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DETECTION OF TRYPANOSOMA EVANSI IN DROMEDARY CAMELS IN LIBYA.

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SUMMARY

The infection of camels with trypanosomosis in Tripoli-Libya was conducted for the first time by this work. Antibodies against T.evansi was detected in 13 (6.2%) of 210 camels serum samples using CATT at dilution 1 : 4 and 10 (4.8%) of 210 camels serum samples by ELISA at dilution 1 : 25. T.evansi was not detected in 210 camels Giemsa stained blood films examination.

Keywords: Trypanosoma evansi; Blood films; CATT; ELISA; Camel

INTRODUCTION

Trypanosomosis caused by Trypanosoma evansi is widely distributed throughout the world in area with hot weather and transmitted mechanically by blood sucking insects. T.evansi infection in camels causes surra disease. The disease can be acute or chronic. The chronic form is the most common presented with progressive weakness, emaciation and decrease in the production capacity,while acute form are characterized by fever, emaciation, anaemia and lacrimation (Chaudhary and Igbal 2000) . Giemsa stained blood smears and serological tests as card agglutination test for trypanosomosis (CATT) and enzyme-linked immunosorbant assay (ELISA) are used for detection of T.evansi infection. The present work aims to know the incidence of Trypanosoma evansi affecting camels in Tripoli-Libya by Blood films examination, detection of antibodies against T.evansi by CATT and ELISA.

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MATERIALS AND METHODS

210 camel blood samples were collected from Tripoli abattoirs. The samples were taken from the jugular vein in clean tubes with anticoagulant for parasite examination and without anticoagulant for obtaining sera.

2.1. Parasitological procedures

Thin and thick blood smears were prepared from each blood sample (210 camel) and stained with Giemsa stain for microscopic examination. The serum samples were also prepared from the same animals for serodiagnosis. Pooled blood samples were inoculated into albino mice to isolate parasite (Each 15 samples were well mixed and immediately 0.3 ml was inoculated intraperitoneally in each of two albino Swiss mice (*Mus musculus*). Wet blood films were prepared daily from the tail vein of each inoculated mouse for a period ranged from 7 - 15 days post infection and examined microscopically at x 400).

2.2. Procedure of CATT

Antigen was reconstituted with 2.5ml of CATT buffer. Also positive and negative controls were reconstituted with 0.5 ml of CATT buffer. The tested sera were diluted 1 : 4 with CATT buffer. Fifty µl of the well homogenized CATT antigen was added per test circle on the card and mixed with 25µl of diluted serum by stirring rode. Then the card agitated for 5 minute on circular motion on electric rotator. The samples with blue granular agglutination were considered positive (Bajyana-Songa and Hamers,1988).

2.3. Determination of the end point titre of the investigated T.evansi positive camel sera.

Each positive camel serum was serially diluted from 1 : 8 to 1 : 16 using CATT buffer for detection of end point titre.

2.4. Antigen preparation for ELISA:

Trypanosoma evansi parasites were separated from experimentally infected albino mice as following:

At the peak of parasitaemia the mice were bled and the collected blood was centrifuged at 3000 r.p.m. for 15 minutes. The plasma was removed and several washing using phosphate buffer solution pH. 7.2 were accomplished. The erythrocytes were lysed by using distal water. The suspension was centrifuged at 3000 rpm for 15 minutes and washed several times with distal water. The supernatant was discarded and the sediment was kept in ice bag. The sediment was sonicated with ultrasonicator for 3 minutes with 30 second interval then centrifuge for 45 minutes at 4°C at 14000 r.p.m. The supernatant was used as antigen and stored frozen until used in ELISA. The concentration of protein in the antigen was determined by the method described by (Lowry et al., 1951) .

2.5. Procedure of ELISA:

Elisa plates were coated with 100µ / well of

T.evansi antigen at the concentration 40 µg protein / ml coating buffer after checker board titration which applied to determine the optimal antigen concentration and serum dilution.The plates were incubated for 2 hours at 37°C. Plates were washed three times with washing buffer then blocked with the blocking buffer (200µl / well) and incubated at room temperature for 2 hours. The plates were then washed five times with washing buffer. 100µl / well of each diluted serum samples (1 : 25) was added to selected well. 100µl / well of diluted Positive and negative control serum (1 : 25) from experimentally infected and parasitic free mice respectively was added to selected well. The plates were incubated for one hour at 37°C with shaking. The plates washed five times with washing buffer. 100µl / well of Protein A Peroxidase conjugate (Sigma) diluted in 1 : 500 in PBS was added and incubated for one hour at 37°C with shaking. The plates were washed five times with washing buffer. 50µl / well of substrate solution were added to all wells and the plates were incubated for 30 minutes at 37°C. 50µl / well of 1% SDS were added to stop the reaction. The optical densities (OD) were read at 405 nm with micro-ELISA reader system. The sera were considered positive with absorbance value more than the cut off (cut off = double fold of the mean negative sera).

