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RESEARCH ARTICLE

Seroprevalence of *Trypanosoma evansi* infections among dromedary camels (*Camelus dromedaries*) in North Ash-Sharqiya Governorate, Sultanate of Oman

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الانتشار المصلي لعدوى المِثْقَبِيَّة الإيفانسيَّة بين الإبل وحيدة السنام في محافظة شمال الشرقية، سلطنة عمان

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Abstract. *Trypanosoma evansi* is a well-known hemoprotozoa that infects diverse domestic and wild animals worldwide and causes a devastating disease called surra. This research aimed to investigate seroprevalence of *Trypanosoma evansi* and its associated risk factors in dromedary camels in North Ash-Sharqiya Governorate, Sultanate of Oman. A total of 4364 serum samples was collected from three Willayat in North Ash-Sharqiya Governorate of Sultanate of Oman; Ibra (926, 21.2%), Qabil (1119, 25.6%) and Bidiyah (2319, 53.1%). Samples were examined for the presence of antibodies against T. evansi using card agglutination test for *T. evansi* (CATT/*T. evansi*). Binary logistic regression was used to study the association of *T. evansi* seroprevalence and risk factors such as location, gender, purpose and age of camels. The overall seroprevalence of *T. evansi* detected by CATT/*T. evansi* test was 38% (1659/4364, CI: 36.6-39.5%). There was a significant difference (p=0.001) between location and *T. evansi* seroprevalence, whereas the highest seroprevalence was found in Ibra (49.9%, CI: 46.7-53.1%) followed by Bidiyah (35%, CI: 33-36.9%) and Qabil (34.5%, CI: 31.8-37%). Camels from Ibra were almost two times more likely to have circulating antibodies of *T. evansi* than camels from Bidiyah (OR=1.89, CI: 1.591-2.168). The results of this study showed a significant difference between seroprevalence and sex (p=0.023), whereas the age of camels did not show any significant difference (p>0.05). To our knowledge, this is the initial research that indicated that *T. evansi* antibodies were circulating among camels in Oman, and further research needs to be tackled to study the molecular characterization of *T. evansi* and its prevalence in other animal species. Furthermore, cross-sectional studies of *T. evansi* from different regions in Oman warrant further investigation.

Keywords: Camels, *T. evansi*, CATT, Seroprevalence, Oman

الملخص: المِثْقَبِيَّة الإيفانسيَّة هو نوع معروف من الأوليات وحيدة الخلية يصيب الحيوانات الأليفة والبرية المتنوعة في جميع أنحاء العالم ويسبب مرضًا يسمى الزنبور. يهدف هذا البحث إلى دراسة الانتشار المصلي للمثقبيات الإيفانسيَّة وعوامل الخطر المرتبطة بها في الإبل وحيدة السنام بمحافظة شمال الشرقية، سلطنة عمان. تم جمع ٤٣٦٤ عينة من مصل الدم من ثلاث ولايات في محافظة شمال الشرقية سلطنة عمان. إبراء (926، 21.2٪)، القابل (1119، 25.6٪)، بدية (2319، 53.1٪) تم فحص وجود الأجسام المضادة ضد المِثْقَبِيَّة الإيفانسيَّة باستخدام اختبار تراص البطاقة للمِثْقَبِيَّة الإيفانسيَّة (CATT). تم استخدام الانحدار اللوجستي الثنائي لدراسة الارتباط بين الانتشار المصلي للمِثْقَبِيَّة الإيفانسيَّة وعوامل الخطر مثل الموقع والجنس والغرض والعمر للإبل. كان الانتشار المصلي الكلي للمِثْقَبِيَّة الإيفانسيَّة المكتشف بواسطة اختبار تراص البطاقة للمِثْقَبِيَّة الإيفانسيَّة (38% (1659/4364, CI: 36.6–39.5%) (CATT). كان هناك فرق إحصائي (p = 0.001) بين الموقع والانتشار المصلي للمِثْقَبِيَّة الإيفانسيَّة، حيث تم العثور على أعلى معدل انتشار مصلي في إبراء (49.9٪، CI: 46.7–53.1٪) تليها بدية (35٪، CI: 33–36.9٪) والقابل (34.5٪، CI: 31.8–37٪). كانت الإبل من إبراء أكثر عرضة بمرتين لوجود الأجسام المضادة المنتشرة من المِثْقَبِيَّة الإيفانسيَّة مقارنة بجمال بدية (OR = 1.89، CI: 1.591–2.168). أظهرت النتائج في هذه الدراسة فرقاً احصائيا بين الانتشار المصلي والجنس (p = 0.023)، بينما لم يظهر عمر الإبل أي فرق إحصائي (p> 0.05). على حد علمنا، هذا هو البحث الأولي الذي أشار إلى أن الأجسام المضادة للمِثْقَبِيَّة الإيفانسيَّة توجد بين الإبل في عمان، وهناك الحاجة إلى المزيد من البحث لدراسة خصائص المِثْقَبِيَّة الإيفانسيَّة وانتشارها في أنواع الحيوانات الأخرى، علاوة على ذلك، يتطلب مزيدا من البحث والدراسات للمِثْقَبِيَّة الإيفانسيَّة في مناطق مختلفة من عمان.

