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DIAGNOSIS OF CHRONIC TRYPANOSOMA EVANSI INFECTION AMONG SEROLOGICALLY POSITIVE CAMELS USING A LATEX AGGLUTINATION TEST FOR THE DETECTION OF CIRCULATING TRYPANOSOMAL ANTIGENS

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SUMMARY

A total of 10^4 imported Sudanese camels were tested for Trypanosoma evansi infection aiming to determine the prevalence of camels with chronic latent disease that may act as an exotic source of infection. The research employed direct blood smear examination, micro-haematocrit centrifugation technique (MHCT), card agglutination test (CATT/T. evansi) for antibody detection and latex agglutination test for detection of circulating trypanosomal antigens. Blood smear examination and MHCT have detected patent parasitemia in 5 camels (4.8%). Thirty (28.84%) serologically positive camels had also circulating trypanosomal antigens. Eight (7.69%) serological reactors were negative for antigens, while 12 (11.5%) camels with detectable levels of circulating antigens, tested negative for antibodies. A total of 42 (40.30%) camels were positive for circulating antigens, an indication of current infection. It was evident that the existence of circulating trypanosomal antigens dose not correlate with the presence of specific antibodies. Detection of circulating T.evansi antigens using latex agglutination test was found to be a more sensitive and reliable means of practical diagnosis of carrier camels suffering chronic latent infection. The epidemiological importance of results is discussed.

INTRODUCTION

Among the different parasitic diseases affecting camels, trypanosomiasis, caused by Trypanosoma evansi is the most serious and devastating infection. The disease is widespread among camel populations throughout the tropical and subtropical regions of the world, resulting in high morbidity and mortality (Olaho Mukani et al., 1966b). The disease, surra, is manifested by a variable clinical course that ranges from acute fulminating fatal infection to chronic insidious disease that persists for years (Luckins, 1992).

Acute infections terminating in death are self-limiting. Thus, animals with acute infection play a minor role in the spread of the disease. On the contrary, camels with chronic infection, the most common form of the disease, sustain a low level parasitemia which is microscopically undetactable. These camels serve as reservoirs of the parasite and maintain infection among the vector population that in turn maintain lateral transmission of infection to susceptible camels (Lossos, 1986).

Cryptic trypanosoma infection is ascribed to the fact that T.evansi is predominately a tissue-dwelling parasite. Parasites are sequestered in internal organs as liver , spleen, heart, kidney and bone marrow (Nantulya, 1994). Sequestered parasites are continuously shedding into the circulation several antigenic components as a result of their metabolism or due to parasite death and disintegration. The detection of such a state of antigenaemia indicates the presence of active, infection (Nantulya, 1990).

The preparation of monoclonal antibodies against non-variable antigens of African trypanosomes has enabled the development of antigen-capture enzyme linked immunosorbent assay (antigen-ELISA) for the detection of circulating trypanosomal antigens. The technique has been successfully used for the accurate diagnosis of infection in cattle (Nantulya and Lindqvist, 1989); dogs , goats , pigs, water buffalos (Nantulya et al., 1989) and camels (Waithanji et al., 1993; Pathak et al., 1997), as well as for the diagnosis of rhodesiense sleeping sickness in humans (Nantulya, 1989).

Recently, a simple, field-oriented latex agglutination test was developed for the detection of circulating invariant T.evansi antigens in camel sera (Nantulya, 1994; Olaho-Mukani et al., 1996a). Not only this antigen detection assay was more sensitive than parasitological methods for the diagnosis of non-patent infetions, but was also useful as a tool for evaluation of the treatment efficacy of camel trypanosomiasis (Olaho-Mukani et al., 1996b).

In the present research we surveyed a random sample of Sudanese camels imported to Egypt for latent chronic T.evansi infection using card agglutination test (CATT/T. evansi) for demonstration of trypanosomal antibodies as well as latex agglutination test (Suratex) for the detection of circulating trypanosomal antigens aiming to determine the prevalence of camels harboring T.evansi infection and may act as a source of lateral transmission of the disease to our indigenous camels.

