomes. It is especially pathogenic in camelids and equids. *T. evansi* also has a wide range of domestic and wild host's worldwide. The loss of maxicircle kinetoplast DNA was responsible for the large range of hosts of *T. evansi*, but the same effects did not lead to the same results in *T. equiperdum*. While almost all mammalian species are susceptible, their susceptibility not only is highly variable from one species to another but may also be variable from one geographical area to another. In Africa and the Middle East, *T. evansi* is mainly a parasite of camels (*Camelus dromedarius*), horses (*Equus caballus*), donkeys (*Equus asinus*) crossbreeds (mules), cattle (*Bos taurus*), sheep (*Ovis aries*) goats (*Capra hircus*), African buffalo (*Syncerus caffer*), cats (*Felis domesticus*) and dogs (*Canis familiaris*) (Desquesnes et al., 2013). In Asia, *T. evansi* is a major parasite for water buffaloes (*Bubalus bubalis*), elephants (*Elephas maximus indicus*), and antelope deer (*Cervus unicolor*) (Tuntasuvan and Luckins, 1998).

### **1.6. Clinical Signs**

The pathogenic effects of *T. evansi* are classical such as any other pathogenic mammal trypanosomes, including fever anemia, loss of appetite and weight, loss of condition and productivity, nervous signs and/or abortion, cachexia, and death,

with or without more peculiar signs related to the host species (Gardiner and Mahmoud, 1990). However, what is quite surprising is the variable intensity of these signs, from totally unapparent to lethal, from one to another host species, but sometimes trypanosomosis due to *T. evansi* has long been considered as a mild, chronic, or asymptomatic disease in Bovinae (*Bos bubalus*, *syncerus*, and *poephagus*). In Africa and Latin America, surra is still an important disease in cattle. In Indonesia, Surra infection results in anemia, losses in weight, milk and meat production, and losses in draught power (Pholpark et al., 1999; Kashiwazaki et al., 1998). In beef cattle, when surra occurs for the first time in a new area, high mortality can be recorded. In all cases, if the clinical signs recede, it is suspected that surra exacerbates other latent infections (Desquesnes et al., 2013).

### **1.7. Serodiagnosis of *T.evansi* infection**

Serological diagnosis is still remains as a common method for large-scale epidemiological surveillance (Verloo et al., 2000). Serological tests are able to reveal ongoing or past trypanosome infections based on antibody detection. For surra, the most specific antibody detection tests make use of the *T. evansi* specific variant surface glycoprotein (VSG) RoTat 1.2 as antigen. The CATT/*T.evansi* is such a test in the form of a direct agglutination test and is the only rapid diagnostic test for surra that is recommended by the World Organization for Animal Health (OIE, 2012; Bajyana and Hamers, 1988). The recombinant variable surface

glycoprotein RoTat1.2 showed no differences to the native antigen in serological diagnostic tests of *T. evansi* infection in dromedary camels (Lejon et al. 2005). Nevertheless, it failed to detect *T. evansi* type B which lacks or does not express RoTat1.2 (Tran et al. 2009; Ngaira et al., 2005). According to the World Organization for Animal Health (OIE, 2012), antibody detection ELISA using trypanosome crude antigen is considered as a conventional and standard method for the diagnosis of animal trypanosomosis. In addition to the conventional tests, the development of recombinant technology has resulted in the introduction of a number of new recombinant antigens, including *T. evansi* GM6 which consisted of 4 repeat domains (rTeGM6-4r) for use in disease diagnostic surveillance (Nguyen et al., 2014; Thuy et al., 2012; Goto et al., 2011). Nguyen and colleges have recently developed rTeGM6-4r based ELISA that is capable of detecting antibodies to animal trypanosomes in water buffaloes (Nguyen et al., 2014).

## Chapter two

### Materials and Methods

#### 2.1 Study area:

This cross sectional study was conducted in Omdurman, Khartoum and Khartoum Bahry, Khartoum State Sudan, in the period between May and December 2016.

#### 2.2. Samples

Blood samples were collected from 304 apparently healthy cattle from Omdurman, Khartoum and Khartoum Bahry, Khartoum State, Sudan in the period from May to December 2016. Samples were collected after obtaining consent from the cattle owners; 5-7 ml of blood was drawn from the jugular vein into plain vacutainer tubes (Terumo, Japan). Samples were labelled with a unique code and were placed in a cool box at 4°C and transported to a laboratory where serum was harvested in 1.5 ml tubes. Sera were then kept at -20°C for further use. All sera were tested with CATT/*T. evansi*.