RESULTS

T.evansi was not detected in Giemsa stained blood film examination. Antibodies against T.evansi was detected in 13 (6.2%) by CATT at dilution 1 : 4 and 10 (4.8%) by ELISA at dilution 1 : 25 (Table 1). The end point titres of the antibodies against T.evansi in 13 positive camels by CATT were 9 and 4 had dilutions 1 : 4 and 1 : 8 respectively. Inoculation of mice with pooled blood from the examined camels revealed the presence of *T.evansi* in the blood of mice at seven days post infection.

Table (1): Number and percent of blood films positive,ELISA positive,CATT positive and end point titres by CATT.

No. of animals examined	Blood films		ELISA		CATT		end point titres	
	+ve	%	+ve	%	+ve	%	1:4	1:8
210	0	0%	10	%4.8	13	6.2	9	4

DISCUSSION

In the present work, *T.evansi* was not detected in 210 camels by Giemsa stained blood film examination. Similar result (0%) were recorded by Abdel-Wahab 1988 in Egypt and Ibrahim et al., (1992) in Bahrain, and El-Magrabi (2007) in Lybia. This might be due to low parasitaemia. However in chronic stage of trypanosomosis, the number of trypanosomes in the peripheral circulation is generally low and difficult to be detected microscopically (Robson and Ashkar, 1972). Mice inoculation in present work, revealed the presence of this parasite in the blood films prepared from the inoculated mice seven days post infection, this positive results by mice inoculation while it was negative by blood smears examination could be due to the fact that camels were chronically infected. Also, Richardson (1948) said that inoculation of blood into rates was essential for detecting of Trypanosoma infection in such cases. The results of this work was nearly similar to that obtained by Fikry (1978) who mentioned that camels samples negative by blood smear examination provide to be infected with trypanosomes when the blood samples were inoculated into the albino rates and the trypanosomes were detected in the rats peripheral blood sixth day after intra-peritoneal inoculation.

Serological examination of 210 camels serum samples by CATT detected antibodies against T.evansi and revealed that 13 (6.2%) of camels were found infected. Also, by this serological test many authors in different countries found high incidence of *T.evansi* in camels. In Morroco, 14.1% (Atarhouch, Atarhouch et al., 2003) ; in Mali, 30.6% (Diall et al., 1994) ; in west Niger, 12.0% (Pacholek et al. 2000) ; in Egypt, 28.8% (Elsaid et al.,1998) and 43% (Abdel-Rady, 2006) in Kenya, 32.2% (Ngaira et al., 2003) and 45.9% (Njiru et al., 2004) ; in Chad, 30.52% Delafosse and Doutoum, 2004) . The examination of the same number of camels serum samples by ELISA detected antibodies against T.evansi in 10 (4.8%) out of the 210 examined camels. This indicates that the serological tests have more ability in determining chronic infection by detection of antibodies against this parasite beside its sensitivity and specificity. The previous studies demonstrated high percents in different countries, in Egypt, 55.96% (Awad 1996) and 30.55% (Salah EL-Din 1995) ; in Sudan, 31.3% (Elamin et al. (1998) ; in Kenya, 13% (Waithanji et al. 1993) and 49% (Olaho-Mukani et al. 1993) , in Saudi Arabia, 13.8% (Omer et al. 1998) ; in Morroco, 18.2% (Atarhouch et al. (2003) ; in Somalia, 57.65 (Caille 1987) and 56.4% Baumann and Zessin 1992) ; in India, 85.32% (Jain et al.2000).The possible explanation of the lower percent of animal infected in the urban camels may be due to better awareness to chemoprophylaxis, better feeding and management availability of medicine and related advice (Singh et al.2004). The serological methods, which detect the circulating antibodies against Trypanosoma are more effective

for diagnosis of the disease (Molyneux, 1975). The difference in the results by serological tests observed in this work, might be due to specificity of the CATT. Even the number of infected camels with *T.evansi* seems to be low, treatment of infected animals should be used for controlling infection from camel reservoir the parasite's camel .

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