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Evaluation of mono- and polyclonal antibody-based antigen detection immunoassays for diagnosis of *Trypanosoma evansi* infection in the dromedary camel

DIALL (O.), NANTULYA (V.M.), LUCKINS (A.G.), DIARRA (B.), KOUYATE (B.). Evaluation des tests immuno-enzymatiques de détection des antigènes au moyen des anticorps mono- et polyclonaux pour le diagnostic de l'infection à *Trypanosoma evansi* chez le dromadaire (*Camelus dromedarius*). *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (2) : 149-153

L'aptitude de deux tests ELISA d'absorption immuno-enzymatique utilisant, l'un un anticorps monoclonal spécifique anti-*Trypanosoma brucei* obtenu sur souris, l'autre des anticorps polyclonaux spécifiques de *Trypanosoma evansi* produits sur lapin, a été évaluée en vue de la détection des antigènes circulants comme méthode de diagnostic des infections à *Trypanosoma evansi* dans le sérum des dromadaires. Quatre vingt onze sérums d'un troupeau camelin témoin du Kenya indemne de *T. evansi* ont tous donné des résultats négatifs au test ELISA des anticorps monoclonaux et seuls deux d'entre eux (2,2 p.100) ont donné des résultats faussement positifs avec les anticorps polyclonaux. Lors d'analyses ultérieures des sérums d'animaux infectés (décelés par inoculation à la souris), les anticorps monoclonaux ont décelé les antigènes dans 90 sérums sur 108 testés (83,3 p.100). Cette proportion s'est révélée inférieure pour les polyclonaux qui ont détecté les antigènes dans 67 des 110 sérums testés soit 60,9 p.100. Les deux tests ont détecté des antigènes différents, mais dans la combinaison des résultats, 99 sérums sur 107 se sont révélés positifs, soit 92,5 p.100. Dans une enquête portant sur 316 sérums provenant des régions de Gao et Nara au Mali, une forte proportion a réagi positivement aux antigènes (43,5 p.100 pour le monoclonal et 42,9 p. 100 pour le polyclonal). Parmi ces sérums, 22 (7,0 p. 100) seulement sont issus de sujets à parasitologie positive. Ainsi, les tests ELISA se sont montrés au moins six fois plus sensibles que la technique de centrifugation de l'hématocrite. Au vu de ces résultats qui démontrent que dans une proportion importante des sujets peuvent être à la fois antigène-positifs et parasitologiquement négatifs, les auteurs recommandent dans la pratique courante du "surra", l'emploi de ces deux tests immuno-enzymatiques en complément des techniques basées sur la parasitologie chez le dromadaire.

Mots clés : Dromadaire - *Camelus dromedarius* - *Trypanosoma evansi* - Trypanosomose - Antigène - Sérologie - Diagnostic - Test ELISA - Mali - Kenya.

INTRODUCTION

Trypanosomosis caused by *Trypanosoma evansi*, commonly known in Africa as "surra" is a major protozoal disease affecting camels (7). Although "surra", has been reported in all the camel breeding areas, its distribution and economic importance are not yet satisfactorily established. This is largely because the detection of trypanosomes in peripheral blood, which is the principal method of diagnosis, is not sensitive enough especially when applied to chronic infections (3, 4, 7, 8).

The search for more sensitive diagnostic tests has previously focused on the detection of anti-trypanosome antibodies in sera of infected animals. The main tests developed are the enzyme-linked immunosorbent assay (ELISA), the immunofluorescent antibody test (IFAT) and the card agglutination test (CATT) (1, 2, 6, 16, 17). However, using these tests it is not possible to differentiate between past and current infections because antibodies can persist for several months following cure (5).

New assays have recently been developed which detect circulating trypanosome antigens in camel sera as a means for diagnosis (10, 12, 15). The test developed by RAE and LUCKINS (15) utilizes polyvalent rabbit antisera to *T. evansi* lysates while the assays described by NANTULYA *et al* (10, 12) are based on monoclonal antibodies against a *T. brucei* group-specific antigen. This article reports the comparison of both tests for the diagnosis of camel trypanosomosis.