MATERIALS AND METHODS

Samples: Blood samples were collected in heparinized vacuatiners (Becton & Dickinson, USA). Samples were randomly collected from a total of 10^4 Sudanese camels at Cairo (El-Basaten) and Giza (El-Warak) abattoirs. Animals ranged in age from 4 to 20 years old. All animals were apparently healthy and showed no signs suspeicious of trypanosomiasis.

Direct detection of trypanosomes: Trypanosoma evansi parasitaemia was detected

by examination of Giemsa-stained blood films and micro-haematocrit centrifugation technique (MHCT), as described by Woo, (1970).

Card agglutination test for trypanosomiasis (CATT/ T.evansi):

Plasma samples were tested for the presence of antibodies to T.evansi with the CATT kit developed at the Institute of tropical Medicine, Laboratory of Serology, Antwerp, Belgium. The kit was kindly supplied by Dr.Nestor V.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). Fifty ul antigen were mixed with 25 ul of camel plasma sample (diluted 1:2 with PBS) 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 Dr. Venand M. Nantulya through the manufacturer AccuPharma Inc., Montreal, Canada. The test reagent is a suspension of latex particles which have been sensitized with a monoclonal antibody against T.evansi invariant internal antigen according to the method of Buscher et al. (1991). The circulating trypanosomal antigen molecules in the serum, plasma or heparinized whole blood samples are captured by the specific monoclonal antibody on the latex partricles leading to agglutination of the particles. Fifty ul of test plasma was mixed with equal volume of test reagent on a test circle on a special test slide. The test slide was then titled 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.

RESULTS

Table 1 is illustrating the results of the different tests employed in the study. Using Giemsa-stained blood smears and MHCT, out of 104 camels, five camels (4.80%) were detected with acute T.evansi parasitemia. Anti-trypanosomal antibodies were evident in 38 camels (36.53%) using CATT, while 42 (40.38%) were positive for circulating T.evansi antigens using the latex agglutination antigen test, Suratex.

Combining the results of both CATT and Suratex, reveals that 30 (28.84%) camels were positive for antibodies and circulating antigens, while 8 (7.69%) camels showed only anti trypanosomal antibodies, but were negative for antigen. On the other hand , 12 (11.53%) camels had detectable levels of circulating antigens, but tested negative for antibodies. The remaining 54 (51.92%) camels did not evidence either T.evansi-specific antibodies or circulating antigens.

Table (1): Results of card agglutination test (CATT), antigen detection latex agglutination (Suratex) and direct parasite detection on 104 camels.

Test	No.Positive%	Positive	Total
Blood smear	5	4.80	104
Mhct	5	4.80	104
Catt/Suratex	30	28.84	104
Catt	8	7.69	104
Suratex	12	11.53	

DISCUSSION

Successful programs for the control of infectious diseases are founded on accurate epidemiological information. Unfortunately, the available information about cameline trypanosomiasis in Egypt is insufficient for the establishment of a comprehensive view about the epidemiology of the disease nationwide . The instability of the local enzootic situation is accentuated through the massive inflow of imported Sudanese camels that act as a continuous source of exotic T.evansi infection.

Furthermore, the traditional diagnosis of camel trypanosomiasis has been based on outdated methodology including blood smear examination, the mercuric chloride test (Bennett, 1929; Abdel-Latif, 1957), the formol gel test (Knowles, 1924; Abdel-Latif, 1957) and the thymol turbidity test (Abdel-Ghaffar, 1960). These methods are not sufficiently reliable for definite diagnosis of latent infections (Pegram and Scott, 1976; Boid et al., 1985). Laboratory animal inoculation is of diagnostic value in such cases, but this technique is of limited use as a field test for the survey of large numbers of animals.

In this research, using direct blood smear examination and MHCT, acute parasitemia was confirmed in five camels (4.80%). This result is in agreement with previous reports of El-Sawalhy and Ebeid (1994) who reported a prevalence of 5% and 4.17% respectively among Sudanese camels slaughtered at Cairo and Kalyobia abattoirs, while Abdel-Latif (1957) reported a prevalence of 4.7% among imported Sudanese camels.

