Trypanosoma evansi infection in camels in Jordan

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Key words

Camels - *Trypanosoma evansi* - Blood - Epidemiology - Jordan.

Summary

A total of 257 camels in different districts of Jordan were used in the present study. These areas included Jordan Valley (140 camels), Al-Safawi (51 camels), Ramtha (36 camels) and Amman (30 camels). Blood samples were collected from camels into tubes containing anticoagulant. Blood smears were prepared from each blood sample and stained. Blood samples were also inoculated in immunosuppressed mice. Results indicated that *Trypanosoma evansi* was present in 132 camels. Infection rate of trypanosomiasis was 33% by direct smear stain technique and 51% by mouse inoculation. The highest rate of infection was reported in Jordan Valley from April to September. All 65 affected camels in the acute form of the disease showed signs of fever, anorexia, weakness, severe anemia and leukocytosis. Hematological examination revealed that all 65 camels were heavily infested with *T. evansi*. Sixty-seven camels showed low parasitemia with no evidence of clinical trypanosomiasis. In 125 camels, clinical signs were not observed, nor was *T. evansi* found in the blood. All camels treated with melarsomine in a dose of 0.25 mg/kg body weight recovered although 18% required 2-3 treatments.

■ INTRODUCTION

Trypanosoma evansi was the first trypanosome to be described and identified as a causative agent of mammalian trypanosomiasis. The earliest reports on the trypanosome were published by Evans (9) who associated it with an endemic disease in equines and camels known as *Surra* in the Dera Ismail Khan in the Punjab province (13). Since then, the disease has been reported quite frequently in North Africa, Sudan, Ethiopia and East Africa, through the Middle East into the Indian subcontinent and south East Asia, including all the islands of the Indonesian archipelago and the Philippines (1, 2, 4, 10, 13). It is also found in Central and South America and in countries belonging to the former Soviet Union (17). In Middle East countries, the main hosts are camels and, to a lesser extent, horses (4). *T. evansi* epidemics tend to involve different animal hosts in different parts of the world (2, 4, 10, 15, 22). In Indochina horses are mainly affected, followed by camels, bovine and buffaloes, whereas in Middle Asia the main hosts are camels and, to a lesser extent, horses. In certain parts of Africa such as Somalia, Kenya, Ethiopia, Sudan, Chad, Nigeria and West Africa, camels are affected most (10,13). In Argentina, horses are the main hosts, causing a disease known as *Mal de Caderas*, but the parasite has also been found in dogs (15). The sensitivity of parasitological diagnosis varies according to the technique used and a combination of the hematocrit method together with inoculation of blood into rodents is recommended as being the most effective procedure (18). Different serological methods have been used (8, 20, 22). However, these techniques are not always capable of distinguishing current from past infection due to the prolonged persistence of antibodies in the blood of treated animals (12, 16). The effect of the disease is most noticeable in pregnant and lactating camels and weaners. Anemia is commonly associated with trypanosome infections (18, 21, 22). Giemsa staining can detect the parasite 6-10 days after infection. The purpose of this study was to present the clinical and hematological picture, diagnosis and the effect of melarsomine treatment on camels affected by *Trypanosoma evansi* infection in Jordan.

■ MATERIALS AND METHODS

Sampling method

A total of 257 camels belonging to sixteen different farms in four different districts of Jordan were sampled between April 1996 and March 1997 (figure 1). Data were collected and pooled from camels from four different locations which represent camel

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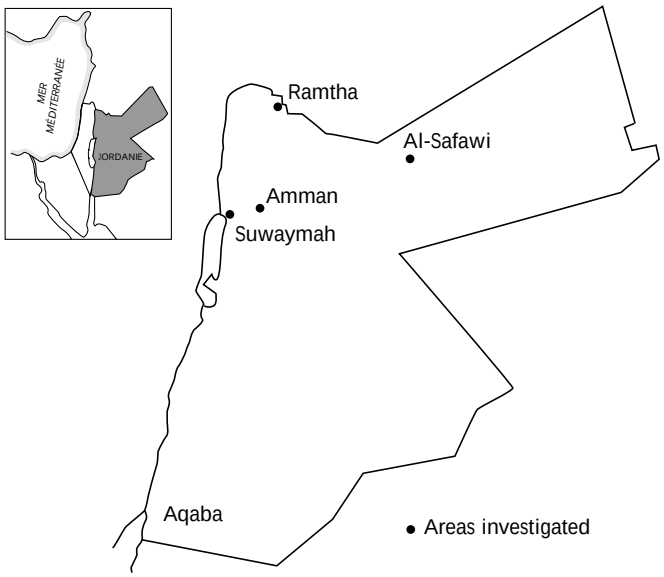

Figure 1: *The areas investigated in Jordan.*

population in Jordan (Jordan Valley, Suwaymah area, warm desert, n = 140; Al-Safawi, cool desert, n = 51; Ramtha, warm steppe, n = 36; and Amman, warm temperate, n = 30). Herd size ranged between 12 and 250 camels with 4 herds classified as small (12-50 camels), 5 herds as medium (51-99 camels) and 7 herds as large (≥ 100 camels). Twenty percent of camels in small herds, 15% in medium herds and 10% in large herds were sampled using systematic random sampling method (14). Based on the table random number, the first animal in each herd was chosen (14). The selected camels were examined clinically and recorded on a special form. Each animal was sampled only once during the study period.

