Molecular detection of equine trypanosomes in the Sudan

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A B S T R A C T

Equine trypanosomosis (ET) is a protozoan disease affecting equines in many parts of the world. We examined 509 samples collected from geographically distinct regions in eastern, central and western Sudan to estimate the endemicity of ET using the generic ITS1-PCR diagnostic methods. Results revealed that horses and donkeys were infected by Trypanosoma brucei subgroup, Trypanosoma vivax, Trypanosoma simiae and Trypanosoma congolense. The prevalence of Trypanosoma spp. was higher in horses (12.7%, n = 393) than in donkeys (3.4%, n = 116). The highest prevalence was observed in South Darfur State (19.3%, n = 202), followed by Kassala State (15.1%, n = 86), Gadaref State (3.7%, n = 82), and Khartoum State (2.6%, n = 76). No trypanosomes were detected in the 63 samples collected from North Kordofan State. We report for the first time the presence of T. simiae and T. congolense in horses in the Sudan. This study should alert veterinary services, authorized bodies to take action toward ET by undertaking countrywide epidemiological studies of the disease and adopting control strategies.

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1. Introduction

Equine trypanosomosis (ET) is an economically important arthropod-borne disease caused by Trypanosoma species. In particular, equines are susceptible to infection caused by members of the subgenus Trypanozoon. Recent findings on the evolution of this subgenus and the adaptations of Trypanosoma brucei to gradual loss of kinetoplast DNA suggested that Trypanosoma equiperdum and Trypanosoma evansi are indeed petite mutants of T. brucei and do not qualify to the status of separate species (Lai et al., 2008). Hence, the names Trypanosoma brucei brucei, Trypanosoma brucei equiperdum and Trypanosoma brucei evansi were proposed. These three species are considered to be the most pathogenic trypanosomes infecting equines and causing diseases that are referred to as nagana, dourine and surra, respectively (Claes et al., 2003).

While tsetse fly (Glossina species) acts as biological vectors for T.b. brucei, the subspecies T.b. equiperdum and T.b. evansi have adapted to non-cyclical transmission. Tsetse fly also transmits Trypanosoma congolense and Trypanosoma vivax to equines; however, natural infection with these two species is rarely seen in horses (Kihurani et al., 1994).

Non-tsetse transmitted ET can be caused also by T. vivax, which is present both in South America and in tsetse depopulated areas of Africa (Touratier, 2000). Such transmission can be through Tabanus, Stomoxys, Lyperosia and other biting-flies (FAO, 1998).

The role of carrier animals can be emphasized by the fact that severe form of the disease occurs in horses and camels, whereas, cattle and buffalo are considered important reservoirs of the infection for equines (Soulsby, 1982). Another important factor is the presence of abundant vectors in the areas of equine populations (Yagi and Razig,
In recent years, DNA-based technologies including polymerase chain reaction (PCR) have been increasingly used for the diagnosis of trypanosomosis, and for large scale analysis of trypanosomes samples from camels and cattle in the Sudan (OIE, 2010; Salim et al., 2011a,b).

To our knowledge, no report of ET using conventional or molecular techniques has been documented in the Sudan so far. This study aimed to provide information on the prevalence of ET, its local enzootic situation and the possible causative trypanosome species in the Sudan using the generic ITS1-PCR.

2. Materials and methods

2.1. Samples collection

In a surveillance conducted in 2010 (October 1st to November 15th), 509 blood samples were collected from horses and donkeys in FTA cards (Whatman FTA® elute, Whatman, UK). Clinically healthy animals were randomly sampled from geographically distinct areas from five states in the Sudan, namely Kassala, Gadaref, Khartoum, North Kordofan and South Darfur (Table 1). Emphasis was given to South Darfur State and larger numbers of animals were sampled due to its higher equine population.