Several serological tests have been applied for the diagnosis of camel trypanosomiasis, including passive haemagglutination test (PHA) (Jatkar and Singh, 1971), immunofluorescent technique (IFAT) (Luckins et al., 1979), enzyme linked imunosorbent assay, (ELISA)(Luckins, 1977; Luckins et al., 1979) and recently the card agglutination test (CATT/T. evansi) (Diall et al., 1994). Limited trials have been done for the application of serological methods for diagnosing camel trypanosomiasis in Egypt, including PHA (Elsaid, 1992) and ELISA (El-Sawalhy and Ebeid, 1994).

Recently developed (Diall et al., 1994), the card agglutination test (CATT) showed superior 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. This antigen was found ubiquitous among all T.evansi stocks and does not suffer from interference by other infections (Songa and Hamers, 1988; Pathak et al., 1997).

On applying CATT, 38 camels (36.53%) had detectable levels of trypanosome-specific antibodies. In agreement with our results El-Sawalhy and Ebeid (1994) reported that 40.8% of camels slaughtered at Cairo and Kalyobia abattoirs had specific antibodies as detected by ELISA. It is worthy to mention that in studies conducted by Diall et al. (1994) CATT revealed a global serological prevalence of 30.6%, whereas trypanosomes were found in only 5.85% of the corresponding animals.

The prevalence of serological reactors reflects the frequency of animals that have been actually exposed to T.evansi infection and developed a detectable specific humoral antibody response. By excluding the blood smear positive cases, our data revealed that 33 cases (31.73%) were parasitologically negative, though had detectable levels of trypanosome -specific antibodies. Similar results were reported by Luckins et al. (1979) in the Sudan, who found that 34.7% of blood smear negative camels had detectable antibodies when tested by ELISA.

It is plausible to assume that this group of aparasitaemic serological reactors, which constitutes one third of the tested population , may depict camels that are likely suffering asymptomatic chronic T.evansi infection. However, serological reactivity may not necessarily reflect an existing trypanosomal infection (Rae and Luckins, 1984; Nantulya, 1990).

By applying antigen detection test to serologically positive animals, 30 (28.84%) cases with evident antibodies had also circulating trypanosomal antigens, an indication of current infection. This group , including 5 camels with acute parasitemia, constitute the actual focus of exotic T.evansi infection.

Meanwhile, 8 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 (Luckins, 1992).

On the other hand, by applying Suratex to serologically negative animals, it was interesting to find that 12 cases had evident T.evansi antigenaemia, in spite of the absence of detectable levels of specific antibodies. The presence of circulating antigens in this group of camels clearly confirms the existence of active carrier infection.

A similar finding has been recently recorded by

Pathak et al., (1997). In a herd of Indian camels, antigen-ELISA was able to detect 93 cases with evident antigenaemia, while CATT detected only 78 cases with specific antibodies. On the contrary, Olaho-Mukani et al., (1993) reported that ELISA detected 90 camels with specific antibodies, while antigen ELISA detected only 63 with evident antigenaemia among a herd of Kenyan camels suspected of trypanosomiasis Evidently, this discrepancy clearly demonstrates the propensity of variation between the results of antibody-based immmunoassays, while confirming the accuracy of antigen-detection assays.

From the aforementioned discussion, our results clearly demonstrate the worthiness of circulating antigen detection for the diagnosis of T.evansi latent infection. It is evident that the existence of specific antibodies does not correlate with the presence of circulating trypanosomal antigens. In the tested population, the prevalence of blood smear negative, serological reactors did not represent the actual incidence of carrier camels suffering latent T.evansi antigenaemia.

The continuous importation of Sudanese camels to Egypt, that introduces more carrier animals, is expected to increase the parasite burden of the local tabanidae fly populations which in turn infect indigenous camels, thus accentuating the instability of the local enzootic conditions. Therefore, in order to control the disease, carrier camels must be detected and either subjected to chemotherapy or elimninated by slaughter.

Detection of circulating antigens of T.evansi using latex agglutination test has proven merit in discerning camels with active latent infection, regardless of blood smear examination or serological reactivity. As an epidemiological tool for use in the survey of trypanosomiasis, the test could prove to be a useful immunodiagnostic assay for detecting active carriers of infection in Camels and other species. The test is simple, field- oriented and unlike ELISA, it does not require special sophisticated equipment.

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