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Original article

Utilization of crude and recombinant ELISAs for serodiagnosis of camel trypanosomosis in Sudan

Ehab Mossaad[a,b,*], Bashir Salim[c], Keisuke Suganuma[b,d,*], Mohammed A. Hassan[e], Batdorj Davaasuren[b], Elgailani A. Elamin[c], Amel O. Bakhiet[a], Rawan A. Satti[a], Xuenan Xuan[b,d], Simon Peter Musinguzi[b,g], Noboru Inoue[f]

[a] Department of Pathology, Parasitology and Microbiology, College of Veterinary Medicine, Sudan University of Science and Technology, P.O.Box 204, Khartoum, Sudan
[b] National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
[c] Faculty of Veterinary Medicine, University of Khartoum, 13314 Khartoum-North, Sudan
[d] Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
[e] Tsetse and Trypanosomosis Control Department, Central Veterinary Research Laboratory, Animal Resources Research Corporation, Ministry of Livestock, Fisheries and Rangelands, Khartoum, Sudan
[f] Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
[g] Department of Microbiology, Faculty of Biomedical Sciences, Kampala International University - Western Campus, P.O.Box 71, Bushenyi, Uganda

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ABSTRACT

This study was carried out to evaluate the application of CATT/*T. evansi*, crude and recombinant (TeGM6-4r) antigen ELISAs in the diagnosis of camel trypanosomosis caused by two trypanosome species, *T. evansi* and *T. vivax,* in Sudan. Concurrently, the current situation of camel trypanosomosis was investigated based on the results of a serological analysis. The recombinant tandem repeat antigen TeGM6-4r is conserved among salivarian trypanosome species and was highly sensitive in the detection *Trypanozoon*, and *T. vivax*. It has been validated in the diagnosis of surra in cattle and water buffalo but not in camels. A comparative evaluation of a crude antigen ELISA and a recombinant antigen GM6 (rTeGM6-4r) ELISA was performed using 189 blood samples, which included 148 samples obtained from different camel herds in Eastern Sudan and 41 samples from camels that had been brought from Western Sudan to local markets. The results showed that the rTeGM6-4r ELISA detected the greatest number of positive samples (n = 118, 62%), while CATT/*T. evansi* and the crude antigen ELISA detected the lowest number of positive samples (n = 73, 39%). The kappa value of rTeGM6-4r as compared to TeCA ELISA was 0.5515, which indicated moderate agreement. We concluded that the rTeGM6-4r ELISA is the test of choice for use in screening camel for trypanosomosis caused by *T. evansi* and *T. vivax* in Sudan.

1. Introduction

Trypanosoma evansi, which is found worldwide, emerged on two independent occasions from a West African *T. brucei* ancestor. The host and route of transmission are shared by *T. evansi* types A and B due to parallel but independent evolutions, which established paraphyletic groups (Cuypers et al., 2017). The parasite is mechanically transmitted to camels by tabanids and stomoxys in sub-Saharan Africa (Desquesnes et al., 2013). It causes a major enzootic disease in dromedary camels and was first reported in Sudan in 1904 (Shommein and Osman, 1987; Elkarib, 1961).

Recently, microscopy- and PCR-based techniques have been used to estimate the prevalence of camel trypanosomosis in Sudan were by (Mossaad et al., 2017; Babeker and Hassab Elrasoul, 2014; Salim et al., 2011; Ali et al., 2011; Aradaib and Majid, 2006; Elamin et al., 1998; Dafaalla, 1988). However, a card agglutination test (CATT/*T. evansi*) has also been used for serological investigations (Babeker and Hassab Elrasoul, 2014; Ali et al., 2011). The microscopic detection of the parasite is highly specific, but shows low sensitivity (OIE, 2008). However, the sensitivity of a parasitological diagnosis is limited by low parasitemia, because the disease is chronic in most cases. Molecular diagnostic assays have been developed to enhance the diagnostic resolution and shorten the diagnostic window (Desquesnes and Davila, 2002; Thekisoe et al., 2005). PCR-based detection methods can

* Corresponding authors at: National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan.
E-mail addresses: ehabmssd7@gmail.com (E. Mossaad), k.suganuma@obihiro.ac.jp (K. Suganuma).

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detect1–5 parasites/ml of blood in most species and 10 parasites/ml of blood in buffaloes (OIE, 2012; Claes et al., 2004; Desquesnes and Davila, 2002; Desquesnes et al., 2001), while loop-mediated isothermal amplification assays are sensitive enough to detect 0.1 parasite/ml blood (Ngaira et al., 2003; Reid and Copeman, 2002).