2.2. DNA extraction

Genomic DNA was extracted from FTA® elute (Whatman Inc, USA) using a previously published protocol (Salim et al., 2011b). To enhance the recovery of DNA and to ensure correct estimation of prevalence, six punches were used per sample (Cox et al., 2010; Salim et al., 2011a,b). Briefly, blood samples collected on FTA cards were dried thoroughly at room temperature. Using a sterile punch tool, each FTA card was punched out at 6 different positions each was 3 mm in diameter. These were placed into sterile microcentrifuge tubes and rinsed 3 times each in 750 µL deionized water by vortexing for 5 s and discarding of water. DNA was eluted using a buffer that contained 90 µL deionized water plus 10 µL 10× Thermopol Reaction Buffer (Biolabs, Inc, England). Elution was performed by heating the sample at 95°C for 30 min using a heat block. Eluted DNA concentration ranged between 100 and 250 ng/µL. DNA was stored at −20°C until used.

2.3. ITS1-PCR for trypanosome detections in equines

Isolated DNA of 509 samples was subjected to PCR, which amplified the ITS1 region of the rDNA gene of all African trypanosomes using ITS1 CF and BR primers ITS1 CF: 5′-CCCGAAAGTCACGGATATTG, BR: 5′-TGCTGCTTCTTCAAGCAA (Njiru et al., 2005). The 250 bp, 400 bp, 480 and 700 bp for T. vivax, Trypanosoma simiae, T. brucei subspecies and T. congolense savannah, respectively, were amplified using GoTaq® Colorless Master Mix, 2× (Promega Co, USA) in a 10 µL total volume. Each reaction included 5 µL GoTaq® Colorless Master Mix, 0.5 µL of each 10 mM primer, 2 µL RNase-free water and 2 µL extracted DNA of 50 ng/µL concentration. Thermocycling profile started with initial hold for 2 min at 95°C, followed by 35 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 1 min. A final extension step followed, which was for 5 min at 72°C.

3. Results

3.1. Detection of equine trypanosomes by generic ITS1-PCR

Four Trypanosoma species were found to infect horses and donkeys in Sudan. Those were T. brucei subgroup, T. vivax, T. simiae and T. congolense.

Table 1

<table>
<thead>
<tr>
<th>State/town</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Horses</th>
<th>Donkeys</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>South Darfur State</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shearia/Taisha</td>
<td>12.39°N</td>
<td>25.27°E</td>
<td>18</td>
<td>0</td>
<td>18</td>
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<tr>
<td>Nyala</td>
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<td>24.56°E</td>
<td>17</td>
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<td>17</td>
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<tr>
<td>Adayla/Tomat</td>
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<td>27.01°E</td>
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<td>51</td>
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<tr>
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<td>24.11°E</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Tulas/Jidad</td>
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<td>24.44°E</td>
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<tr>
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<td>25.11°E</td>
<td>44</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
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<td></td>
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<td></td>
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<tr>
<td>El Obeid</td>
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<td>30.10°E</td>
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<td>19</td>
<td>23</td>
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<tr>
<td>Khowai</td>
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<td>40</td>
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<tr>
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<td>32.28°E</td>
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<td>32.45°E</td>
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<td>Khartoum North</td>
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<td>32.54°E</td>
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<td>20</td>
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<tr>
<td>Gadaref State</td>
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<tr>
<td>Gadaref</td>
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<td>35.28°E</td>
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<td>23</td>
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<tr>
<td>El Fao</td>
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<td>34.08°E</td>
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<td>18</td>
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<tr>
<td>Showak</td>
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<td>16</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
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<td>36.00°E</td>
<td>53</td>
<td>14</td>
<td>67</td>
</tr>
<tr>
<td>Halfa</td>
<td>15.32°N</td>
<td>35.59°E</td>
<td>10</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>393</td>
<td>116</td>
<td>509</td>
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</table>
Table 2
Number and prevalence of Trypanosoma species in horses and donkeys.

