# Application of crude and recombinant ELISAs and immunochromatographic test for serodiagnosis of animal trypanosomosis in the Umkhanyakude district of KwaZulu-Natal province, South Africa

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ABSTRACT.  A total of 231 serum samples were collected from sheep (n=9), goats (n=99) and cattle (n=123) in northeastern KwaZulu-Natal, South Africa. Trypanosome infection was detected using *Trypanosoma brucei brucei* crude antigen (TbbCA) and *T. congolense* crude antigen (TcoCA) ELISA assays. Recombinant antigen (*T. evansi* GM6 which consisted of 4 repeat domains, TeGM6-4r) ELISA and immunochromatographic test (ICT) were also used. Crude antigen ELISA, TeGM6-4r-ELISA and ICT detected 27.3%, 29% and 19.9% of trypanosome seropositive samples, respectively. Trypanosome infection prevalence in cattle and goats was 35.8–46.3% and 0–9.1%, respectively. Out of 9 sheep serum samples, 2–4 sera (22.2–44.4%) were positive. The detection performance of crude and recombinant antigen ELISAs was relatively similar (K=0.6–0.7); both are recommended for reference diagnosis and large scale epidemiological surveys. There is potential application for ICT in on-site diagnosis, but its sensitivity should be improved.
KEY WORDS:  animal African trypanosomosis, ELISA, immunochromatographic test, serodiagnosis, South Africa

Nagana is the form of animal African trypanosomosis caused by *Trypanosoma brucei brucei, T. congolense* and *T. vivax* in domestic animals including cattle, dogs, donkeys, goats, horses and sheep [10, 13, 17]. These trypanosomes are mainly transmitted to their host by arthropod vectors of the genus *Glossina*, which are commonly known as tsetse flies [2, 7, 12].

Historically, South Africa occupies an important position with regard to animal African trypanosomosis. David Bruce's discovery of the parasite in animals from South Africas' Zululand in 1895 [1], resulted in the formulation of continent-wide control strategies. There have been four tsetse species in South Africa: *G. morsitans morsitans, G. pallidipes, G. brevipalpis* and *G. austeni* [1, 12]. *G. m. morsitans* was completely disappeared from South Africa after the rinderpest epizootic in 1897, while *G. pallidipes* was totally eliminated in an extensive air spraying and animal

dipping in insecticides during 1950s [7]. To date, only *G. brevipalpis* and *G. austeni* remain and are confined to the indigenous forests, river beds, protected nature reserves and game parks located in KwaZulu-Natal Province [12, 20]. Recent studies on the prevalence of Nagana in South Africa reported the presence of *T. congolense* and *T. vivax* infections in the insect vector and livestock in the area [2, 11, 12]. In 1990, an outbreak of Nagana in northeastern KwaZulu-Natal led to the implementation of emergency control measures [7], and it indicated that this disease had been neglected in South Africa. As a result, it highlighted the need for frequent epidemiological surveys to monitor the prevalence of trypanosome infections.

All recent prevalence and epidemiological studies of Nagana in South Africa have been determined by microscopy and PCR based techniques [2, 8, 11, 12, 20]. Serological assays, however, are useful tools for large-scale epidemiological surveillance [21]. According to the World Organization for Animal Health (OIE 2012), antibody detection ELISA using trypanosome crude antigen is regarded as a conventional and standard method for the diagnosis of animal trypanosomosis. In addition to the conventional tests, the development of recombinant technology has led to the introduction of a number of new recombinant antigens, including *T. evansi* GM6 which consisted of 4 repeat domains (TeGM6-4r) for use in disease diagnostic surveillance [3, 14, 19]. We recently developed two immuno-diagnostic assays that are capable of

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detecting antibodies to animal trypanosomes: TeGM6-4r-based ELISA and an immunochromatographic test (ICT) [14]. This study therefore sought to evaluate the application of crude and recombinant antigen ELISAs and ICT in the serodiagnosis of animal trypanosomosis in livestock in the Umkhanyakude District of KwaZulu-Natal Province, South Africa.