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). 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), an antibody detection ELISA using a trypanosome crude antigen is considered to be the 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 consists of 4 repeat domains (rTeGM6-4r), for use in disease surveillance (Nguyen et al., 2014; Thuy et al., 2012; Goto et al., 2011). We recently developed an rTeGM6-4r-based ELISA that is capable of detecting antibodies to animal trypanosomes in water buffaloes (Nguyen et al., 2014). We have also evaluated the application of crude and recombinant antigen ELISAs in the serodiagnosis of animal trypanosomosis in cattle, sheep and goats in South Africa (Nguyen et al., 2015). Most recently, we updated the molecular epidemiology of camel trypanosomosis in Sudan and showed—using molecular and parasitological methods—that the disease is caused by both *T. evansi* and *T. vivax*, (Mossaad et al., 2017). It is known that camelids antibodies lack L-chains and that the heavy-chain antibodies are γ-isotypes and are functional in antigen binding (Conrath et al., 2003). We therefore sought to evaluate the application of recombinant antigen ELISAs in the serodiagnosis of camel trypanosomosis in Sudan.

2. Materials and methods

2.1. Study area

Blood samples were obtained from 189 camels from two states in Eastern Sudan: El-Showak district, Kassala State, where three herds were sampled in the villages of Wd-Alhlio, Alshagrab and Khor Wd-Omer ($n = 148$); and from 41 camels that were brought from Western Sudan to El-Gazira State, Tumbool market. These camels crossed the natural barrier of the White Nile (WN) (Fig. 1). 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 a *T. evansi* crude antigen ELISA (TeCA-ELISA), a recombinant (rTeGM6-4r)-based ELISA and a card agglutination test for *T. evansi* - CATT/*T. evansi*.

2.2. Enzyme linked immunosorbent assay (ELISA)

All serum samples were tested with a TeCA-ELISA and rTeGM6-4r based ELISA. Trypanosome crude antigens were prepared according to the OIE manual (OIE, 2012). The recombinant antigen rTeGM6-4r was produced and the ELISA was conducted as described previously (Thuy et al., 2012), with minor modifications. In this study, peroxidase-conjugated protein A (Invitrogen, Japan) was used as the secondary antibody after being diluted 16,000-times while the primary antibody was optimized at 1,000-times. The optical density (OD) was assessed at 5 min after the addition of the substrate by measuring the OD_{450} using a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, Shanghai, China).

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 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 Songa and Hamers, 1988; Verloo et al., 2000).

2.4. Data analysis

Cohen's kappa coefficient was calculated to measure the agreement between the tests using VassarStats: Website for Statistical Computation (http://www.vassarstats.net/kappa.html); the results were interpreted according to the method previously described by Viera and Garrett (2005). The *P* values for the comparison of the seroprevalence between the study areas were determined using the GraphPad Prism software program (GraphPad Software Inc., CA, USA). *P* values of < 0.05 were considered to indicate statistical significance.

3. Results

The three serological tests showed varying levels of sensitivity in estimating the seroprevalence of trypanosome infection in camels in both areas (Table 1).

3.1. CATT/T. evansi

In the present study the CATT/*T. evansi* was used to detect the active infections as it is suitable for detection of early infections with *T. evansi* (OIE, 2012). This test estimated that the overall seroprevalence in all sampled camels was 39% ($n = 73$); the seroprevalence in the Eastern and Western WN areas was 40% ($n = 59$) and 34% ($n = 14$), respectively (Table 1). The seroprevalence of trypanosome infection in the two study areas did not differ to a statistically significant extent ($p > 0.05$).

3.2. ELISAs

The two crude and recombinant ELISAs showed different immunoreactivity in the detection of antibodies against camel trypanosomes (Fig. 2). The cutoff ELISA values were calculated as mean ± 3SD of OD values using 16 negative control sera. These negative control sera were from camels that tested negative for trypanosomes by microscopy, CATT/*T. evansi* and a PCR (Mossaad et al., 2017). The cutoff crude antigen and recombinant antigen ELISA values were 0.663 and 0.306, respectively. The results show that the rTeGM6-4r ELISA detected the highest number of trypanosome-positive samples ($n = 118$; 62%) (Fig. 2), of which, 100 (68%) and 18 (44%) positive samples came from the Eastern and Western WN areas, respectively (Table 1). The seroprevalence in the two areas did not differ to a statistically significant extent. However, the TeCA ELISA detected 73 (39%) trypanosome-positive samples (Fig. 2), of which 62 (41%) and 12 (29%) positive samples came from the Eastern and Western WN areas, respectively (Table 1). The seroprevalence in the two areas did not differ to a statistically significant extent. In comparison to TeCA, which is recommended by OIE, the kappa value of the rTeGM6-4r ELISA was calculated to be 0.5515, which indicates moderate agreement.

