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Seroprevalence, risk factors and haematology of *Trypanosoma evansi* in dromedary camels in northern Oman

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Abstract

Trypanosoma evansi is a blood parasite that is mechanically transmitted by hematophagous flies and causes a serious disease in most mammals in the tropics and subtropics. This study aimed to investigate the seroprevalence of *Trypanosoma evansi* and the associated risk factors in dromedary camels from the northern region of Oman. Serum samples were collected from 388 dromedary camels and examined using a card agglutination test (CATT/*T. evansi)*. Haematological parameters were also assessed using EDTA blood samples. Binary logistic regression was used to examine the association of *T. evansi* seroprevalence with potential risk factors, including location, age, sex, mixing with other animals, deworming, tick presence, prophylaxis usage, vector control and abortions in she camels. The overall seroprevalence of *T. evansi* detected by the CATT/*T. evansi* test was 26.3% (102/388, CI: 21.9-30.9%). There was a significant relationship between *T. evansi* seroprevalence and the location (x^2=11.99, p=0.017). Camels younger than four years of age were 3.79 times more likely to have circulating antibodies against *T. evansi* than camels between four to ten years of age. The mean MCV and eosinophil count in the seropositive camels were significantly lower than in non-infected ones. Further research is needed to investigate the active infection of *T. evansi* in other animal species and locations in the country.

Key words: Oman, Camels, *T. evansi*, CATT, Seroprevalence, Haematology

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Introduction

Trypanosomosis is a parasitic disease that affects camels in many parts of the world. There are several factors associated with the disease prevalence, such as animal location, sex, age, and fly control (Shafqaat, 2004). *Trypanosoma evansi* is the most common cause of Trypanosomosis in dromedary camels in tropical and subtropical countries. The disease has a significant impact on camels' health, production, and workability (Shafqaat, 2004).

Variations in haematological parameters are a consequence of diseases like Trypanosomosis (Pandey et al. 2015; Ohaeri and Eluwa 2011; Khan 2008). In the field, a card agglutination test (CATT/*T. evansi*) was found to be sufficiently sensitive to detect *Trypanosoma evansi* infection in animal herds (Jaiswal et al., 2015). In Oman, the major aims for raising camels are racing and milk and meat production. Camel racing is a flourishing industry in the Arabian Gulf region, including Oman where large amounts of money are invested in purchasing prime healthy animals. The industry also employs many personnel for camel rearing and health care. Therefore, to understand the disease impact on a country, it is important to conduct field surveys covering different regions and collect relevant data such as haematological parameters and risk factors.

In the current study, the prevalence of *Trypanosoma evansi* in camels was investigated in five northern Omani regions (Al Buraimi, Ad Dakhiliyah, Al Batinah, Ash Sharqiyah, and Al Dhahirah) using a serological card agglutination test (CATT/*T. evansi*). In addition, haematological parameters were studied to determine whether there are significant differences between seropositive and seronegative *T. evansi* camels. Additionally, we performed an analysis of possible risk factors in relation to Trypanosoma infection.

Materials and Methods

Study area and sampling

Five regions in the north of the Sultanate of Oman (Al Buraimi, Ad Dakhiliyah, Al Batinah, Ash Sharqiyah and Al Dhahirah) were targeted to investigate the seroprevalence of *T. evansi* in camels. The blood of a total of 388 camels was collected in this study, using 95% confidence levels and an expected prevalence of 50% (Thrusfield 2007). Samples were collected from camels of different ages and sex in the target areas.

Ten ml of blood samples were collected from each animal via jugular vein puncture into two vacutainers, one containing ethylene diamine tetra acetic acid (EDTA) and the second was a plain vacutainer. Blood samples were transported in a cool box at 4 ºC for laboratory testing. Blood in the EDTA vacutainers was used for a blood cell count, whereas serum recovered from the plain vacutainers after centrifugation was kept at -20 °C until used for the card agglutination test (CATT/ *T. evansi*).