MATERIALS AND METHODS

Camel sera

Three groups of sera were analyzed. The first one consisted of 112 sera obtained from camels in Northern Kenya. All these animals had a parasitologically confirmed diagnosis as determined by mouse inoculation (4).

The second one was obtained from a control herd of 91 camels from a farm at Athi River, Kenya. All camels on this farm were parasitologically negative as determined by mouse inoculation (4) and by buffy coat examination (8). None of these sera was positive for anti-trypanosome antibodies as revealed by ELISA (J.N. WAITUMBI, personal communication).

The third group came from a trypanosomosis survey carried out in the Nara and Gao areas in Mali. A total of 316 animals were sampled, 7 % of which were found to harbour *T. evansi*-like trypanosomes by thin and thick blood films and by buffy coat examination.

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Techniques for antigen detection

Monoclonal antibody-based ELISA

This technique was used as described previously (10, 12). Briefly, flat bottom micro-ELISA plates were coated with 0.5 µg/ml of a partially purified IgM fraction of monoclonal antibody TR7/47.34.16 in carbonate-bicarbonate buffer, pH 9.6, with 0.02 % sodium azide and kept at 4 °C until use. This antibody is *T. brucei* group-specific (14). The excess coating antibody was drained off and washing buffer (Dulbecco's PBS pH 7.4 containing 0.5 % Tween 80) was added to all the wells in volumes of 50 µl/well. An equal volume for each serum sample was tested in duplicate. The plates were incubated for 15 min at room temperature, the excess reactants drained off and the conjugate (the same monoclonal antibody labeled with horse radish peroxidase), diluted 1:250 in washing buffer containing 1 % normal mouse serum was added without prior washing of the plate.

The plate was incubated for 15 min, the buffer drained off, after which the wells were filled with washing buffer and left for 10 min. This procedure was repeated twice. After the last wash, the buffer was drained off and the chromogen and substrate (100 µl/well) were added. The chromogen used consisted of 250 µl/ml of 2,2'azino bis (3 ethyl)-benzothiazoline - 6 - sulfonic acid (ABTS) in 50 mM citric acid buffer, pH 4.0, containing 0.01 % substrate (hydrogen peroxide). The optical densities (O.D.'s) were read after 30 min at 414 nm wave length, using a Titertek Multiskan micro ELISA auto-reader (type MCC 340). The threshold of O.D. regarded as positive was 0.050.

Polyclonal antibody-based ELISA

Micro ELISA plates were treated with 1 % glutaraldehyde (100 µl/well). After 30 min incubation, plates were rinsed twice then filled with distilled water and left for 15 min. The water was poured off and the plates coated with rabbit anti-*T. evansi* IgG diluted 1:1700 in carbonate -bicarbonate buffer, pH 9.6, (100 µl/ml) and incubated overnight at 4 °C.

The excess coating antibody was drained off and the plates rinsed once, then washed 3 x 3 min with washing buffer (PBS/0.05 %, Tween 20). Washing buffer containing 1 % bovine serum albumin (diluting and blocking buffer) was then dispensed in volumes of 100 µl/well and the plates incubated for 30 min at 37 °C. After incubation, the plates were rinsed and washed as indicated above. Then the test sera diluted 1:5 in diluting buffer were dispensed (100 µl/well).

The plates were washed again in the same way and the conjugate (rabbit anti-*T. evansi* IgG coupled with horse, radish peroxidase) diluted 1:1000 in diluting buffer was dispensed (100 µl/well). Plates were incubated at 37 °C. After an extensive washing as indicated for the first test, chromogen in substrate buffer was dispensed (100 µl/well) and the optical densities read as before. As in the previous test the threshold of O.D. regarded as positive was 0.050.

RESULTS

Sera from camels with parasitologically confirmed diagnosis (Northern Kenya)

In the monoclonal antibody-based ELISA, 83,3 % of the sera from animals with parasitologically confirmed diagnosis, were antigen-positive, while the polyclonal antibody-based ELISA was able to detect antigens in 60.9 % of the sera. When both tests were applied to the same samples, the proportion of positive animals detected rose to 92.5 % (table I). The distribution of O.D. values is shown in figure 1.