الكلمات المفتاحية: الإبل، المِثْقَبِيَّة الإيفانسيَّة، اختبار تراص البطاقة، الانتشار المصلي، سلطنة عمان

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Introduction

Parasite remains a major constraint for optimum animal production throughout the world. *Trypanosoma evansi*, which belongs to the genus *Trypanosoma*, subgenus Trypanozoon is a blood parasite that infects domestic livestock and wild animals; and caused disease called surra (Bargash et al., 2014). The parasite has a wide geographical distribution in many

tropical and subtropical countries due to the adaptation of mechanical transmission by different types of hematophagous flies, such as *Tabanus*, *Stomoxys*, *Lyperosia* and *Haematobia* species (Desquenes et al., 2013). The disease was reported in camels from different regions of the Kingdom of Saudi Arabia (AlTaqi et al., 2011; Amoudi et al., 2011; El-Wathiq et al., 2016), in Kuwait (Al-Taqi, 1989) and United Arab Emirates (Chaudhary and Iqbal, 2000). Camels along with horses and dogs are considered the principal hosts to the parasite and this may show symptoms, such as fever, anorexia, and edema (Abdel-Rady, 2008). The disease has a significant impact on animal's health, production and efficiency and can result in death of animals if no timely treatment is offered. Some biochemical changes have been documented in camels related to *Trypanosoma* infection in dromedary camels. (Sazmand et al., 2011). The disease causes infertility in the dromedary camels by changing plasma steroids concentration and semen characteristics (Al-Qarawi et al., 2004).

The disease can be diagnosed by parasitological methods, such as microhematocrit centrifugation technique and microscopic examination of thin blood smears (Babeker and Hassab, 2014; Bennoune et al., 2013; Bogale et al., 2012; Hagos, 2009) and serological methods such as ELISA and card agglutination test for *T. evansi* (CATT/T. *evansi*) (Babeker et al., 2014; Zayed et al., 2010; Hagos, 2009). The introduction of molecular diagnosis enhanced the accuracy of *T. evansi* detection due to the dynamics of parasitaemia (Baraghash et al., 2014; Tehseen et al., 2015). The Card agglutination test was more common to be used for screening a large numbers of animals under field conditions (Uilenberg, 1998). On the other hand, the CATT test can render false positive results due to the circulation of antibodies.

The estimated number of camels in the Sultanate of Oman is 242833 heads (Agriculture Censuses, 2013). The highest density of the camel population in Oman is located in the Dhofar Governorates, as most people use camels for milk and meat production. The second highest density of camels is in Ash-Sharqiya North Governorate with a total of 21577 camels and mostly are raced camels. Since the first detection of the parasite in 1984 (Srivastava,1984) and the continuing reports of the disease from the Ministry of Agriculture, Fisheries Wealth and Water Resources, *Trypanosoma evansi* was wandering in the country and negligible cohort study has been done to identify the frequency and risk factors of the disease in livestock. Due to lack of work on seroprevalence of *Trypanosoma* among camel population in Oman, the present study was planned to determine the seroprevalence and risk factors of *T. evansi* in Omani camels in North Ash-Sharqiya Governorate using serological techniques.

Materials and Methods

Study Area and Sampling Collection

This study was conducted in three Willayat in North Ash-Sharqiyah (Ibra, Qabil and Bidiyah). A total of 4364 camel blood samples were collected between July 2014 and November 2015 from Ibra (283 farms), Qabil (260 farms) and Bidiyah (394 farms). Ten millilitres of blood was collected from jugular vein of each camel in a plain tube and transported in a cool box to the lab for screening *T. evansi* using card agglutinin test (CATT/ *T. evansi*). Information on location, sex, age and camel purpose were collected during blood sample collection. In this study, camels were divided into three groups according to age (<5, 5-10 and >10 years old).