Questionnaire

A questionnaire was conducted to identify risk factors associated with *Trypanosoma evansi* infection in camels. The camels' owners were interviewed, and consent was obtained during the collection of blood samples. The questionnaire included ten categories: location, age, sex, mixing camels with other animals, deworming, tick presence, Trypanosoma prophylaxis used, methods of fly control, she-camel abortion history, and the presence of other animal species such sheep, goats, cattle, dogs and horses.

Card Agglutination Test (CATT/ T. evansi)

CATT/*T. evansi* was used to detect anti-trypanosome antibodies in the serum of infected animals by direct agglutination. The process was performed according to the manufacturer's instruction (Institute of Tropical Medicine, Antwerp, Belgium). About 2.5 ml of CATT buffer was added to a vial of freeze-dried CATT antigen and the vial was immediately shaken for a few seconds to obtain a homogeneous suspension. Following that, 0.5 ml of CATT buffer was added to the positive and negative controls. One drop (45 µl) of the homogenized CATT antigen was added to each test area and 25 µl of the serum was added. The reaction was

mixed using a stirring rod and the test card was rotated for 5 minutes and then read.

Estimation of blood haematology parameters

Haematological parameters were determined using CEL-DYN 3700 (Germany). The following haematology values were determined: haemoglobin (Hgb), mean corpuscular volume (MCV), white blood cells (WBC) count, red blood cells (RBC) count, mean corpuscular haemoglobin concentration (MCHC), and neutrophil, lymphocyte, monocyte, basophil, and eosinophil counts.

Statistical Analysis

Statistical analysis was conducted using the SPSS PC package Version 20 (IBM, SPSS) at alpha= 0.05 significance level. Seroprevalence association with the potential risk factors was conducted at a 95% confidence level. Univariate analyses for individual risk factors to identify the association between the seroprevalence and the potential risk factors were conducted using a Pearson Chi-square Test or a Fisher Exact Test. A binary logistic regression model was used to examine the significance revealed by the univariate analyses. A student *t*-test was used to examine the differences in mean haematological parameters between the seroprevalence of *T. evansi* positive and negative cases.

Results

Seroprevalence of T. evansi and risk factors in Omani camels

Out of the 388 serum samples examined using CATT/ *T. evansi*, 102 (26.3%, CI: 21.9-30.9%) were positive for *T. evansi*. The frequency of infection was high in Al Dhahirah (50%, 15/30) followed by Ash Sharqiyah (28.1%, 54/192), Al Batinah (20.9%, 18/86), Ad Dakhiliyah (20.4%, 10/49) and Al Buraimi (16.17%, 5/31) (Table 1).

Potential risk factors were analysed using the chi-square test at $P<0.05$ and a 95% confidence interval. There were no significant relationships between the seroprevalence of *T. evansi* and sex, camels mixing with other animals, other animal species in the farm, deworming, *Trypanosoma* prophylaxis treatment, the method of fly control, or abortion history. There was, however, a significant relationship between disease prevalence and location (x^2=11.99, p=0.017).

Based on logistic regression, camels that were sampled from the Al Dhahirah region were 2.22, 1.96, 2.5, and 1.64 times more likely to have circulating antibodies against *T. evansi* than camels sampled from Al Batinah, Ad Dakhiliyah, Ash Sharqiyah, and Al Buraimi regions, respectively (Table 2). Moreover, there was a significant relationship between the seroprevalence of *T. evansi* and age (x^2=12.05, p=0.002). Camels less than four years of age were 3.79 times more likely to have circulating antibodies against *T. evansi* than camels between four to ten years of age (Table 3).

Table 1. Association of *T. evansi* seroprevalence and some potential risk factors using a card agglutination test (CATT/ *T. evansi*)

Risk factors	Number of camels examined	CATT/T. evansi		Chi- square	P-value
		Positive	Negative (%)		
Location					
Al Batinah	86	18	68 (79.1)	11.99	0.017
Ad Dakhiliyah	49	10	39 (79.6)		
Ash Sharqiyah	192	54	138(71.9)		
Al Buraimi	31	5 (16.1)	26 (83.9)		
Al Dhahirah	30	15	15 (50.0)		
Total	388	102	286 (73.7)		
Age					
<4 years	99	39	60 (60.6)	12.05	0.002
4-10 years	220	46	174 (79.1)		
>10 years	50	16	34 (68.0)		
Total	369	101	268 (72.6)		
Sex					
Male	47	13	34 (72.3)	0.02	0.96
Female	318	89	229 (72.0)		

