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Diagnosis Of *Trypanosoma evansi* Infection Among Sudanese Camels Imported To Egypt Using Card Agglutination Test (CATT) And Antigen Detection Latex Agglutination Test (Suratex)

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ABSTRACT

A total of 125 imported Sudanese camels were tested for *Trypanosoma evansi* infection to determine the prevalence of camels with chronic disease that may act as an exotic source of infection. The research employed direct blood smear examination, card agglutination test (CATT/*T. evansi*) for antibody detection and latex agglutination test (Suratex) for the detection of circulating trypanosomal antigens. Blood smear examination detected patent parasitemia in five camels (4.0%) (two cases were CATT negative). Thirty six camels (28.8%) were CATT positive, while forty five (36.0%) were positive for circulating antigens. Thirty one cases (24.8%) were positive for both tests. Five (4.0%) CATT reactors were negative for trypanosomal antigens. However, 14 (11.2%) camels with circulating trypanosomal antigens, including one case with patent parasitemia, tested CATT negative. Samples of these camels, as well as camels with parasitemia were tested for antibodies to common trypanosomal antigens using indirect hemagglutination test (IHA) using *T. brucei gambiense* antigen. Eleven (78.60%) of the 14 CATT-negative camels had IHA titers ranging from 1/64-1/2048 (including the case with parasitemia) giving concurrence with Suratex results. All five cases with patent parasitemia were positive for IHA. Detection of circulating *T. evansi* antigens using Suratex was found to be a more sensitive and reliable means of practical diagnosis of camels with chronic latent infection.

INTRODUCTION

Cameline trypanosomiasis (surra), caused by *Trypanisoma evansi*, is considered the most serious disease affecting camels in Egypt. It is assumed that the instability of the local enzootic situation may be accentuated through the massive inflow of imported Sudanese camels that may act as a continuous source of exotic *T. evansi* infection. However, the available information about the prevalence of the disease among the native and imported camel populations, as well as other livestock species, is insufficient for the establishment of a comprehensive view about the epizootiology of the disease nationwide.

Furthermore, the traditional diagnosis of camel trypanosomiasis in Egypt has been based on outdated methodology including, the mercuric chloride test and the formal gel test (Abdel-Latif 1957; Safwat 1980; Fayed et al. 1984) and the thymol turbidity test (Abdel-Ghaffar 1960). These methods are not sufficiently reliable for the accurate diagnosis of latent infections (Pegram and Scott 1976). Limited trails of serological diagnosis of camel surra have been carried out in Egypt. These include indirect hemagglutination test (IHA) (Elsaid 1992) and ELISA (El-Sawalhy and Ebeid 1994).

Recently, several field-oriented diagnostic procedures have been developed for the diagnosis of surra in camels. The card agglutination test (CATT/*T. evansi*) (Diall et al.

1994), showed high sensitivity for detecting *T. evansi*-specific antibodies, since it is based on the trypanosome variable antigen types (VAT) RoTat 1.2 which is expressed early during infection.

Moreover, a simple latex agglutination test (Suratex) was developed for the detection of circulating invariant *T. evansi* antigens in camel blood. This antigen-detection assay was more sensitive than parasitological methods for the diagnosis of non-patent infections (Nantulya 1994; Olaho-Mukani et al. 1996).

In the present research we surveyed a random sample of Sudanese camels imported into Egypt using CATT for the demonstration of trypanosomal antibodies as well as latex agglutination test (Suratex) for the detection of circulating trypanosomal antigens, aiming to determine an accurate prevalence of *T. evansi* infections and to identify animals that may act as a source of infection to our indigenous animals.

MATERIALS AND METHODS

Samples: Blood samples were collected in heparinized vacutainers (Becton & Dickinson, USA). Samples were randomly collected from a total of 125 Sudanese camels at Cairo (El-Basaten) abattoir. Samples were collected during the winter months, November and December, to insure minimal insect transmission of infection. Animals were apparently healthy and showed no signs suspicious of trypanosomiasis.

Blood smear examination: *Trypanosoma evansi* parasitaemia was detected by examination of Giemsa-stained blood films.

Card agglutination test for trypanosomiasis (CATT): The CATT/*T. evansi* kit was kindly supplied by Dr. Nestor van Meirvenne Institute of Tropical Medicine, Antwerp, Belgium. *Trypanosoma evansi* RoTat 1.2 antigen was reconstituted with the supplied phosphate buffered saline (PBS, pH 7.2). Test plasma samples were diluted 1:4 with supplied PBS. Fifty μl antigen were mixed with 25 μl of diluted sample on the supplied card and agitated in a circular motion on electric rotator at 60 rotations per minute for 5 min. Samples with blue granular agglutination were considered positive.