<table>
<thead>
<tr>
<th>Host species</th>
<th>T. brucei subgroup</th>
<th>T. vivax</th>
<th>T. simiae</th>
<th>T. congolense</th>
<th>Total no. (prevalence %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horses</td>
<td>17</td>
<td>14</td>
<td>16</td>
<td>6</td>
<td>50 (12.7)</td>
</tr>
<tr>
<td>Donkeys</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4 (3.4)</td>
</tr>
</tbody>
</table>

* Three cases of mixed infection (53 trypanosomes in 50 horses).

3.2. Prevalence of trypanosomes in horses and donkeys

Infection with trypanosomes was found to be more prevalent in horses (12.7%, n = 393) than in donkeys (3.4%, n = 116) (Table 2). All the four species were detected in samples collected from horses, in which T. brucei subgroup had the highest prevalence (4.3%, n = 393) followed by T. simiae (4.1%, n = 393), T. vivax (3.6%, n = 393) and T. congolense savannah (1.5%, n = 393). In donkeys, only T. vivax was detected (3.4%, n = 116).

3.3. Prevalence of trypanosomes according to locations

The highest prevalence of trypanosomes was observed in South Darfur State (19.3%, n = 202), in which three animals showed mixed infection. This was followed by Kassala State (15.1%, n = 86), Gadaref State 3 (3.7%, n = 82) and Khartoum State (2.6%, n = 76). No parasites were detected in the 63 samples collected from North Kurdofan.

According to locality, the highest prevalence was observed in Buram/Dimasoya (36.4%, n = 44) followed by Ed Al Fursan (29.2%, n = 24), Nyala (23.5%, n = 17) and Shearia/Taisha (22.2%, n = 18) all in South Darfur State. These were followed by Showak in Gadaref State (18.8%, n = 16), Kassala in Kassala State (16.4%, n = 67), Khartoum in Khartoum State, 2 (11.8%, n = 17), Halfa in Kassala State (10.5%, n = 19) and Tulus/Jijad (10.4%, n = 48) and Adayla/Tomat (9.5%, n = 51) in South Darfur State (Table 3).

No trypanosomes were detected in samples collected from the localities El Obied, Khowai, Omdurman, Gedaref and El Fao (Table 3). T. vivax in donkeys was detected in two localities, Showak (18.8%, n = 16) and Halfa (11.1%, n = 9).

T. vivax has the highest overall prevalence among the species detected. This species was found in all States investigated except North Kurdofan. The highest prevalence was recorded in Kassala State (14.9%, n = 67).

T. brucei subgroup and T. simiae were exclusively found in South Darfur State. Mixed infection were observed in one horse from Ed Al Fursan and two horses from Tulus/Jijad, all harboring T. brucei subspecies and T. simiae.

On the other hand, 6 T. congolense savannah cases were detected, five of which were from South Darfur State and one case from Kassala, a region known to be free from tsetse fly (Table 3).

4. Discussion

Sudan represents the world’s 25th highest equine population (Fidiel, 2007). The value of equine in transport support in agriculture, race, rescue missions as well as display of social pride is undeniable. Along with equine piroplasmosis and African horse sickness, equine trypanosomosis (ET) is one of the most serious equine diseases in Sudan. It remains frequently reported in veterinary clinics, especially at the season of flies’ burden. However, little is known on the prevalence and distribution of...
Trypanosoma species causing ET in Sudan. Previous to this work, no systematic studies on ET had been performed and, thus, the epizootiological and epidemiological situation of ET in Sudan remained unclear.

In an attempt to explore the situation of ET in Sudan and the Trypanosoma species that cause it, we undertook this study and applied the generic ITS1-PCR method to analyze 509 samples collected from equines in five States of the country. The ITS1-PCR method is increasingly used in surveys to study trypanosomosis in different African countries (Desquesnes et al., 2001; Njiru et al., 2005; Thumbi et al., 2008; Pinchbeck et al., 2008; Salim et al., 2011a,b). However, the method has drawbacks such as the low analytical sensitivity of the ITS1 CF and BR primers, which range between 10 pg (100 trypanosomes) for Trypanozoon, T. vivax and T. congolense clades to 100 pg (1000 trypanosomes) for T. simiae and T. godfreyi (Njiru et al., 2005), and its inability to distinguish T. brucei subgroup at its sub-species level.

