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Molecular detection of equine trypanosomes in the Sudan

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ABSTRACT

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 Kurdofan 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.

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,

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1976). 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 Kurdofan 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-CCGGAAGTTCACCGATATTG, BR: 5-TGCTGCGTTCTTCAACGAA (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
Number of samples collected from horses and donkeys and their locations and coordinates.

State/town	Latitude	Longitude	Horses	Donkeys	Total
South Darfur State					
Shearia/Taisha	12°39′ N	25°27′ E	18	0	18
Nyala	11°56′ N	24°56′ E	17	0	17
Adayla/Tomat	11°29′ N	27°01′ E	51	0	51
Ed Al Fursan	11°54′ N	24°11′ E	24	0	24
Tulus/Jidad	11°05′ N	24°44′ E	48	0	48
Buram/Dimasoya	11°04′ N	25°11′ E	44	0	44
North Kurdofan State					
El Obied	13°08′ N	30°10′ E	4	19	23
Khowai	13°00′ N	29°21′ E	40	0	40
Khartoum State					
Omdurman	15°40′ N	32°28′ E	39	0	39
Khartoum	15°31′ N	32°45′ E	17	0	17
Khartoum North	15°65′ N	32°54′ E	5	15	20
Gadaref State					
Gadaref	14°02′ N	35°28′ E	23	25	48
El Fao	14°11′ N	34°08′ E	0	18	18
Showak	14°39′ N	35°87′ E	0	16	16
Kassala State					
Kassala	15°30′ N	36°00′ E	53	14	67
Halfa	15°32′ N	35°59′ E	10	9	19
Total			393	116	509