Blood collection and analysis

Blood samples were collected from the jugular vein of all camels under the study. The blood was placed in two 10-ml partial-vacuum tubes. One tube contained ethylendiaminetetraacetic acid (EDTA) at a concentration of 1 mg/ml blood; the second tube contained no anticoagulant and sera were separated from the tube following blood coagulation, and stored at –20°C until analysis. Blood smears were prepared from each blood sample and stained with Giemsa stain. Total white blood cell counts (WBC), total red blood cell counts (RBC), hemoglobin concentration (Hb) and packed cell volume (PCV) were determined using the standard hematological methods described by Coles (7). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also calculated (7). Serum was analyzed for total protein (TP) concentration.

Animal grouping

For statistical analysis, the animals were divided into three different groups. Group 1 included the heavily positive animals by direct smear technique. Group 2 included the animals with few parasites. Group 3 included animals negative for the parasite detection. General clinical examinations were recorded. Sick animals were carefully examined for their body temperature, respiration rates, presence of edema and the color of the mucous membranes. Regional and seasonal occurrence of the disease was also recorded.

Mice inoculation test

Randomly bred white mice were used in this experiment. They were housed in plastic cages (24 cm x 18 cm) and were supplied with food and water *ad libitum*. Each mouse received 0.5 ml of dexamethasone intramuscularly plus 0.5 ml of camel blood intraperitoneally. The mice were closely observed for 7 days and then sacrificed. From each mouse, blood smears were stained with Giemsa stain and examined under light microscopy with x 400 magnification for the presence of the parasites. The blood smears prepared from the camel samples were similarly observed.

Treatment

The camels of group 1 were treated with melarsomine (Cymelarsan, Rhône Mérieux, France) at a dose of 0.25 mg/kg body weight by deep intramuscular injection of freshly prepared aqueous solution. Clinical and parasitological examination of treated camels were made on a weekly basis. Twelve camels, which did not recover quickly, received repeated doses of melarsomine until recovery occurred.

Statistical analysis

The analysis of variance, Student's t-test and χ^2 test were used. Differences were considered significant ($p < 0.05$). Data were expressed as mean ± SD.

■ RESULTS

Epidemiological findings

Trypanosoma evansi was diagnosed in 132 camels (51%). The infection rate was 33% by direct smear stain technique and 51% by mouse inoculation method. The results of diagnostic tests are given in table I. Out of 257 camels examined, mice inoculation revealed 47 infections not detected by the direct smear method. The mice inoculation test was found more sensitive than the blood smear stain technique ($p < 0.05$). All the samples positive by blood smears were also positive by mice inoculation. A significant ($p < 0.05$) difference in the rate of infection between Jordan Valley and the other areas under the study was found (table I). The highest rate of infection in camels in the Jordan Valley was recorded from April to October 1996 and then decreased significantly ($p < 0.001$) from November 1996 to March 1997 (table II).

Clinical and hematological findings

In group 1, the total of 65 camels showed signs of fever, anorexia, weakness and severe anemia. Two camels developed subcutaneous edema and one camel aborted. Blood smear examination revealed the presence of a high number of *T. evansi* (table I). In group 2 (67 camels) no evidence of clinical trypanosomiasis was noticed except that the animals had slight anorexia. When blood smears were stained and examined, they were found to be parasitized by a few *T. evansi*. In group 3 (125 camels), neither clinical signs of trypanosomiasis were seen nor could the parasite be found in their stained blood smears and mice inoculation test.

Table III shows the mean values of WBC, RBC, Hb, PCV and TP. Blood indexes include MCV, MCH, and MCHC are also shown. All camels in group 1 had a significant increase in mean value of WBC, MCV and MCH ($p < 0.05$), while the mean values of RBC,

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Table I

Prevalence of *Trypanosoma evansi* in camels by direct smear and mice inoculation tests