[a] Significant differences (P<0.05)

There was no significant association between the seroprevalence of *T. evansi* and sex (x^2=0.02, p=0.96). The females were 28% (89/318) and the males were 27.7% (13/47) seropositive. The percentage of infected camels, when mixed with other animal species, was 25.7% (54/210) (Table 3). On the other hand, the percentages of infected camels in mixed farms, with ruminants, horses, and dogs, were 70%, 23.1%, and 25.6%, respectively. Out of 151 farms, 45 farms (29.8%) had camels only.

There was no significant relationship between the seroprevalence of *T. evansi* and camel deworming (x^2=0.11, p=0.74). However, there was a significant relationship between the seroprevalence of *T. evansi* and tick presence (x^2=4.23, p=0.040).

Table 2. Binary logistic regressions of sample location and age with the frequency of CATT/*T. evansi* seroprevalence.

Risk factors	β	SE - β	AOR	P-value
Location				
Al Dhahirah	0.000		1.000	
Al Batinah	-1.33	0.45	0.265 (0.109-0.641	0.003
Ad Dakhiliyah	-1.36	0.51	0.256 (0.095-0.695)	0.007
Ash Sharqiyah	-0.93	0.40	0.391 (0.179-0.855)	0.019
Al Buraimi	-1.65	0.61	0.192 (0.058-0.635)	0.007
Age				
<4 years	0.000		1.000	
4-10 years	-0.900	0.264	0.407 (0.242-0.683)	0.001
>10 years	-0.323	0.366	0.724 (0.353-1.484)	0.37

β: logistic coefficients; SE: standard error; AOR: adjusted odds ratio; CI: confidence interval;
[a]: Significant association (P<0.05).

The findings of this study showed no significant relationship between the seroprevalence of *T. evansi* and the use of *Trypanosoma* prophylaxis and the method of fly control (p=0.52, p=0.66, respectively). Most owners used the spraying method for fly control, rather than the pour-on method. There was no significant relationship between the seroprevalence of *T. evansi* and she-camel abortions (x^2=0.47, p=0.49).

Haematological parameters in infected and non-infected camels

There were no significant differences between *T. evansi* seropositive and seronegative camels in the haematological parameters, except for MCV and the eosinophil count. The mean MCV and the eosinophil count in *T. evansi* seropositive camels were significantly lower than in seronegative camels (Table 4).

Table 3. Association of the potential risk factors and the seroprevalence of *Trypanosoma evansi* using a card agglutination test (CATT)

Risk factors	No. examined	CATT/T. evansi		Chi-square	P-value
		Positive (%)	Negative (%)		
Camel mixed					
Yes	210	54 (25.7)	156 (74.3)	0.55	0.46
No	147	43 (29.3)	104 (70.7)		
Total	357	97 (27.2)	260 (72.8)		
Other animals on					
Ruminants	26	6 (23.1)	20 (76.9)	1.01	0.80
Ruminants	164	42 (25.6)	122 (74.4)		
Ruminants	20	14 (70.0)	6 (30.0)		
Camels only	151	45 (29.8)	106 (70.2)		
Total	361	99 (27.4)	262 (72.6)		
Camel dewormed					
Yes	173	46 (26.6)	127 (73.4)	0.11	0.74
No	192	54 (28.1)	138 (71.9)		
Total	365	100 (27.4)	265 (72.6)		
Tick presence					
Yes	329	84 (25.5)	245 (74.5)	4.23	0.040
No	39	16 (41.0)	23 (59.0)		
Total	368	100 (27.2)	268 (72.8)		
Trypanosoma					
Yes	283	75 (26.5)	208 (73.5)	1.34	0.52
No	73	20 (27.4)	53 (72.6)		
Not sure	12	5 (41.7)	7 (58.3)		
Methods of fly					
Spray	347	93 (26.8)	254 (73.2)	0.848	0.66
Pour-on	7	2 (28.6)	5 (71.4)		
No control	13	5 (38.5)	8 (61.5)		
She-camel					
Yes	78	19 (24.4)	59 (75.6)	0.47	0.49
No	240	68 (28.3)	172 (71.7)		
Total	318	87 (27.4)	231 (72.6)		