4. Discussion

The results of this study showed that camel trypanosomosis is highly

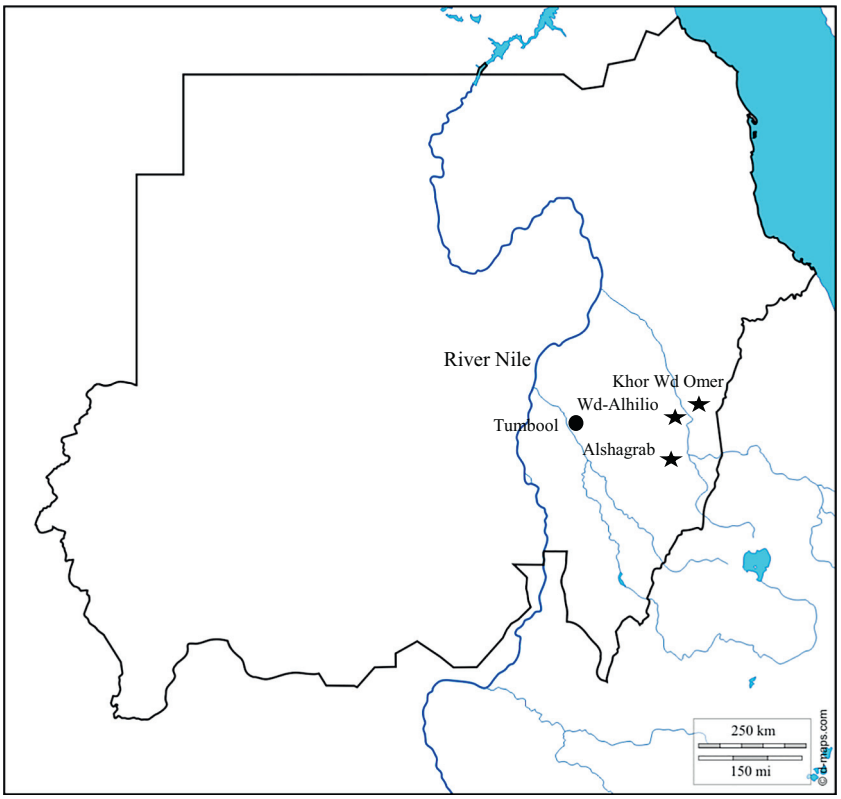

Fig. 1. A map of Sudan: The locations of the sampling areas from different herds are shown with black stars. The black dot indicates the Tumbool slaughterhouse. Source: http://www.d-maps.com/carte.php?num_car=1310&lang=en. With some modifications.

Table 1
Trypanosome infection in camels in the East Nile and West Nile areas of Sudan.

	rGM6-4r	TeCA	CATT/*T. evansi*
East Nile	100/148 (68%)	61/148 (41%)	59/148 (40%)
West Nile	18/41 (44%)	12/41 (29%)	14/41 (34%)
Total	118/189 (62%)	73/189 (39%)	73/189 (39%)

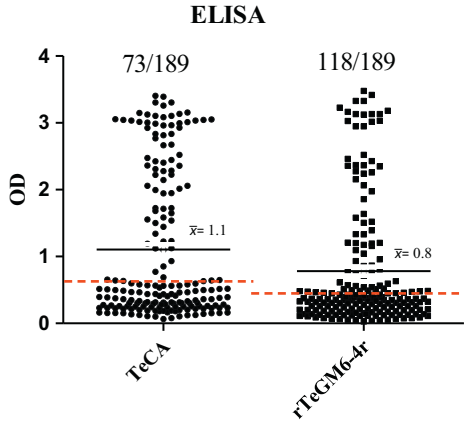

Fig. 2. The ELISA results of serum samples (n = 189) collected from camels from the East Nile and West Nile areas of Sudan. Sera were tested using *T. evansi* recombinant protein (rTeGM6-4r) and *T. evansi* crude antigen (TeCA). The dotted red lines indicate the cutoff ELISA value (0.306) for rTeGM6-4r and (0.663) for TeCA. ($\bar{x}$) indicates the mean OD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