Area	Animal group	No. of camels examined	No. (%) of positive cases by stained smear method	No. (%) of positive cases by mouse inoculation method
Jordan Valley				
	G1	61	61	61
	G2	48	12	48
	G3	31	0	0
	Total	140	73 (52%)[a]	109 (77.8%)[a]
Al-Safawi				
	G1	0	0	0
	G2	5	2	5
	G3	46	0	0
	Total	51	2 (3.9%)[b]	5 (9.8%)[b]
Ramtha				
	G1	0	0	0
	G2	5	3	5
	G3	31	0	0
	Total	36	3 (8.3%)[b]	5 (13.8%)[b]
Amman				
	G1	4	4	4
	G2	9	3	9
	G3	17	0	0
	Total	30	7 (23.3%)[b]	13 (43.3%)[b]
Total				
	G1	65	65	65
	G2	67	20	67
	G3	125	0	0
	Total	257	85 (33%)[A]	132 (51.4%)[B]

a, b: differ within columns by χ^2 test; A, B: differ within rows by χ^2 test

Table II

Diagnosed cases of trypanosomiasis by the direct stain method and mice inoculation method according to months of the years 1996-1997

	Number Positives/Number Tested			
Months	**Jordan Valley**	**Al-Safawi***	**Amman***	**Ramtha***
April 1996 to October 1996	104/117[a]	5/31	12/19	4/20
November 1996 to March 1997	5/23[b]	0/20	1/11	1/16
Total	109/140	5/51	13/30	5/36

a, b: differ within column by χ^2 test
* The sample size in the these districts is too low for statistical analysis of seasonal variations

Hb, PCV, and MCHC were significantly decreased ($p < 0.05$). The total protein remained within the normal range ($p > 0.05$).

Results of the treatment

All animals in group 1 (65 camels) recovered completely following melarsomine treatment. 82% of the camels recovered following the first dose. However, 18% of camels with clinical signs of trypanosomiasis needed a second or even a third dose of melarsomine. Signs of edema started to disappear gradually and affected camels started to consume their feed normally.

■ DISCUSSION

The present investigation showed that *T. evansi* is a common parasite of camels in the Jordan Valley. The overall infection rate among camels in Jordan Valley alone was 77.8%. It is well known

Table III

WBC, RBC, Hb, PCV and total protein values in groups 1, 2 and 3

Variables	Group 1	Group 2	Group 3
WBC (µl-1)	12,300[a] ± 1160	9,330[b] ± 720	9,100[b] ± 1704
RBC (10^6 cell/µl-1)	4.50[a] ± 0.580	6.20[b] ± 0.667	7.00[b] ± 0.600
Hb (g dl-1)	9.2[a] ± 0.833	11.33[b] ± 1.030	12.30[b] ± 0.992
PCV (%)	27.66[a] ± 0.78	29.70[b] ± 1.560	31.20[b] ± 1.300
MCV (fl)	62[a] ± 7.07	48[b] ± 4.90	44[b] ± 3.25
MCH (pg)	20.54[a] ± 1.77	18.26[b] ± 0.79	17.58[b] ± 1.19
MCHC (g^{dl-1})	33.20[a] ± 2.23	38.1[b] ± 3.16	39.36[b] ± 2.15
TP (gdl-1)	7.84[a] ± 0.68	7.5[a] ± 0.25	7.10[a] ± 0.60

Data are express as mean ± SD
a, b: means in a row with the same superscript letter are not significantly different ($p > 0.05$)

that the Jordan Valley, especially Suwaymah lies at the lowest point on the earth (about 406 m below sea level). It is influenced by the Mediterranean bioclimate, and is situated in the warm desert-climate zone (3). In this area tabanid flies were found in abundance and we observed the flies feeding on the camels. In Sudan camel trypanosomiasis has been found to be seasonal; epidemics occurred especially after the long rains of April, with sporadic infections during the dry season (6, 13). Mechanical transmission from camel to camel by biting flies including *Tabanus*, *Stomoxys* and *Haematopota* has been reported (13). A feeding time of five seconds is enough to acquire infection in a tabanid fly and a similar time feeding on a new host is sufficient to transmit the infection; a single fly can infect at least two clean individuals following an interrupted infective meal (11).

In the present study, the disease exhibits two different clinical forms, the acute form and the chronic form. It has been reported that in the acute form, *T. evansi* is invariably present in the blood and the disease is almost always fatal (13). The disease, however, generally takes a chronic form resulting in heavy economic losses due to lower milk and meat yields (11). Abortion, premature births and reduced milk production greatly reduce reproductive potential in affected herds. Chronically infected animals may survive for three to four years and act as a source of the infection.

The use of immunosuppressed mice described in this paper showed a higher diagnostic sensitivity for *T. evansi* than the direct stain method. This information is wholly in agreement with previous work. Serological methods are used as complementary diagnostic tools in many clinical or epidemiological situations. However, these techniques are not always capable of distinguishing current from past infection due to the prolonged persistence of antibodies in the blood of treated animals (12, 16, 17).