[a] Significant association ($P<0.05$)

Table 4. Mean Comparison of some haematological parameters between seropositive and seronegative *T. evansi*

Parameters	CATT/*T. evansi*		T-test	P-value
	Positive (Mean ±SD)	Negative (Mean ±SD)		
RBC	102 (8.44 ± 1.48)	285 (8.52 ± 1.28)	0.510	0.61
Hgb	102 (12.61 ± 2.03)	285 (12.93 ± 2.00)	1.391	0.17
HCT	102 (36.01 ± 4.83)	285 (36.83 ± 4.85)	1.47	0.14
MCV	102 (42.42 ± 2.77)	285 (43.41 ± 2.90)	3.054	0.003
MCHC	102 (35.16 ± 1.22)	285 (35.04 ± 1.52)	0.672	0.502
WBC	99 (11.24 ± 7.96)	283 (10.98 ± 5.12)	0.373	0.71
Neutrophils	99 (6.74 ± 4.43)	283 (6.89 ± 3.95)	0.330	0.742
Lymphocytes	99 (3.15±5.84)	283 (2.77 ± 2.52)	0.878	0.38
Monocytes	99 (0.42 ± 0.33)	283 (0.42 ± 0.30)	0.319	0.75
Basophils	99 (0.21 ± 0.25)	283 (0.22 ± 0.18)	0.233	0.82
Eosinophils	99 (0.49 ± 0.34)	283 (0.64 ± 0.43)	3.39	0.001

Discussion

The seroprevalence of *T. evansi* in camels in this study (26.3%) was lower than the 38% reported in North Ash-Sharqiya Governorate, Oman (AlKharusi et al., 2021). However, it was higher than the prevalence reported in Morocco, Mauritania, Egypt, Somalia and Ethiopia (14.1%. 14.2%, 15.5%, 15.9% and 13.7%, respectively) (Atarhouch et al., 2003; Dia et al., 2011; Abo-Aziza et al., 2017; Mohamoud, 2017 and Birhanu et al., 2015), and lower than those reported in other countries: Sudan, Egypt, Saudi Arabia, Algeria, Chad, and Somalia (52.2%, 43.5%, 39.4%, 32.4%, 30.5%, and 26.4% respectively), reported by Babeker and Hassab Elrasoul (2014), Abdel-Rady (2008), Al Afaleq et al. (2015), Boushaki (2019), Delafoss and Doutoum (2004) and Salah et al. (2019).

The significant relationship between the seroprevalence of *T. evansi* and location in Oman can be attributed to climate variability in correlation with topography. Oman's geography is made up of variable terrains, ranging from deserts to coastal plains and mountainous areas. This would affect disease vector availability and activity. Also, in Algeria they found that the seroprevalence was variable between areas in the same country (Benaissa et al., 2020). The variation in the seroprevalence of disease between different locations could be attributed to differences in camel breed, and the age of animals, as well as to differences in camel management systems. Some research reported that seroprevalence was increased in older animals (Atarhouch et al., 2003; Bogale et al.,

2012). The management system has an effect on *T. evansi* infection and the seroprevalence of Trypanosoma was higher in semi-intensive and intensive management systems than extensive systems (Benaissa et al., 2020).

The seroprevalence levels of the disease were higher in the youngest group of camels (<4yr), followed by the adult group (>10yr), at 39.4% and 32%, respectively. This finding is similar to previous studies in that younger camels were more susceptible to the disease than adult camels (Lemecha, et al. 2008). The participation of younger camels in racing activities in different locations in the country may increase their chance of being infected due to close contact with other animals. The interaction of *T. evansi* among different hosts varied from an acute condition in horses and camels to a mild condition in sheep and goats. Therefore, the presence of reservoir and susceptible hosts in the same environment could complicate the epidemiology of the disease (Desquesnes et al., 2013).