TABLE I Detection of circulating antigens in sera of parasitologically confirmed cases (from Northern Kenya).

Tests	Number of sera tested	Number of sera positive for antigens	% positive
Monoclonal antibody-based ELISA	108	90	83.3
Polyclonal antibody-based ELISA	110	67	60.9
Combination of both tests	107	99	92.5

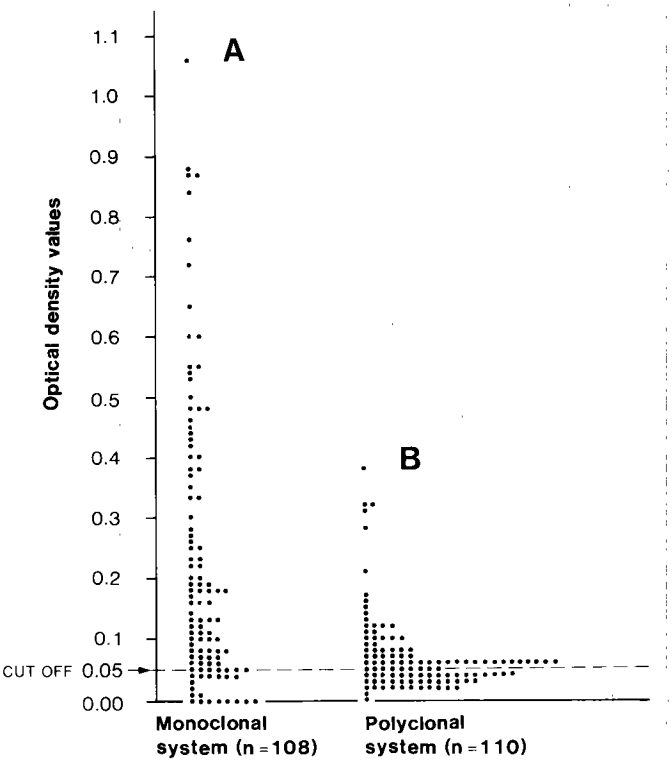

Fig. 1 : Optical density values in a population of camels with a parasitologically confirmed diagnosis.

Sera from a control negative herd (Athi River, Kenya)

All the camel sera from a trypanosomosis-free farm gave negative readings when the monoclonal antibody-based ELISA was used, and only 2 of the 91 (2.2 %) sera gave positive reactions when the polyclonal system was used.

Sera from the field survey

Of the sera collected from camels in the field survey, 43.5 % were antigen positive by the monoclonal antibody-based ELISA and 42.9 % positive by the polyclonal antibody based ELISA (table II). In contrast, infections were detected in only 7 % of the animals by parasitological techniques. The sera of 19 of these parasitologically positive animals were tested with both ELISAs : 18 were positive in the monoclonal system, 14 in the polyclonal system. The distribution of O.D. values for the antigen positive samples is shown in figure 2.

TABLE II Results of a trypanosomosis survey carried out on camels in an endemic area of Mali using three different tests.

Test	Number of samples	Number of positive	% positive
Parasite detection (HCT, smears)	316	22	7.0
Antigen detection (monoclonal antibody-based ELISA)	308	134	43.5
Antigen detection (polyclonal antibody-based ELISA)	298	128	42.9
Combination of both ELISA tests	296	184	62.0