Blood sample collection was conducted out in 3 local municipalities of Umkhanyakude district in northeastern KwaZulu-Natal Province, namely Hlabisa (28.1333° S, 31.8667° E), Mtubatuba (28.4167° S, 32.1833° E) and The Big 5 False Bay (28.0189° S, 32.2675° E) between March and May 2013 (Fig. 1). In the sampling areas, the farming system is called rural communal farming which is not for commercial purposes, but for family consumption and a sign of wealth. Sample animals were from communal farmers for which owners did not have information on the age of animals. The cattle breed was Nguni; and there was no information on goat and sheep breeds. Blood was collected from the jugular vein of sheep, goats and cattle into silicone coated vacutainers (BD Vacutainer System, Oxford, U.K.), allowed to clot before serum was harvested and then stored at −20°C until further use. A total of 231 serum samples were obtained from 9 sheep, 99 goats and 123 cattle (Table 1). Sheep are not preferred as domestic livestock in KwaZulu-Natal Province, hence, only a few were available during the sampling period. Three serum samples for use as positive controls were obtained from mice experimentally infected with *T. congolense* IL3000. Five sheep, ten goat and ten cattle serum samples from KwaZulu-Natal Province, which were confirmed to be negative following microscopy and PCR, were included as negative controls.

All serum samples were tested with TbbCA-ELISA, TcoCA-ELISA and recombinant TeGM6-4r based ELISA. Trypanosome crude antigens were prepared according to the OIE manual [15], from *T. b. brucei* GUTat 3.1 bloodstream forms and *T. congolense* IL3000 procyclic forms propagated in HMI-9 and TVM-1 *in vitro* cultures as described previously [5, 6]. The recombinant antigen TeGM6-4r was produced using the protocol described by Thuy *et al.* [19] and ELISA conducted using the method of Thuy *et al.* [19] with minor modifications. Five min after adding the substrate, the optical density (OD) values were determined by measuring $OD_{620}$ using a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, Shanghai, China).

The TeGM6-4r based ICT was developed using the recombinant antigen TeGM6-4r [14, 19]. The antigen TeGM6-4r was immobilized at the test line, and anti-TeGM6-4r polyclonal antibodies were immobilized at the control line on the ICT strip. For the color indicator, TeGM6-4r was conjugated with gold colloid (BBI Solutions, Cardiff, U.K.) and sprayed on the conjugate part. To test the samples, serum samples were diluted 1:5 times in phosphate buffered saline and applied onto the sample part of the ICT strips. The results were observed by naked eye after 10–20 min. The sample was recorded as positive if both the control and test lines indicated red coloration or as negative if only the control line turned red. Data were analyzed using Graphpad (http://



Fig. 1. Maps showing the sampled area. (A) South Africa with its nine provinces. (B) KwaZulu-Natal Province. (C) Umkhanyakude district with its five local municipalities. Samples were collected in Hlabisa, Mtubatuba and The Big 5 False Bay local municipalities.

graphpad.com/quickcalcs/). Kappa values were calculated and interpreted according to the method of Viera & Garrett [22].