Latex agglutination antigen test (Suratex) for the detection of circulating trypanosomal antigens: The latex agglutination antigen test (Suratex) was kindly supplied by the manufacturer, AccuPharma Inc., Montreal, Canada. Fifty μl of test plasma were mixed with one drop of the latex reagent on a test circle of the supplied slide. The test slide was then tilted and rotated manually for 2 min. and examined for agglutination, then rotated for further 3 min. to allow weak reactions to develop and examined again.

Indirect hemagglutination test (IHA) : Cellognost Trypanosomiasis IHA was performed according to the instructions of the manufacturer (Behring, Germany). The antigen for IHA is prepared from the trypomastigotes of *T. b. gambiense*. The antigen is adsorbed on formolized sheep erythrocytes. Plasma samples were geometrically diluted with Tris-buffered saline pH 8.0 in V shaped microtiter plates. To 50 μl diluted plasma 25 μl of test antigen were added. Cell controls, antiserum and normal serum controls were also included. Plates were mixed by tapping and incubated at room temperature for 3 hours.

Samples were tested before and after overnight absorption (4℃) with non-sensitized sheep red cells (Behring, Germany) to confirm the specificity of the agglutination reaction.

RESULTS

The results of the different tests employed in the study are presented in Table 1. Using Giemsa-stained blood smears, five camels (4.0%) with patent *T. evansi* parasitemia were

detected. The anti-VAT RoTat 1.2 antibodies were evident in 36 camels (28.80%), while 45 (36.0%) were positive for circulating *T. evansi* antigens. Out of five camels with patent infection only three cases were CATT positive, while 4 camels were positive for Suratex. One camel was negative for both tests (Table 2).

Table 1. Results of card agglutination test (CATT), antigen detection latex agglutination (Suratex) and blood smear examination of 125 camels.

Test	No. Positive	% Positive
Blood smear	5	4.00
CATT	36	28.80
Suratex	45	36.00
CATT/Suratex	31	24.80

Table 2. Results of CATT, Suratex, IHA and blood smear examination of 25 camels.

#	CATT	Suratex	IHA*	BS
1	--	+	--	--
2	--	+	--	--
3	--	+	--	--
4	--	+	64	+
5	--	+	64	--
6	--	+	128	--
7	--	+	128	--
8	--	+	128	--
9	--	+	256	--
10	--	+	256	--
11	--	+	512	--
12	--	+	2048	--
13	--	+	2048	--
14	--	+	2048	--
15	+	+	128	+
16	+	+	256	+
17	+	+	128	+
18	--	--	2048	+
19	--	--	--	--
20	--	--	--	–
21	--	--	--	–
22	--	--	--	–
23	--	--	--	–
24	--	--	--	–
25	--	--	--	–

* IHA titers; BS = Blood Smear.

Combining the results of CATT and Suratex, 31 (24.80%) camels were positive for

both assays, while 5 (4.0%) camels positive for antibodies were negative for circulating antigens. On the other hand, 14 (11.20%) camels with detectable levels of circulating antigens, were devoid of anti-VAT antibodies (including a blood smear positive case). The remaining 75 (60.0%) camels did not evidence either *T.evansi*-specific antibodies or circulating antigens.

Samples of the CATT-/Suratex+ camels, blood smear positive cases, as well as six CATT-/Suratex- control cases, were tested for the presence of antibodies to common trypanosomal antigens by IHA using *T. b. gambiense* antigen.

Out of the 14 CATT-/Suratex+ cases, 11 were IHA positive with titers ranging from 1:64-1:2048 (including a blood smear positive case). Three cases were IHA negative. When the undiluted sera of these cases were CATT tested, 5 cases showed weak positive reaction. All the blood smear positive cases were IHA positive, including a case negative for both CATT and Suratex. All CATT-/Suratex- camels were IHA negative. No change in IHA titers was observed after overnight absorption of samples with non-sensitized sheep erythrocytes (Table 2).

DISCUSSION

Using blood smear examination patent parasitemia was confirmed in five camels (4.0%). Previous reports have recorded similar prevalence rates among imported Sudanese camels, 4.7% (Abdel-Latif 1957), 3.3% (Nessiem 1987), 4.5% (Elsaid 1992) and 5% (El-Sawalhy and Ebeid 1994).

Although the prevalence of animals with overt infection did not significantly increase over the past forty years, the actual number of camels introducing infection may have increased due to the increase in the total numbers of imported camels.