endemic in the study area. This confirmed our previous data, which were obtained using parasitological and molecular techniques (Mossaad et al., 2017). The ELISA used in this study was derived from a system established with an antiglobulin conjugated with horseradish peroxidase (HRPO) that is specific to sheep or cattle immunoglobulins (Nguyen et al., 2014). Given the lack of a specific anti-camel HRPO conjugate, peroxidase-conjugated protein Awas applied in this study and proved to be quite efficient for the assessment of camel samples; this is in line with the findings of other authors (Desquesnes et al., 2009; Rae et al., 1989; Zweygarth et al., 1986). The application of peroxidase-conjugated protein Aovercomes the difficulties associated with the production of specific anti-dromedary immunoglobulin, which has been used in other studies (Molina et al., 1999). The rTeGM6-4r ELISA detected 62% (118/189) of the positive samples, while the CATT/*T. evansi* and TeCA ELISA detected the same number of the positive samples 39% (73/189). It is worth mentioning that the camels in this study were previously confirmed to be harboring *T. vivax* as well as *T. evansi* (Mossaad et al., 2017). This confirmed that rTeGM6-4r ELISA assays applied in this study are not species-specific, since the TeGM6 antigen is 100% identical to *T. b. brucei* GM6 and is highly conserved among salivarian trypanosomes (Nguyen et al., 2014; Pillay et al., 2013; Thuy et al., 2012; Li et al., 2005). The results of the present study suggests that the rTeGM6-4r ELISA may have an application as a universal diagnostic test, as it would detect salivarian trypanosome species, including *T. b. brucei*, *T. congolense* and *T. vivax*. One possible explanation for why the rTeGM6-4r ELISA detected a greater number of positive samples in comparison to the CATT/*T. evansi,* is the absence of the RoTat 1.2 VSG gene—the marker of CATT/*T. evansi*—in some *T. evansi* isolates from Sudanese camels (Mossaad et al., 2017; Salim et al., 2011) as well as that CATT/*T. evansi* principally detects IgM. The rTeGM6-4r ELISA has detected a greater number of positive samples in comparison to the TeCA ELISA, this most probably due to the ability of the rTeGM6-4r ELISA to detect the antibodies against *T. vaivax* as well

as *T. evansi*. The moderate agreement between the TeCA ELISA and rTeGM6-4r ELISA is because of the ability of the rTeGM6-4r ELISA to detect *T. vivax* positive sera which could not be detected by the TeCA ELISA. However, the rTeGM6-4r ELISA has detected all positive samples detected by the TeCA ELISA. The results of this study showed that ELISAs utilizing TeCA could be efficiently applied in the serodiagnosis of camel trypanosomosis caused by only *T. evansi*.

The present study established and validated ELISAs for camels, based on crude (TeCA) and recombinant (rTeGM6-4r) antigens, using a commercially-available protein A conjugate. The ELISA test demonstrated satisfactory performance. ELISA utilizing recombinant (rTeGM6-4r) antigens could be used for large-scale seroprevalence studies of camel trypanosomosis caused by *T. evansi* and *T. vivax,* while TeCA ELISA could be used for large scale seroprevalence of *T. evansi*. The CATT/*T. evansi* can be used to target individual animals for treatment with trypanocidal drugs as recommended by OIE (OIE, 2012).

5. Conclusion

This is the first report on crude and recombinant antigen ELISAs that can be applied in investigating the seroprevalence of camel trypanosomosis in Sudan. In addition, the rTeGM6-4r ELISA was found to be a suitable test for the mass screening of camel trypanosomosis caused by both *T. evansi* and *T. vivax*. This study emphasizes the need for further research with regard to the diagnosis and curative treatment of camel trypanosomosis, since the rTeGM6-4r ELISA revealed that 62% of the serum samples that were assessed in the present study were positive for trypanosomes (including *T. vivax*); this seroprevalence is very high in comparison to that reported in a previous study on *T. vivax* and co-infection with *T. vivax* and *T. evansi* (Mossaad et al., 2017). Further studies in several host species are anticipated, as co-herding and apparently healthy carriers might play a vital role in the transmission of camel trypanosomosis. The results of the present study also suggest that camel owners and veterinary authorities should no longer neglect camel trypanosomosis.

Ethics approval and consent to participate

Permission to conduct this study was obtained according to the standards of animal experimentation at the Obihiro University of Agriculture and Veterinary Medicine (Approval no. 28-46).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests in association with the present study.

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