In this study, the seroprevalence of *Trypanosoma* was not different between females and males (28% and 27.7%, respectively). This pattern is not in agreement with previous studies carried out in Sudan, where females had a higher rate of infection (Babeker and Hassab Elrasoul, 2014), and Somalia (Mohamoud, 2017). It also disagrees with a study carried out in Ethiopia that showed a higher infection in males (20.25%) compared to female camels (17.72%) (Bogale et al., 2012).

The percentage of infected camels on farms where they were mixed with ruminants, horses, and dogs were 70%, 23.1%, and 25.6%, respectively. Mixing horses with camels was a risk factor, so when horses are present, camels are 2.49 times more likely to be positive for the disease (Benfodil et al., 2019). This should be taken into consideration when raising camels, especially for racing activities.

The results of the current study also showed a significant relationship between the seroprevalence of *T. evansi* and the presence of ticks. Tick presence is most probably due to poor management systems, which may lead to an increase in parasite infections in camels as a consequence of host blood losses and other pathogen transmission. The majority of camel owners use spraying methods for fly control rather than the pour-on method, which is less effective than other methods (Drummond, 1983).

Haematology parameters of animals are an important indicator of health (Ohaeri and Eluwa, 2011). Various studies worldwide have been conducted to study the link between camel age, gender, diseases, lactation and reproduction, and haematological and biochemical profiles (Islam *et al*., 2019; Maher *et al*., 2017; Derakhshanfar *et al*., 2010; Kamal, 2008; Al-Busada and Osman 2000; Ghafoor *et al*., 2018; Ayoub *et al*., 2003; Metawie *et al*., 2000; Abd El-Slam and Arafa 2018; Al-Busada 2007; Al-Sultan 2008; Al-Shamisi *et al*., 2013; Jalali *et al*., 2018).

All haematology parameters decreased ($P<0.05$) in camels that have parasitic infections, with an increase of eosinophils and monocytes (Momenah, 2014). In this study, MCV was significantly lower in infected camels. Although these values were still within the normal range for camels, they are in agreement with previous studies such as the one done by Kamal (2008). Haematology parameters were significantly different ($P<0.05$) between infected and non-infected camels (Ghafoor et al., 2018). In animals that have a parasitic disease, this mostly leads to anaemia, since trypanosomes affect red blood cells and cause haemolysis (Benoit et al., 1997). When MCV is decreased, that means that erythrophagocytosis is occurring in camels infected with Trypanosoma (Ohaeri and Eluwa, 2011). There was no significant difference in the

mean values of RBC, WBC and MCHC between camels infected with Trypanosoma and non-infected camels. This finding agrees with the results reported by Siham et al. (2022). The Hb value is within range in this study and this disagreed with other research (Siham et al., 2022). However, the major symptom of trypanosomiases in animals is anaemia (Enwezor and Sachey, 2005). In a study done in Iran with female camels, camels with a chronic infection had low monocytosis and haemoglobin and PCV was an indication of anaemia in camels infected with Trypanosomiases (Derakhshanfar et al., 2010). Parasitic disease can cause eosinophilia, as it was noticed in acute *T. evansi* infections in dromedary camels (Njiru *et al*., 2000). On the other hand, eosinopoenia was observed in calves infected with *T. evansi*. The reduction of the leuckocyte count during trypanosomes infection could be due to the enhancement of erythropoiesis and the reduction of myelopoiesis to compensate the massive destruction of erythrocytes by the parasite (Weiss and Wardrop, 2010). However, our results should be interpreted with caution since active detection of *T. evansi* was not attained.

Conclusion

This study provides data on the seroprevalence of *Trypanosoma evansi* in dromedary camels in five northern regions of Oman. It indicates that there are several risk factors affecting the prevalence of Trypanosomoses in Omani camels. It also demonstrates that parasitic diseases can influence some blood parameters. However, further studies are required to cover the southern region of Oman. To reduce cases of Trypanosomiases in Oman, we recommend treating animals and controlling the vector.

Conflict of interest

The authors declare that they have no conflict of interest.

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