On applying CATT, 36 camels (28.80%) had detectable levels of anti-RoTat 1.2 antibodies. Using a crude *T. evansi* antigen in an ELISA system, El-Sawalhy and Ebeid (1994) reported a higher prevalence of 40.80% serological reactors among Sudanese camels slaughtered at Cairo and Kalyobia abattoirs. It is noteworthy that in a study conducted by Diall et al. (1994) on camels raised in northern Mali, CATT revealed a serological prevalence of 30.60%, whereas trypanosomes were found in only 5.85% of the corresponding animals.

The prevalence of serological reactors may reflect the frequency of animals that have been exposed to *T. evansi* infection and developed a specific humoral antibody response. By excluding the blood smear positive cases, our data reveals that 33 cases (26.40%) were parasitologically negative, though had detectable levels of VAT-specific antibodies.

It is uncertain to assume that this group of serological reactors, which constitute about one third of the tested population, may represent camels that are actually carrying *T. evansi* infection. Serological reactivity may not necessarily reflect an existing trypanosomal infection (Rae and Luckins 1984; Nantulya 1990).

By applying Suratex to CATT-positive, blood smear negative animals, 28 (82.40%) of 34 cases with evident antibodies had also circulating trypanosomal antigens. This group of camels therefore may be harboring *T. evansi* infection and may constitute an exotic source of infection.

On the other hand, by applying Suratex to CATT-negative animals, 14 cases were positive for circulating antigens, in spite of the absence of detectable levels of anti-RoTat 1.2 antibodies. Several studies have recorded inconsistencies between antibody and antigen detection tests. Franke et al. (1994) found a significant agreement between antibody-ELISA

and CATT results but did not find agreement between antibody tests and antigen detection ELISA for the diagnosis of *T. evansi* infection in horses, cattle and dogs.

A similar finding has been recorded by Pathak et al. (1997) among Indian camels suspected of trypanosomiasis, where CATT detected less reactors than antigen-ELISA. On the contrary, Olaho-Mukani et al. (1993) reported that antibody-ELISA detected more camels with trypanosoma-specific antibodies, while antigen-ELISA detected less numbers of camels with antigenaemia among a herd of Kenyan camels.

Evidently, this discrepancy clearly demonstrates the propensity of variation between the results of antibody and antigen detection assays. This may not be surprising in view of the fact that such results represent two distinct serological procedures. It is understandable to find cases with trypanosoma-specific antibodies devoid of parasite antigens. However, it may be peculiar to find cases with circulating antigens in the absence of the antigen-specific antibodies, unless the antigen detection system is prone to non-specific reactions.

It was tempting to test such samples devoid of antibodies to the VAT RoTat 1.2, for the presence of antibodies to invariant antigens common with *T. b. gambiense*. Test systems employing *T. b. gambiense* antigens have been effectively used for the detection of *T. evansi*-infected camels (Zweygarth et al. 1984; Elsaid 1992).

It was interesting to find that samples of 11 (78.60%) of 14 camels lacking anti-RoTat 1.2 activity had IHA titers to *T. b. gambiense* antigens (including a blood smear positive case), an indication that these cases were exposed to trypanosomal infection. Moreover, when the undiluted sera of these cases were CATT tested, they showed weak positive reactions. These results confirm the specificity of Suratex results. Also, it may indicate that antibodies to invariant trypanosomal antigens are more prevalent than antibodies to the early VAT RoTat 1.2. The absence of antibodies to the VAT RoTat 1.2 may not, therefore preclude the presence of antibodies to other variant antigens.

Meanwhile, 5 serological reactors failed to show evidence of circulating antigens. This result is in accordance with those of Rae and Luckins (1984) and Nantulya (1990) that antibody levels decline slowly and remain high for several months following the elimination of trypanosomes either through chemotherapy or due to spontaneous recovery.

All the blood smear positive cases were IHA positive, including a CATT-, Suratex- case. This case may present an early *T. evansi* infection. Similar cases have been previously reported among parasitologically-positive camels tested with antigen ELISA (Waitumbi and Nantulya 1993) and Suratex (Nantulya 1994). Animals with acute infection are expected to test negative for antigen as during trypanosome multiplication phase, there may be insufficient parasite destruction to produce detectable levels of circulating antigens (Waitumbi and Nantulya 1993).

From the previous discussion, our results demonstrate the worthiness of complementing antibody-based assays with tests for antigen detection for the diagnosis of *T. evansi* infection. Further investigations are needed for the evaluation of their sensitivity and specificity on camels at distinct phases of infection.

Detection of circulating trypanosomal antigens using Suratex was found to be more sensitive for the detection of camels that have been exposed to *T. evansi* infection. The test can be a useful epidemiological tool for the survey of trypanosomiasis among camels and other animal species. The test is simple, field-oriented and unlike ELISA, it does not require special sophisticated equipment.

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