The serum samples from sheep, goats and cattle demonstrated immunoreactivity in ELISA assays (Fig. 2). Cutoff values for each ELISA test were calculated using the negative controls. The cutoff values of crude antigen and recombinant antigen ELISAs for sheep were 0.25 and 0.22, respectively; while the cutoff value of all the ELISA assays for goat was 0.34 and cattle was 0.22, respectively. The results show that TeGM6-4r ELISA detected the highest number of trypanosome-positive samples at 29% (67/231). The two crude antigen based ELISA assays, TbbCA and TcoCA, both detected 27.3% (63/231) trypanosome-positive samples. Using the ICT, only 19.9% (46/231) of samples were found to be trypanosome-positive. In comparison to the reference tests, TbbCA and TcoCA ELISAs, the TeGM6-4r ELISA demonstrated high sensitivity and specificity ranging from 73.0–79.4% and from 87.5–89.9%, respectively with a kappa value of 0.6–0.7, indicating substantial agreement [22]. The ICT had a relatively low sensitivity (30.2–36.5%) and moderate specificity (83.9–86.3%) with a kappa value of 0.2–0.3 (low agreement). Seroprevalence of animal trypanosome infections was variably estimated by different serodiagnostic tests applied in this study (Fig. 3). The prevalence in goats and cattle was 0–9.1% and 35.8–46.3%, respectively. No trypanosome-positive goat samples were detected by ICT; this compared to 6–9% goat serum samples which were positive with the other 3 diagnostic assays. Out of 9 sheep serum samples, crude antigen based ELISAs, TeGM6-4r ELISA and ICT detected 3, 4 and 2 trypanosome-positive sera, respectively.

The results of this study showed that TeGM6-4r ELISA was relatively sensitive (73.0–79.4%) and specific (87.5–89.9%) in the detection of animal trypanosomes in sheep, goats and cattle. In comparison with crude antigen-based

Table 1. Trypanosome infection in sheep, goat and cattle serum samples detected by ELISAs and ICT

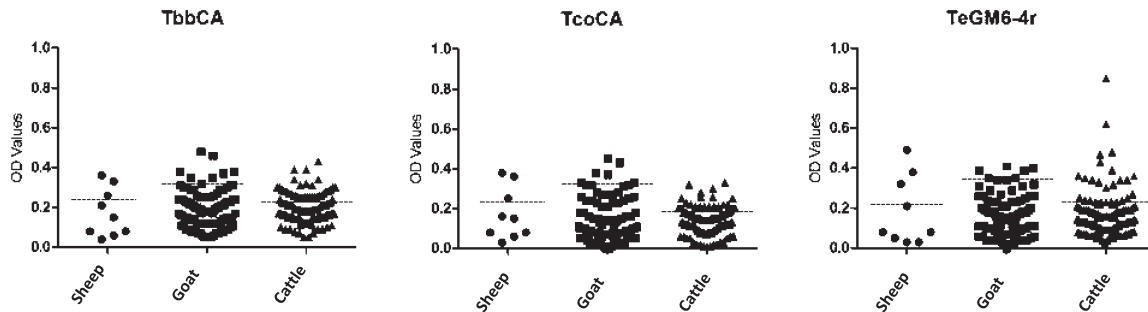| Animal species | Sample number | TbbCA/ELISA | TcoCA/ELISA | TeGM6-4r/ELISA | TeGM6-4r/ICT |
|---|---|---|---|---|---|
| Sheep | 9 | 3 (33.3) | 3 (33.3) | 4 (44.4) | 2 (22.2) |
| Goat | 99 | 7 (7.1) | 9 (9.1) | 6 (6.1) | 0 (0) |
| Cattle | 123 | 53 (43.1) | 51 (41.5) | 57 (46.3) | 44 (35.8) |
| Total | 231 | 63 | 63 | 67 | 46 |
| Percentage (%) | – | 27.3 | 27.3 | 29 | 19.9 |