There are few trypanocidal drugs available for treatment of camel trypanosomiasis. For many years suramine has been the drug of choice, a single dose of 10 mg/kg body weight usually being effective. In our study all camels in the clinically sick group responded satisfactorily to melarsomine treatment. It has been reported that melarsomine has a high efficacy against *T. evansi* infection in the dromedary camel (19).

The significant decrease in blood RBC, Hb, MCHC and PCV associated with increase in MCV and MCH values may be considered as microcytic hypochromic type anemia. Also, the present study revealed that the infected group of camels had higher values of total proteins although not significantly different. Boid *et al.* (5) found that total protein concentration was increased above normal values in camels experimentally and naturally infected with *T. evansi*. It has been stated that protein levels varied during *T. evansi* infection of Indian camels (19). In acute infection, albumin remained unchanged but it fell below normal during chronic disease, alpha-globulin fell during acute infection and increased during chronic disease, beta-globulin increased during acute infection but remained unchanged during chronic disease and gamma-globulin increased during both acute and chronic diseases.

■ CONCLUSION

Trypanosomiasis is a common camel disease in Jordan Valley but is of low prevalence in the other areas investigated (Al-Safawi, Ramtha, Amman). Variations in the percentage of infection were seen from month to month with a peak started from April to October. Affected camels can be treated safely with melarsomine, although 18% of the animals required 2-3 treatments.

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Résumé

Al-Rawashdeh O. F., Sharif L.A., Al-Qudah K., Al-Ani F.K. Infections à *Trypanosoma evansi* chez des chameaux en Jordanie

Dans cette étude, 257 chameaux provenant de différents districts de Jordanie ont été examinés : 140 dans la vallée du Jourdain, 51 dans le secteur d'Al-Safawi, 36 dans celui de Ramtha et 30 dans celui d'Amman. Les prélèvements sanguins ont été collectés dans des tubes contenant un anticoagulant. Les frottis ont été préparés à partir de chaque échantillon et colorés. Les échantillons ont également été inoculés à des souris immunodéprimées. Les résultats ont montré la présence de *Trypanosoma evansi* chez 132 chameaux. Le taux d'infection atteignait 33 % avec la technique de coloration des plaques et 51 % avec celle de l'inoculation aux souris. Le taux le plus important a été trouvé dans la vallée du Jourdain, d'avril à septembre. Parmi tous les chameaux, 65 étaient atteints de la forme aigüe de la maladie et présentaient de la fièvre, de l'anorexie, de l'asthénie, une anémie sévère et une leucocytose. Un examen hématologique a démontré qu'ils étaient gravement contaminés par *T. evansi.* D'autres chameaux (67) étaient plus légèrement infectés et n'avaient pas de manifestations cliniques de la trypanosomose. Enfin, 125 chameaux ne présentaient aucun signe de la maladie ni aucune trace de *T. evansi* dans le sang. Tous les chameaux malades, traités à la mélarsomine à la dose de 0,25 mg par kg ont été guéris, bien que 18 % d'entre eux aient eu besoin de deux à trois traitements successifs.

Mots-clés : Chameau - *Trypanosoma evansi* - Sang - Epidémiologie - Jordanie.

Resumen

Al-Rawashdeh O. F., Sharif L.A., Al-Qudah K., Al-Ani F.K. Infección de *Trypanosoma evansi* en camellos en Jordania

El presente estudio incluyó un total de 257 camellos en diferentes distritos de Jordania. Estas áreas incluyeron el Valle del Jordán (140 camellos), Al-Safawi (51 camellos), Ramtha (36 camellos) y Amman (30 camellos). Se recolectaron muestras sanguíneas de los camellos en tubos conteniendo anticoagulante. Se prepararon frotamientos sanguíneos a partir de cada muestra de sangre y se tiñeron con colorante. También se inocularon muestras de sangre en ratones inmunosuprimidos. Los resultados indican que el *Trypanosoma evansi* estuvo presente en 132 camellos. La tasa de infección de la trypanosomosis fue de 33% por medio de la técnica de tinción de frotamiento directo y 51% mediante la inoculación de ratones. La mayor tasa de infección se reportó en el Valle del Jordán, entre abril y septiembre. Los 65 camellos afectados por la forma aguda de la enfermedad mostraron signos de fiebre, anorexia, debilidad, anemia severa y leucocitosis. Los exámenes hematológicos revelaron que los 65 camellos estaban fuertemente infestados con *T. evansi.* Sesenta y siete camellos mostraron una parasitemia baja, sin evidencia clínica de trypanosomosis. No se observaron signos clínicos ni *T. evansi* en sangre de 125 otros camellos. Todos los camellos tratados con melarsormina a dosis de 0.25mg/kg de peso se recuperaron, aunque 18% requirieron dos o tres tratamientos.

Palabras clave: Camello - *Trypanosoma evansi* - Sangre - Epidemiología - Jordania.