#### 2.3. The card agglutination test for *T. evansi* - CATT/*T. evansi*

All sera were subjected to antibody detection with the CATT/*T. evansi*. The CATT/*T. evansi* was performed according to the manufacturer's instructions (Institute of Tropical Medicine, Antwerp, Belgium). Briefly, 25 µl of cattle serum

was diluted (1:4) in CATT-buffer and dispensed onto the reaction zone of a plastic test card. After adding one drop (approximately 45  $\mu$ l) of CATT reagent, the reaction mixture was spread by a stirring rod and allowed to react on a CATT rotator for 5 min at 70 rpm. A specimen was considered positive when blue agglutinates were visible (Bajyana and Hamers, 1988; Verloo et al. 2000).

#### **2.4. Data analysis**

The seroprevalence of *T. evansi* was calculated based on the following formula:  
$$P (\%) = \text{Number of positive samples} / \text{total number of animals} \times 100.$$
A chi-squared test to investigate the differences in the prevalence of trypanosome infections within different variables was performed using SPSS version 20 (SPSS, IBM). *P*-values of  $P < 0.05$  were considered to indicate statistically significant differences.

## Chapter three

### Results

A total of 304 serum samples were collected randomly from apparently healthy cattle to assess the prevalence of *T. evansi* infection in cattle in Khartoum state, Sudan. Within the samples collected, there were 62 (20.4%) male samples and 242 (79.6%) female samples. With regard to the age, the animals were grouped into 3 groups. The proportion of sampled animals within different age groups was as follows: <1year old: 140 (46%), 1-3years old: 33 (10.9%) and >3years old: 131(43.1%). While in terms of area, samples were collected from 3 different areas. The proportion of sampled animals within different areas was as follows: 100 (32.9%) samples were collected from Omdurman, 102 (33.6%) samples from Khartoum Bahry and 102 (33.6%) samples from Khartoum (Table1).

Out of 304 samples, antibodies against *T. evansi* were detected in 91 samples with overall prevalence of (29.9%) using CATT/*T. evansi* (Table 1).

Three risk factors including sex, area and age were investigated in this study to assess the association of these risk factors and the prevalence of *T. evansi* infection. Antibodies against *T. evansi* were recorded in 13 (21%) male's samples and in 78 (32.2%) female's samples. There was no significant difference was observed in the prevalence between males and females ( $P \geq 0.05$ ) (Table 2).

Antibodies against *T. evansi* were recorded in 31(31%) in Omdurman samples, in 31 (30.4%) in Khartoum Bahry samples and in 29 (28.4%) in Khartoum samples. The prevalence in the three areas did not differ to a statistically significant extent ( $P \geq 0.05$ ) (Table 2).

Antibodies against *T. evansi* were recorded in 36 (25.7%) in <1year old samples, in 10 (30.3%) 1-3years old samples and in 45 (34.4%) in >3 years samples. The prevalence within different age groups did not differ to a statistically significant extent ( $P \geq 0.05$ ) (Table 2)



**Table 1.** Proportion of cattle sampled in each group of variables

Variables	Frequency	%
Sex		
Male	62	20.4
Female	242	79.6
Total	304	100
Age		
<1 year	140	46.1
1-3 years	33	10.9
>3 years	131	43.1
Total	304	100
Location		
Omdurman	100	32.9
Khartoum Bahry	102	33.6
Khartoum	102	33.6
Total	304	100
CATT outcomes		
Positive	91	29.9
Negative	213	70.1
Total	304	100

**Table 2.** Univariate analysis for testing the association of the risk factors with *T. evansi* seroprevalence

Risk factors	CATT/ <i>T. evansi</i>		Chi-square values	df	P-value
	Positive No. (%)	Negative No. (%)			
<u>Sex</u>					
Male	13 (21)	49 (79)	2.99	1	0.084
Female	78 (32.2)	164 (67.8)			
<u>Area</u>					
Omdurman	31 (31)	69 (69)	0.92	2	0.17
Khartoum Bahry	31 (30.4)	71 (69.6)			
Khartoum	29 (28.4)	73 (71.6)			
<u>Age (years)</u>					
<1 year	36 (25.7)	104 (74.3)	2.41	2	0.30
1-3 years	10 (30.3)	23 (69.7)			
>3 years	45 (34.4)	86 (65.6)			

