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A seroprevelence of *Trypanosoma evansi* infection in dairy cattle in Khartoum State, Sudan, using the card agglutination test for *Trypanosoma evansi* - CATT/*T. evansi*

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Abstract

Trypanosome 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 recorded 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 trypanosomoisi caused by *T. evansi* infection in Khartoum State, Sudan.

Keywords: Dairy cattle, serodiagnosis, Sudan, surra, trypanosomosis.

Introduction

Trypanosoma evansi, the agent of "surra," is a salivarian trypanosome, originating from 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. 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).

Materials and Methods

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.

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 camels' 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*.

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 camel 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 µl) 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).

Data analysis: The seroprevalence of *T. evansi* was calculated based on the following formula: P (%) = Number of positive samples/total number of animals x 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 < 0.05 were considered to indicate statistically significant differences.

Results

In total, 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 samples collected, there were 62(20.4%) male samples and 242(79.6%) female samples. With regard to the age, 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 East Nile 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* (Table1).

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
Area		
Omdurman	100	32.9
East Nile	102	33.6
Khartoum	102	33.6
Total	304	100
CATT/*T. evansi* outcome		
Positive	91	29.9
Negative	213	70.1
Total	304	100

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≥0.05) (Table 2).

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

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 seroprevelence 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 Locality, (n =31; 30.4%) in East Nile Locality and (n =29; 28.4) in Khartoum Locality. This indicates that 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 of the environment.

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

Risk factors	CATT/*T. evansi*		*Chi*-square values	*df*	*P*-value
	Positive No. (%)	Negative No. (%)			
Sex					
Male	13 (21)	49 (79)	2.99	1	0.084
Female	78 (32.2)	164 (67.8)			
Area					
Omdurman	31 (31)	69 (69)	0.92	2	0.17
East Nile	31 (30.4)	71 (69.6)			
Khartoum	29 (28.4)	73 (71.6)			
Age (years)					
<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)			

With regard to sex as risk factor, we recorded (n =13; 21%) positive mal samples and (n =78; 32.2%). Although the prevalence within females was higher than within males, the seroprevalence in the two 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 the 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, 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.

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تقصي مصلي لنسبة الاصابة بطفيل التربانوسوما ايفانساي وسط ابقار الحليب بولاية الخرطوم، السودان بإستعمال اختبار كات

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المستخلص

طفيل التربانوسوما 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 بثقب 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 مذيد 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.