Diagnosis of Serum by Serological Examination

Card agglutination test for *T. evansi* kit (CATT/ *T. evansi*) was used to detected anti-trypanosome antibodies in serum of infected animal by direct agglutination method according to the manufacturer's instructions (Institute of Tropical Medicine, Antwerp, Belgium). The antigen contains fixed, freeze-dried and stained bloodstream from trypanosomes type RoTat 1.2, a predominant variable antigen type of *Trypanosome evansi* expressed during the early course of infection (Bajyana-Songa and Hamers, 1988). About 2.5 ml of CATT/*T. evansi* buffer was added to a vial of freeze dried CATT/ *T. evansi* antigen and shake the vial immediately for a few seconds to obtain homogeneous suspension. Then 0.5 ml of CATT/*T. evansi* buffer was added to a positive and negative control. On the test area of the card, one drop (45 µl) of the homogenized CATT/*T. evansi* antigen was added in each test area and 25 µl of the serum diluted at 1:4 with PBS pH 7.2 according to the manufacturer's instructions was then added. The reaction was mixed using a stirring rod and the test card was rotated for 5 minutes for subsequent reading of the results.

Statistical Analysis

The statistical analysis was conducted using SPSS version 20 (IBM, SPSS) at a = 0.05 significance level. Seroprevalence and associated risk factors were conducted at 95% confidence level. Univariate analysis for individual risk factors to identify association between the seroprevalence and the potential risk factors were tested using Pearson Chi-square or Fisher Exact test. A binary logistic regression was used to examine the significance that revealed by the univariate analysis and to determine odds ratio.

Results and Discussion

Out of 4364 blood samples examined using CATT/*T. evansi,* 1659 (38%, CI: 36.6-39.5%) were found to be seropositive for *T. evansi* (Table 1). The frequency of infection was high in Ibra 49.9% (462/926, CI: 46.7-53.1%)

followed by Bidiyah 35% (811/2319, CI: 33-36.9%) and Qabil 34.5% (386/1119, CI: 31.8-37%). There was significant difference between disease seroprevalence and location (x^2 =70.43, p=0.001) (Table 2). Based on logistic regression, camels that sampled from Ibra region were 1.89 times more likely to have circulating antibodies against *T. evansi* than camels sampled from Bidiyah region (OR=1.89, 95%CI: 1.591-2.168).

The results showed a significant difference between seroprevalence and sex (p=0.023). The female had highest infection rate 38.7% (1435/3706) than male 34% (224/658). The female seropositive camels were about 1.2 times more likely than male camels. Moreover, there was no significant association between diseases seroprevalence and the purpose (p=0.053). Despite the non-significance difference, camels used for production 38.6% (1488/3858) had a higher rate of being seropositive than racing camels 34.1% (168/493). On the other hand, there was no statistical significant difference between diseases seroprevalence and the age (p>0.05) (Table 1).

To our knowledge, this is the initial study that provides information about the seroprevalence of *T. evansi* infections in three regions in Oman using serological test (CATT/*T. evansi*). The results showed significant difference between diseases seroprevalence and camel sex and sample location. On the other hand, there is no significant difference between the diseases seroprevalence with camel age and purpose of camels. The overall seroprevalence of *T. evansi* infections was found to be 38%. Our finding is relatively lower than the results reported in Saudi Arabia with a seroprevalence of 39.4% by card agglutination test with considerable differences between eastern and central regions (Al-Afaleq et al., 2015). The seroprevalence of this study was higher than other studies done in Ethiopia by Bogale et al. (2012), Birhanu et al. (2015), Hagos et al. (2009), and in Somalia by Mohamoud (2017), who reported a prevalence of *T. evansi* in camels 18.22%, 13.76%, 24.88%, 15.9% respectively. However, our results of *T. evansi* seroprevalence was relatively lower than that reported in Sudan and Egypt as estimated to be 52.2% and 43.5% respectively (Babeker and Hassab Elrasoul, 2014; Abdel-Rady 2008). Since CATT/*T. evansi* cannot differentiate between current and past infections, the higher seroprevalence of *T. evansi* revealed by this research may be attributed either to genetic variation of camels breeds existed in same farms or weak farm management programs adopted by camel's owner as the causative agent was reported in the country without comprehensive study to reveal its distribution. *Trypanosoma evansi* seroprevalence was higher in Ibra (49.9%) than Qabil and Bidiyah, this might be due to environmental factors as Ibra is a mountainous area and had wetland that may enhance the propagation of the parasite vectors, whereas Qabil and Bidiyah are sand areas with significantly less vector density.

