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Prevalence of *Trypanosoma evansi* in camels in four states of great Butana, Sudan

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Abstract
Surra, a vector borne disease caused by *Trypanosoma evansi*, is considered as a major enzootic disease mainly for the dromedary camel. Therefore, a cross-sectional study was conducted to determine the prevalence of camel trypanosomosis and assess the distribution and dynamics of the vectors responsible for transmission of the disease in camels (five localities); sheep and goats in Sennar state and equines in White Nile state, from November 2014 to October 2015. Parasitological examination was conducted using Giemsa stained blood smears. The positives were reported in camels only and with prevalence of 2.9% by blood smear. The prevalence and percentages of camels' infection in five geographical locations, seasons and animal breeds, animal sex and age were scoured and discussed. It could be suggested that well-planned research program should be performed including the relation of the surra prevalence to its vector dynamics, host susceptibility, grazing system and vector-control practices in all affected areas.

Keywords: *Trypanosoma evansi*, camels, butana, Sudan

1. Introduction

Livestock is the largest subsector of the Sudanese domestic economy and is a growing contributor to exports. To a remarkable extent, the Sudanese economy is based on a combination of mobile and sedentary pastoral and agro-pastoral production by farming and herding households in almost every region and state. Sudan ranks second, after Somalia, in camels world-wide [1]. The protozoan parasite *Trypanosoma evansi* (*T. evansi*) has a large diversity mammalian hosts. It infects both intra- and extra-vascular fluids of mammals and causes the surra disease in Africa, Asia, Europe and Latin America and [2]. Surra constitutes one of the major veterinary problems worldwide [3]. Additionally, since 2008, surra has been asked to be reported to the OIE [4]. Furthermore, recently it was reported the first human trypanosomiasis caused by *Trypanosoma evansi* in India and hence, making it also a potential human pathogen [5].

Tsetse fly is considered as the main vector, however, *T. evansi* is transmitted mechanically, non-cyclically, by Haematophagus flies such as: Tabanus, Stomoxys, Lyperosia, and Chrysops. Because the trypanosomes remain infective for only a short period, such transmission occurs while such biting flies through their mouthparts, feed on more than one host [6]. *T. evansi* affects the health, working capacity and productivity of dromedary camels however, clinical signs are not pathognomonic therefore, diagnosis must be confirmed by laboratory methods. Additionally, assessment of the prevalence of *T. evansi* would be better should it performed by means of parasitological (Giemsa stained thin smear), serological and molecular tests [7].

In Sudan, blood samples obtained from sheep goats and camels owned by nomads in Kasala state, eastern Sudan were examined using parasitiological methods [8]. Trypanosomes were found in camels specimens however the *Trypanosoma evansi* were found in the samples of the three animal using the enzyme immunoassay. They, however, suggested that sheep and goats might be involves in the transmission in Sudan. In Sudan, parasite prevalence of 5.4% using parasitological examination, 31.3% with ELISA and ranged between 33.9 to 42.1% using the molecular epidemiological [9-10]. In such study area, using the parasitological techniques, found the higher infection rate during the dry period (November to May), the lower infection rate among young camels and lower prevalence of infection in herds of camels raised by nomads compared with those kept by agropastoralists. Furthermore, drugs resistance in Sudanese *Trypanosoma evansi* to suramin, a drug that which was attributed to an extensive and repeated use in Kassala (near the east border of Ethiopia) and also to quinapyramine (Trypacide) was reported [11-12]. In Ethiopia, the adjacent international border to the study area, the occurrence of surra is associated with camel rearing areas [13]. Therefore, a similar problem may be expected in the adjacent areas of Ethiopia and vice versa. Under field conditions camels may not show overt clinical signs of trypanosomosis as cattle do [14]. Additionally, *T. evansi* affects the health, working capacity and productivity hence, causes considerable economic losses [15]. The infections of dromedary camels are clinical signs not pathognomonic, therefore, diagnosis must be confirmed by laboratory methods. Additionally, assessment of the prevalence of T. evansi would be better should it be performed by means of parasitological (Giemsa stained thin smear), serological and molecular tests [6, 16]. With limited resources allowed using only the traditional diagnostics method, the present study aimed at investigating the possible occurrence and prevalence of *trypanosomosis evansi* in camels in four states of Great Butana (an area unknown of tsetse (*G.*

tachinoides) challenge); in sheep and goats in Sennar state and in equines in the White Nile state, Sudan as contribution to the epidemiology of the disease.