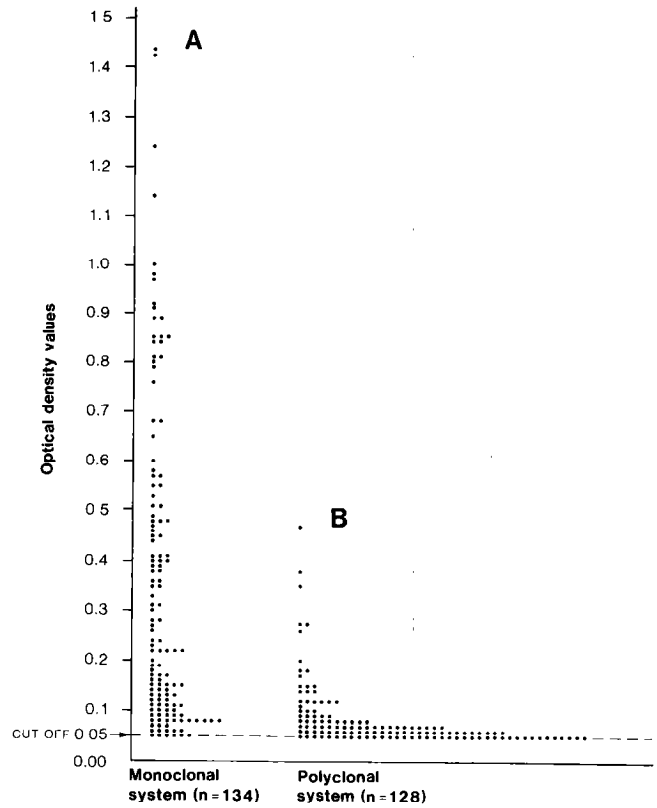

Fig. 2 : Optical density values in a population of camels with antigen-positive diagnosis (field survey).

DISCUSSION

The techniques currently used for diagnosis of camel trypanosomosis involve the detection of trypanosomes in the blood or of anti-trypanosome antibodies in the serum. Demonstration of trypanosomes in the blood of the animal is indeed the best evidence for an infection but the parasite detection techniques are not only tedious, but have also a limited sensitivity especially in the chronic phase of infection which is often aparasitaemic (7). Antibody detection is more sensitive but the presence of antibodies in blood does not always reveal a current infection, since an animal cured can have persistent antibodies for a long time (5) ; besides, in the field, the individual treatment history is usually unreliable.

The immunoassays designed to detect circulating trypanosome antigens appear to provide a significant improvement in the diagnosis of camel trypanosomosis (10, 12, 15). The survey results described here show that these assays were at least 6 times more sensitive than the parasite detection techniques. Thus, whereas only 7 % of the animals examined in the survey had detectable parasitaemia, the antigen detection assays revealed infection rates of 42.9 and 43.5 %, respectively with the polyclonal and monoclonal antibody-based ELISA. Moreover, the advantage of antigen detection assays over antibody assays is that antigen-positivity is a reflection of current infections (11).

Considering the results obtained with the camel population from a trypanosomosis-free farm, which were all negative by the monoclonal antibody system and where only 2 % were positive in the polyclonal antibody system, antigen positivity in the survey samples most likely represented true trypanosome infections.

The correlation of antigenaemia with parasitaemia was evaluated using sera collected from camels with positive parasitological diagnosis as determined by mouse

inoculation. The immunoassay based on monoclonal antibody detected antigens in 83.3 % of the sera, while the polyclonal antibody system detected antigens in 60.9 % of them. The two assays probably identified different antigens. These antigens could not be trapped by one antibody system and revealed by the other (results not shown). A combination of the two assays led to detection of antigens in up to 92.5 % of the animals.

It was noted that some parasite positive animals were antigen ELISA negative. As suggested by NANTULYA (9), failure of the assays to detect antigens in sera from parasite positive animals may be due to the possibility of an early infection in these animals. This is because the antigens detected are structural components of the parasite plasma membrane which are not released until the occurrence of trypanolysis. It has also been suggested that such cases could be due to mopping up of the antigens by the antibody (9). The former is a more plausible explanation obtained under experimental conditions (11, 13).

CONCLUSION

Antigen detection enzyme immunoassays appear to complement the parasite detection techniques by identifying chronic *T. evansi* infections in which parasites are rarely detected. Introduction of these assays into routine diagnosis is likely to lead to a significant improvement in the diagnosis of *T. evansi* infections.