The overall result of this work indicated that, horses in Sudan are infected with T. brucei subgroup, T. vivax, T. congolense savannah and T. simiae. Infection with T. brucei subgroup was exclusively reported in South Darfur State. As both cyclical and mechanical transmissions are present in this State, it could not be resolved which species of T. brucei subgroup specifically infect horses. Future studies should implement methods to differentiate T. brucei subgroup members. For example, the PCR approach based on amplifying the maxicircle kinetoplast DNA (Li et al., 2007) can be used to differentiate T. b. brucei and T. b. equiperdum from T. b. evansi. Similarly, the serum resistance-associated (SRA) gene-based PCR approach (Radwanska et al., 2002) can be used to check whether T. rhodesiense is among the T. brucei subgroup samples from horses in Sudan.

We could not demonstrate the presence of T. b. evansi in horses originating from the other states particularly Kassala and Gadaref, where camel trypanosomosis due to T.b. evansi is well-known. However, in these areas, horse population is significantly lower, and horses are generally reared within urban places for draft power unlike the camels, which are usually reared under nomadic conditions where they are more prone to biting flies.

The infection of horses with T. vivax is expected as horses are known to be susceptible to this species. Moreover, this species is prevalent in Sudan and its mechanical transmission by biting flies and infectivity to cattle has been reported in different regions of the country (Fadl et al., 2000; Rahman, 2005; Mohammed et al., 2010; Salim et al., 2011b). Only few samples were found positive for T. vivax in Omdurman, Khartoum State, and no positive samples were found in North Kurdufan State. In these states, there is a limited exposure of animals to flies during the period of sample collection (after the rainy season). In addition, horses are usually reared in small scales and well monitored by their owner. Larger sampling might be needed to determine the epidemiology of ET in these regions.

High prevalence of T. simiae infection was reported in Darfur State. This parasite is known to infect mainly swine (Stevens and Brisse, 2004), but also other domestic animals such as horses, sheep and cattle (Joshua and Kayit, 1984). We have previously reported the presence of infection with T. simiae in cattle in Sudan (Salim et al., 2011b). The epidemiology of infection with this species in domestic animals in Sudan requires further elucidation.

In this study, infection of horse with the cyclically-transmitted T. congolense savannah was reported in South Darfur State where tsetse transmission is known (Mohamed-Ahmed et al., 1989). However, we also reported T. congolense savannah infection from one horse in Kassala State, a region known to be free of tsetse flies. One explanation for this would be that this horse showing T. congolense savannah infection was imported from a tsetse fly zone. The Northwest part of Ethiopia borders Sudan and there are no restrictions to animal movements between the two countries. One study has indicated the presence of T. congolense savannah, T. brucei, and T. vivax in donkeys originating from Northwest Ethiopia, with T. congolense showing the highest predominance among positive animals (Abebe and Wolde, 2010). Interestingly, in the last decade, mechanical transmission of T. congolense by the tabanid Atylotus agrestiss could be proven (Desquesnes and Dia, 2003). A. agrestiss as well as other tabanid species are prevalent in Sudan (Mohamed-Ahmed and Mihok, 2009), and their involvement in the transmission of T. congolense in the northern part of the country cannot be ruled out.

This is the first report on ET in the Sudan implementing the generic ITS1-PCR. We report for the first time the presence of T. simiae and T. congolense in horses in the Sudan. It is presumed that the study should alert veterinary services and authorized bodies to take action toward ET by undertaking countrywide epidemiological studies of the disease and adopting control strategies.

Competing interests

The authors declare that they have no competing interests.

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