Fig. 2. ELISA results of serum samples (n=231) collected from sheep, goats and cattle in KwaZulu-Natal Province of South Africa. The number of sheep, goats and cattle was n=9, n=99 and n=123, respectively. Sera were tested using 3 different antigens: *T. b. brucei* crude antigen (TbbCA), *T. congolense* crude antigen (TcoCA) and recombinant protein TeGM6-4r. Broken lines indicate the cutoff values: TbbCA/ELISA for sheep, goats and cattle was 0.25, 0.34 and 0.22; TcoCA/ELISA for sheep, goats and cattle was 0.25, 0.34 and 0.22, and TeGM6-4r/ELISA for sheep, goats and cattle was 0.22, 0.34 and 0.22, respectively.
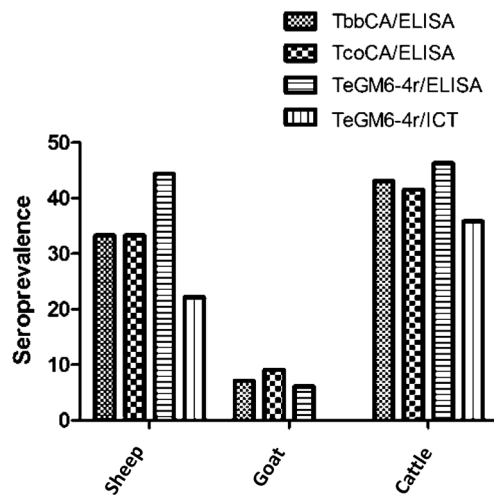


Fig. 3. Seroprevalence of animal trypanosomosis in sheep, goats and cattle detected by ELISAs and ICT. Seroprevalence was variably estimated by different serodiagnostic tests. The prevalence in sheep, goats and cattle was 22.2–44.4%, 0–9.1% and 35.8–46.3%, respectively. No trypanosome-positive goat samples were detected by ICT.

ELISAs, it detected more seropositive sheep and cattle serum samples, indicating a significant sensitivity of the recombinant antigen. This result is consistent with the report of Nguyen *et al.* [14]. The ELISAs and TeGM6-4r ICT 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 [9, 14, 19]. The advantage of the tests is that with the use of a universal diagnostic antigen, it would detect salivarian trypanosome species including *T. b. brucei*, *T. congolense* and *T. vivax*. The GM6 from *T. b. brucei*, *T. congolense* and *T. vivax* could also be used in ELISA. At present, TeGM6-4r and *T. vivax* GM6 ELISA have been evaluated [14, 16]. In this study, sheep, goats and cattle were found to have been exposed to animal trypanosomosis infections. The finding, that the disease is more prevalent in cattle, is in line with previous prevalence reports on animal trypanosomoses in South Africa [2, 8, 11, 12, 20]. Considering the high number of samples collected in this study, goats appear to be less susceptible to animal trypanosome infections; or rather, the tsetse vector has a greater preference for cattle [18]. Goats are considered to be important reservoirs for trypanosomes, and thus, they should be taken into consideration in all programs aimed at controlling the disease [4]. Although the number of sheep samples was limited (n=9) in this study, a relatively high percentage of trypanosome-positive sera (22.2–44.4%) was detected, indicating that there is a need to further investigate the actual prevalence of trypanosomosis in this host.

In conclusion, this is the first report on the evaluation of ICT and recombinant antigen ELISA assays for animal trypanosomosis prevalence studies in South Africa. Reports on the prevalence of animal trypanosomosis and epidemiological studies in South Africa are based on microscopic examination of blood or buffy coat smears for the presence of trypansome parasites or PCR-based diagnostic methods for the presence of trypanosome DNA. Serodiagnostic as-

says have not been widely exploited for diagnosis of animal trypanosomosis. The results of this study showed that ELISA assays utilizing crude and recombinant (TeGM6-4r) trypanosome antigens were highly sensitive and efficient for application in the serodiagnosis of trypanosome infections in livestock. Since the recombinant antigen can be expressed and easily produced in large batches in the laboratory, the TeGM6-4r ELISA could be considered as a useful and reliable supplementary diagnostic technique to microscopy and PCR for the detection of animal trypanosomosis in South Africa. Although the TeGM6-4r ICT was less sensitive than ELISA, it was relatively specific, simple and rapid. With further improvement on its sensitivity, the trypanosome ICT has the potential for use in both research and on-site diagnosis of animal trypanosomosis in trypanosome endemic areas.

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