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DIALL (O.), NANTULYA (V.M.), LUCKINS (A.G.), DIARRA (B.), KOUYATE (B.). Evaluation of mono- and polyclonal antibody-based antigen detection immunoassays for diagnosis of *Trypanosoma evansi* infection in the dromedary camel. *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (2) : 149-153

Two enzyme-linked immunosorbent assays (ELISA), one based on a mouse anti-*Trypanosoma brucei* group-specific monoclonal antibody and the other on rabbit anti-*Trypanosoma evansi* polyclonal antibodies, have been evaluated for their ability to detect circulating trypanosome antigens in camel sera as a means for the diagnosis of *T. evansi* infections. All 91 sera from a negative control camel herd from Kenya gave negative antigen-ELISA results in the monoclonal antibody-based ELISA and only 2 of them (2.2 %) gave false positive results in the polyclonal antibody-based ELISA. In subsequent analyses of sera from infected camels (as determined by mouse inoculation), the monoclonal antibody-based ELISA detected antigens in 90 (83.3 %) out of the 108 sera tested. This percentage was lower for the polyclonal antibody-based ELISA which was able to detect antigens in 67 (60.9 %) out of the 110 sera tested. The two tests detected probably different antigens and when the results were combined, 99 out of 107 (92.5 %) sera were shown to be ELISA positive. In a survey involving 316 camels from the Gao and Nara areas, in Mali, a high proportion of animals tested were antigen positive (43.5 and 42.9 %, respectively for the mono- and polyclonal antibody-based ELISA) compared to only 22 (7.0 %) diagnosed by the parasite detection techniques. Thus, these immunoassays were at least six times more sensitive than the haematocrit centrifugation technique. As a large proportion of cases may be antigen positive but parasite negative, these two of "surra" immunoassays should be used in routine diagnosis in addition to the parasite detection techniques in the dromedary camel. *Key words* : Dromedary camel - *Camelus dromedarius* - *Trypanosoma evansi* - Trypanosomosis - Antigen - Serology - Diagnosis - ELISA - Mali - Kenya.

DIALL (O.), NANTULYA (V.M.), LUCKINS (A.G.), DIARRA (B.), KOUYATE (B.). Evaluación de inmunoensayos para la detección de antígenos basados en anticuerpos mono y policlonales para el diagnóstico de la infección por *Trypanosoma evansi* en el dromedario (*Camelus dromedarius*). *Revue Élev. Méd. vét. Pays trop.*, 1992, **45** (2) : 149-153

La detección de antígenos tripanosómicos circulantes en suero de dromedario (como medio de diagnóstico de infecciones por *T. evansi*), se evaluó mediante dos tests de ELISA : uno basado en anticuerpos monoclonales de ratón, específicos anti-*Trypanosoma brucei* y el otro en anticuerpos policlonales de conejo anti-*T. evansi*. La totalidad de los 91 sueros, provenientes de animales kenianos de un hato control sero-negativo, presentó resultados negativos al ELISA basado en anticuerpos monoclonales y solamente hubo dos (2,2 p. 100) falsos positivos en el ELISA basado en anticuerpos policlonales. En análisis posteriores, realizados con sueros provenientes de animales infectados (determinados por inoculación de ratón), el ELISA basado en anticuerpos monoclonales detectó antígenos en 90 (83,3 p. 100) de los 108 sueros examinados. Este porcentaje fue menor en el ELISA basado en anticuerpos policlonales, ya que detectaron antígenos en 67 (60,9 p. 100) de los 110 sueros examinados. Probablemente cada test detecta antígenos diferentes, al combinar los resultados, 99 (92,5 p. 100) de los 107 sueros fueron ELISA positivos. En una encuesta realizada en las zonas de Gao y Nara (Mali), en 316 dromedario, la proporción de animales seropositivos fue alta (43,5 y 42,9 p. 100 para el ELISA monoclonal y policlonal respectivamente), si se compara con los 22 (7,0 p. 100) diagnósticos determinados por técnicas de detección parasitológicas. Los inmunoensayos fueron seis veces más sensibles que la técnica de centrifugación de microhematocrito. En una gran proporción de los casos, los animales pueden ser seropositivos y "parásito negativos", de manera que estos dos inmunoensayos deben utilizarse en los diagnósticos de rutina, junto con las técnicas parasitológicas de detección en el dromedario. *Palabras claves* : Dromedario - *Camelus dromedarius* - *Trypanosoma evansi* - Trypanosomiasis - Antigeno - Serología - Diagnóstico - ELISA - Mali - Kenia.

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