2. Materials and Methods

2.1 The Study area

The *Trypanosoma evansi* was investigated over an year period during November 2014 and October 2015, in camels in 4 states (5 sites), in sheep and goats in Sennar state (5 sites) and in equine in 4 sites in the White Nile state. In terms of camels availability, the study was performed at the five most important local known areas in Great Butana. As figure 1 shows, these five sites are: Tambul and Wad Elnimir, Gezira state, the known camels' markets. The other three:, Abu Deilaig (Khartoum state); Showak (Gadaref state) and Village 1 Arab (Kassala state). For investigation in sheep and goats the study was carried out at Sennar, Singa, Muzmum, Abu N'ama and Dindir. However, that of equine was at Eldowaim and Kosti (western bank of the West Nile) and Elgitaina and Rabak at the eastern bank. Three distinct seasons exist per year: rainy season (Autumn) is between July and October while the other two dry seasons prevail from November to February (Winter) and from March to June (Summer).

2.2 Sampling Technique and Blood Sources

Within each site, the animals to be sampled were selected randomly. Within the herds, around 30 animals with different animal breed, sex and age were sampled randomly. Sometimes, the number was less than that because the owners refused to give more blood from their animals. The puncture area of the jugular vein was cleaned by 70% ethanol to made the blood smears with a sterile needle. A drop of blood was taken on a clean glass microscope slide, spread by another slide at an acute angel, air dried and fixed by absolute methanol for 1 - 2 second. Each slide was labeled on the smear indicating the site, season, animal breed, animal age, sex, and date of collection.

2.3 Diagnostic Technique and Parasite Identification

The morphological examination on Giemsa-stained thin films were examined under 100 X oil immersion objective using a binocular microscope.

2.4 Data Analysis

The study data was analyzed using SPSS version 16. First, the data was coded appropriately into Microsoft excel spread sheet before been loaded into the SPSS. The prevalence of tick infestation was determined with descriptive statistics. The association between tick distribution and other factors such as location and season, was determined by the chi-square test. The 95% confidence intervals and $p < 0.05$ were set for significance in all cases.

3. Results and Discussion

In the present study, the positive infestation with *Trypanosoma evansi* s was reported in camels only. Camels are one of the main sources of income and food for millions of pastoralists in Africa. Sudan is the second camel's rearing country in the world with camel population estimated at over 4.6 million heads [9]. In general, the camels in Butana area are owned by agro-pastoralists**.** They graze daily in the lands not far from owner's hamlets (Abudelaig); in farms allowed to browse crop residues within family premises/lands during the dry season, semi-intensive, (Skowak and Girba) and two camel's markets (Tamboul and Wad Nimir). The present study was also able to collect data on five variables that could potentially be risk factors for acquisition of trypanosome infections, namely geographical location; seasons and camels' breed, sex and age.

A cross-sectional study was conducted to determine the prevalence of camel trypanosomosis and assess the distribution and dynamics of the vectors responsible for transmission of the disease in camels at Butana (4 states); sheep and goats (Sennar state) and equines (White Nile state) from November 2014 to October 2015. Parasitological examination was conducted using Giemsa stained blood smears. The positives were reported in camels only and with prevalence of 2.9% of camels' blood smears. Different animals' blood specimens from 2766 animals were examined by the morphological method employed for *T. evansi* infection. Camels 828 animals, 717sheep, 697 goats and 524 equines (4 horses and 520 donkeys). Only 24 camel's blood specimens (2.9%)] were found positives (Table 1). It is noteworthy that the camels presented the only prevalence of *T. evansi* infection among all species sampled. Within the camels' specimens, the pattern and prevalence of infection differed according to the locations, seasons, breed, age and sex were shown in Table). In Sudan, the parasite prevalence were reported as of 5.4% using parasitological examination, 31.3% with ELISA [8] and ranged between 33.9 to 42.1% using the molecular epidemiological [9].