## Chapter four

### Discussion

Surra disease, caused by *T. evansi*, causes serious economic losses in various types of animals because it affects their fertility and productivity, and *T. evansi* infections of animals are highly endemic in Africa, Asia, and Latin America (Dobson et al., 2009). *T. evansi* infections are primarily transmitted mechanically by different types of biting flies, including *Tabanus* and *Stomoxys* species (Birhanu et al., 2015; Salim et al., 2011; Mihok et al., 1995; Shommein and Osman, 1987). Many studies reported that these infections are endemic in dromedary camels in Sudan (Mossaad et al, 2017a; Elamin et al., 1998; Babeker and Hassab Elrasoul, 2014; Salim et al., 2011; Ali et al., 2011). However, no data describing the prevalence of the disease in cattle is available in Sudan. In this study, we reported, for the first time the prevalence of the disease in dairy cattle in Khartoum State, Sudan, using CATT/*T. evansi* which is recommended by OIE (OIE, 2012). We recorded overall seroprevalence of (n=91; 29.9%). This is less prevalence as compared to that recorded in camels in Khartoum State (n=115; 52.2%) using the same technique (Babeker and Hassab Elrasoul, 2014). It is worth mentioning that all sampled animals in this study were apparently healthy animals with no clinical symptoms. This is in line with (Malik and Mahmoud, 1978) who claim that cattle, sheep and goats undergo protracted infection in which they may play the role of a

reservoir host. Therefore, co-herding with infected cattle may increase the possibility of infection with *T. evansi* to susceptible animals like camels (Shommein and Osman, 1987). We recorded variable prevalence in dairy cattle in different localities in Khartoum state; this was (n =31; 31%) in Omdurman, (n =31; 30.4%) in Khartoum Bahry and (n =29; 28.4%) in Khartoum. This indicates that almost similar prevalence was recorded in different areas of the study. This could be due to the free movement of animals between different areas along with the possibility of the similar distribution of the vectors within the State due to the similarity in the environment.

With regard to sex as risk factor, we recorded (n =13; 21%) positive male samples and (n =78; 32.2%) femal samples. Although the prevalence within females was higher than within males, the seroprevalence in both sexes did not differ to a statistically significant extent. This most probably indicates that males and females were exposed almost equally to the vector. Similar pattern was reported in camels in Omdurman (Babeker and Hassab Elrasoul, 2014).

Within different age groups, antibodies against *T. evansi* were recorded in (n =36 25.7%) in calves (<1 year), in (n =10; 30.3%) in animals in the age group between (1-3 years) and in (n =45; 34.4%) in adult animals (>3 years). Although the prevalence within animals in the age group (<1 year) was less than other groups of animals, the seroprevalence did not differ to a statistically significant extent. We

also observed that the older the animal the higher is the prevalence. This might be due to the maternal immunity. It worth mentioning that CATT/*T. evansi* is a serological test that detects antibodies which could be accumulated in adult animals due to recurrent infections which may also explain the higher number of positive samples in older animals. However, more studies are needed to better explain this finding.

To the best of our knowledge, there have been no reports from Sudan of the prevalence of *T. evansi* in cattle, whereas its presence in camels, a highly susceptible and severely infected animal species, has been published (Mossaad et al., 2019; Mossaad et al, 2017a; Elamin et al., 1998; Babeker and Hassab Elrasoul, 2014; Salim et al., 2011; Ali et al., 2011). It has also been reported in dogs (Mossaad et al., 2017). *T. evansi* can infect cattle (*Bos taurus*) in Africa; however they are sometimes refractory to the infection (Desquensens et al., 2013). This may explain why infected cattle in this study didn't show clinical signs. However, it could be considered as a reservoir host which can transmit the infection to susceptible animals like camels and horses.

A higher prevalence of the disease as compared to our recorded prevalence has recently been reported in cattle in Egypt (42.2%). Moreover, they have reported coinfection of *T. evansi* with other parasites such as *Babesia bovis*, *Babesia bigemina* and *Anaplasma marginale* (Fereig et al., 2017). It is important to

carryout similar studies to investigate the role of *T. evansi* infection as a predisposing factor of infection with other parasites and in general the effect of concurrent infection on the animal performance.

## **Conclusions**

We concluded that *T. evansi* infection is prevalent in dairy cattle in Khartoum State, Sudan. The disease was overlooked in previous studies that have focused only on the disease in camels as they are known to be more susceptible with serious clinical features. Since we have detected the antibodies in cattle sera, the disease should be neglected in cattle which could play a role as healthy carriers. This should also alert the veterinary authorities that cattle may be clinically infected as they could be a source of the disease to other animal species especially camels. More studies are needed to study the clinical pathology of *T. evansi* in cattle.

## **Recommendation**

- Dairy cattle should be routinely screened for the presence of *T. evansi* and should be treated.
- Co-herding of cattle and camels should be avoided in the animal husbandry to prevent transmission of the parasite.
- More studies to investigate the clinical pathology of *T. evansi* infection in cattle are urgently needed.

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