In the present study, a significant difference was reported in camel sex, the highest seroprevalence of *Trypanosoma evansi* was observed in female 38.7% followed by male 34%. This result in accordance with that found by Babeker and Hassab Elrasoul (2014) and Mohamoud (2017). However, our results are not in line with study done by Bogale et al. (2012) which showed that a higher infection rates in males (20.25%) compared to female (17.72%) with approximately similar prevalence rates among both sexes. Study conducted by Bhutto et

Table 1. : Association of location, age and sex with seroprevalence of *Trypanosoma evansi* using card agglutination test (CATT/ *T. evansi*).

Risk factors	No.	CATT/ *T. evansi*		Chi-square	P-value
		Positive (%)	Negative (%)		
Location					
Ibra	926	462 (49.9)	464 (50.1)	70.43	0.001*
Qabil	1119	386 (34.5)	733 (65.5)		
Bidiyah	2319	811 (35)	1508 (65)		
Age (years)					
<5	1555	608 (39.1)	947 (60.9)	1.49	0.47
5-10	2152	811 (37.7)	1341 (62.3)		
>10	649	237 (36.5)	412 (63.5)		
Sex					
Male	658	224 (34)	434 (66)	5.19	0.023*
Female	3706	1435 (38.7)	2271 (61.3)		
Purpose					
Race	493	168 (34.1)	325 (65.9)	3.74	0.053
Production	3858	1488 (38.6)	2370 (61.4)		

*Significant association (p<0.05)

Table 2. Binary logistic regression of *T. evansi* infections and associated camel's location.

Location	β	SE-β	AOR (95% CI)	*P*-value
Bidiyah	0.000	-	1.000	-
Ibra	0.619	0.079	1. 1.89 (1.591-2.168)	0.001*
Qabil	-0.019	0.077	00.982 (0.845-1.141)	0.810

β: logistic coefficients; SE: standard error; AOR: adjusted odds ratio; CI: confidence interval; *Significant association ($p<0.05$)

al. (2010) indicated that females were more susceptible to the infection with *Trypanosoma* species as host immunity decreased during pregnancy and lactation period. Despite the non-significant result of the age, we observed an increase in *T. evansi seroprevalence* in younger camels with age less than five years (39.1%) as compared to camels with age between five and ten years (37.7%). This study disagreed with previous studies, which found that adult camels were more susceptible to have the diseases than younger camels (Mohamoud, 2017; Atarhouch et al., 2003). Detection of antibodies against *T. evansi* in the host does not necessarily indicate a current infection since antibodies can persist for 2.3–22.6 months after trypanocidal treatment (Monzón et al., 2003). Therefore, younger camels of age less than five years have a higher chance of encountered antibodies against *T. evansi* in their blood compare to the elder one. In addition, younger camels are used to transport to different places in country for racing purposes and exposing these animals to infections. Camels used in production showed higher percentage of *T. evansi* seroprevalence than camels using for race 34.1% and 38.6%, respectively. This might be because camel's keepers care more about racing camels and keep them in open area for grazing, provide them with sufficient complementary diet and treat them against parasites whereas, camels that used for production get less care from camel owner. This could also be due to disparity in management, veterinary care, and parasite control offered to race camels compared to production animals.

Conclusion

To our knowledge, this research was the initial report on *T. evansi* infections in Oman by using card agglutination test (CATT/*T. evansi*). The results showed that the *T. evansi* was circulating in camels within the investigated areas and certain potential risk factors may be associated with the spreading of the disease in the country. The disease might cause great economic losses due to impairment of camel's health, which leads to decrease in production and performance. This study was done in North Ash-Sharqiya Governorate of Sultanate of Oman, so further studies are highly needed to investigate the prevalence of the diseases in different parts of Oman in different livestock using different diagnostic techniques.

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Conflict of interest

The authors declare that they have no conflict of interest.

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