Regarding camels, the the prevalence and percentages were found to be varying between different localities: Abudeliag 20 (80.0 %); Girba 2 (8.0 %); WadNimir 2 (8.0 %); Tambul 1 (4.0 %); and Showak 0.0 (0.0%) (Table 1). The locations showed different camels' management systems: Tamboul and Wad Elnimir (camels' markets); Showak and Girba (ranches) and Abudeliag (rural). Within the 3 rural locations, the relatively high parasitemias at Abudeliag could be established since animals from market and ranches, where seems more concerned about control measures, has displayed the lower rates. Abudeliag is in Khartoum state, the Sudan capital, where animal owners prefer to sell their animals with more higher prices. Apparently, the positive cases observed in such camels' specimens could suggest that they are more exposed to *T. evansi* infection than the other locations sampled. However, the lower prevalence of the disease in the camels' markets could possibly explained as due to better awareness towards better feeding, management, use of medicines and dependence of camel keepers to sell them with good prices. In other words, without ruling out other transmission mechanisms other than bloodsucking flies, the prevalence of *T. evansi* infection during the dry season could suggest that these animals developed a long lasting course of infection in nature.

Table 1: Prevalence and percentages of *T. evansi* in camel's blood at four States of Great Butana, Sudan, from November 2014 and October 2015

Location	Frequency	Percent
Abudeilaig	20	80
Tamboul	1	4
WadNimir	2	8
Girba	2	8
Total	25	100

Breed	Frequency	Percent
Anafi	7	28
Arabi	16	64
Bushari	2	8
Total	25	100

Season	Frequency	Percent
Winter	13	52
Summer	10	40
Autumn	2	8
Total	25	100

Age	Frequency	Percent
<5	9	36
5-10	15	60
11-15	1	4
Total	25	100

Sex	Frequency	Percent
Male	12	48
Female	13	52
Total	25	100

Babesia	location	Season
	Girba100%	Winter 100%
Breed	**Sex**	**Age**
Arabi 100%	Females 100%	<5 = 50% 5-10 = 50%

The present data concerning seasonal distribution of the positives showed that the prevalence and percentages of camels' infection were: in winter 13 (52 %); summer 10 (40 %) and autumn 2 (8 %) (Table 1). This could suggest that these animals may become infected by the end of autumn and during the winter when it could be expected the highest fly count that acts as *T. evansi* vectors during the rainy season. The positive incidence in summer could be explained as the owners in Butana usually take their animals to riverine, Dindir national park or even to the Ethiopian border areas which are also favourable grounds for these flies. That is because *T. evansi* is transmitted mechanically, non-cyclically, by haematophagus flies such as the biting flies under the genus Stomoxys, Tabanus, Chrysops and Lyperosia. Therefore, transmission occurs through their mouthparts when they feed on more than one host within a short interval because the trypanosomes remain infective for only a short period. Regarding camels breeds, the prevalence and percentages were: Arabi 16 (64 %); Anafi 7 (28%); Bushari 2 (8%) and non in the rest of the breeds (Butana, Kenana. Daali, and Darfur). The different habitats and behavior patterns of such animals suggest that there are unknown factors underlying the transmission cycles. The positive infected camels with *T. evansi* could suggest their importance in the maintenance of the parasite in nature. As reported, different factors could be associated to this feature such as host susceptibility, individual nutritional status and difference in strains' virulence. Additionally, animals continuous movement leads to stress that may result in weakness of the infected animals and consequently lower the immunity and hence, augmenting of the quantitative content of parasites in the blood [17].

With regard to the animal sex, the prevalence and percentages were reported almost similar: in females 13 **(**52%) and males 12 (48%) of the positives. This could be explained as due to females' successive pregnancies and stress of lactations.

Regarding the age, the prevalence and percentages scoured (out of 24 positive cases) at different age groups were: <5 years 9 (36%); between 5 and 10 years 15 (60%); between 11-15 years 1 (4%) however non positives were detected above 15 years. Such result could be concurred with the reported results mentioned that surra affects camels of all ages with a higher incidence of disease in younger camels [18]. Therefore, decrease in sensitivity with age could be a reasonable explanation. Additionally, younger are usually more weaker in nutrition. Furthermore, in such pastoral communities animals have to trek for long distances in search of water and pasture especially in the dry season. In general, these results coincided with the reported results performed in this study area, using the parasitological techniques, that found the higher infection rate during the dry period (November to May), the lower infection rate among young camels and lower prevalence of infection in herds of camels raised by nomads compared with those kept by agropastoralists [8].

This year, 2015, the rains are very few that compels the herds to move towards the north (Taka mountains, Kasala state). *Babesia* species were found only at Griba herds only and only during the last collection (September-October, end of the rainy season) when moved towards kassala. Out of 828 camels' specimens tested, only 8 *Babesia* species were detected positives: 100% in Girba location; in winter only (100%); in Arabi breed only; in females only and younger camels (<5 years 50% and 5-10 years 50%). Additionally, *Toxoplasma* was found in these camels' specimens (data in process for publication). However, no *Therileria* or *Anaplasma* were detected in the examined camels' blood specimens. Furthermore, *Therilia, Babesia and Anaplasma* were recorded in sheep, goats and equines (data in process for publication).

The different habitats and behavior patterns of such animals suggest that there are unknown factors underlying the transmission cycles. The positive infected camels with *T. evansi* could suggest their importance in the maintenance of the parasite in nature. As reported, different factors could be associated to this feature such as host susceptibility, individual nutritional status, difference in strains' virulence Additionally, animals continuous movement leads to stress that may result in weakness of the infected animals and consequently lower the immunity, exacerbating of the quantitative content of parasites in the blood [19-20]. Hence, suggest that *T. evansi* infected animals may be involved in the transmission cycle of the parasite Apparently, the positive cases observed in camels'

specimens could suggest that they are more exposed to *T. evansi* infection than the other species sampled. Under field conditions camels may not show overt clinical signs of trypanosomosis as cattle do [14]. That could be more significant in Great Butana due to the expected lower rates of exposure based on the known tsetse habitat in Sudan and its feeding habits. Additionally, without ruling out other transmission mechanisms other than bloodsucking flies, the prevalence of *T. evansi* infection during the dry season could suggest that these animals developed a long lasting course of infection in nature. Meanwhile, the ecological diversity of Sudan could directly interlinked the *T. evansi* transmission between vectors with other parasite's reservoir(s) which could be characterized as a domestic and/or wild enzooty encompassing the entire Sudan due to the freely movement of animals.

On the other hand other domestic and wild animals could involved in such transmission. It was stated that trypanosome infections cause great economic losses occur in small ruminants [21]. Additionally, Sheep and goats have been incriminated as sources/reservoirs of infection to other animals and man. However, it is worth noting that during this study, it was observed that sheep graze together with camels under this agro-pastoral practice. Therefore, it seems as the close proximity between infected and susceptible animals and the availability of the vector population could enhance the prevalence of trypanosomosis. Moreover, It was reported that the oral route may be important in the dispersion of T. evansi infection in dogs and other animals as a consequence of their frequent fights for instance [22]. Therefore, in Sudan, since dogs remain very close to such animals as guards, during the vector season they can play an important role in the epizootiology of such disease. Moreover, without ruling out other transmission mechanisms other than bloodsucking flies, the prevalence of *T. evansi* infection during the dry season could suggest that these animals developed a long lasting course of infection in nature. Therefore, such present results could be inferred as the existence of carrier animals in the vicinity of susceptible camels makes transmission by biting flies possible. Although the importance of wild mammals in the maintenance of *T. evansi* in the natural environment was reported, however a relationship between camels' trypanosomiasis prevalence and proximity of infected wild mammals was not established [23].

Finally, it could be recommended that: because of unexpected infection, camels' owners in the area do not incorporate camels neither in disease detection nor in disease and vector (s) management. Hence, such infected animals would be reservoirs. This could dictate studies also into other domestic and wild animals in a foreseeable future. Additionally, drugs resistance in Sudanese *Trypanosoma evansi* to suramin, a drug that which was attributed to an extensive and repeated use in Kassala (near the east border of Ethiopia) and also to quinapyramine (Trypacide) was reported [11-12]. A similar problem may be expected in the adjacent areas of Ethiopia. Therefore, with the advent of newer diagnostics complemented with traditional ones will be of interesting help as suitable diagnostic tools for determination of the extent of the prevalence, incidence and morbidity [16]. Furthermore, vector control as a solution for surra seems hard to be achieved. That is because a range of non-related biting flies should be targeted. Therefore, formulating and implementing effective management strategies should be focused on. In general, it could be suggested that well-planned research program should be performed including the relation of the surra prevalence to its vector dynamics, host susceptibility, grazing system and tick-control practices in all affected areas.

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