Short Communication

Molecular detection of *Trypanosoma evansi* in camels (*Camelus dromedarius*) in southwestern Saudi Arabia

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Abstract

This cross-sectional study aimed to determine the prevalence of *Trypanosoma evansi* infection and its associated risk factors in camels in the Jazan region, southwestern Saudi Arabia. A total of 679 blood samples were collected from three local breeds of camel – Awadia, Awarkia and Sawahelle – in 11 randomly selected governorates of Jazan region. Blood samples were screened by Giemsa-stained blood film (GSBF) and polymerase chain reaction (PCR) techniques. The overall prevalence of *T. evansi* was 30.9% (210/679, CI: 27.6–34.5%) by PCR, whereas a prevalence of 22.2% (151/679, CI: 19.3–25.5%) was revealed by GSBF. The highest prevalence was identified in Ad-Darb (55.6%) followed by Farasan (44.4%) and Alarda (42.1%) while the lowest prevalence was found in Samtah (10.6%). A significant association between the infection and the location of the camels was found ($P = 0.023$); a high infection rate was recorded in the northern governorates which declined gradually in the central and southern governorates, regardless of the sex, breed or age of the camels. Moreover, a significant interaction of breed and age on disease prevalence was found ($P = 0.012$). Camels of the Awadia breed aged below 5 years were 3.6 times more likely to harbour *T. evansi* than those of the Awarkia breed aged over 15 years. This study demonstrates that *T. evansi* infection is prevalent among camels in southwestern Saudi Arabia. Further studies should be conducted to focus on vectors and hosts, and to evaluate the potential risk factors in order to plan and implement effective control programmes.

Keywords: *Arabia camels, evansi, PCR, prevalence, Saudi surra, Trypanosoma*

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Introduction

Trypanosoma evansi infection, known as surra, is a serious protozoan disease found in livestock, particularly in camels and horses. It is considered the most significant and widespread health problem in livestock globally and it causes substantial economic losses due to the reduction in livestock productivity, high mortality rate, and cost of treatment and control (Dargantes et al., 2009; Desquesnes et al., 2013). *Trypanosoma evansi* was the first pathogenic mammalian trypanosome to be identified in the world; it was detected in the blood of Indian equines and dromedaries in 1880 by Griffith Evans (Hoare, 1972). Subsequently, numerous reports from different countries revealed a wider global distribution and mammalian host range than any other pathogenic *Trypanosoma* species (Franke et al., 1994; Da Silva et al., 2016). Recently, *T. evansi* has emerged as potentially pathogenic for humans, with a few cases of human infection reported in different countries (Joshi et al., 2005; Truc et al., 2013).

T. evansi is mainly mechanically transmitted through blood-sucking insects such as tabanid flies of the genera *Tabanus*, *Stomoxys and Haematopota* (Desquesnes et al., 2013; Rodríguez et al., 2014). However, vertical transmission (transplacental) as well as animal infection through the eating of *T. evansi*-infected tissue have been documented (Campigotto et al., 2015; Raina et al., 1985). Moreover, accidental human infection from a syringe containing *T. evansi*-infected blood and through a wound while butchering infected raw beef have also been reported in India and Vietnam, respectively (Gill, 1977; Truc et al., 2013; Chau et al., 2016).

Surra can be an acute or chronic and asymptomatic or present with fever, anaemia, general weakness, neurologic abnormalities, marked ataxia, blindness, cachexia and often abortion and/or death if left untreated (Rodrigues et al., 2009; Desquesnes et al., 2013). The disease is often fatal in camels, horses and dogs while it is mild or subclinical in cattle, buffaloes, goats and sheep, whereas pigs may serve as reservoirs (Sirivan et al., 1989; Dargantes et al., 2009; Habila et al., 2012). Several surra outbreaks and epidemics have occurred across Asia, Africa, Europe and South America with increasing economic losses reported and studies have revealed that *T. evansi* is the first leading cause of trypanosomosis in camels and horses followed by other trypanosome species such as *T. vivax*, *T. congolense*, *T. brucei* and *T. simiae.* (Desquesnes et al., 2008; Gutierrez et al., 2010; Elshafie et al., 2013; Fikru et al., 2015; Tehseen et al., 2015; Kamidi et al., 2017; Mossaad et al., 2017; Ramírez-Iglesias et al., 2017).

In Saudi Arabia, there is a scarcity of information on the prevalence of ***T. evansi*** infection across the country. Previous studies have revealed that ***T. evansi*** is the predominant blood parasite among camels, with a prevalence ranging from 5% to 44% with the highest rates of prevalence reported in the Jazan, Al-Jouf and Eastern regions (Hussein et al., 1991; Omer et al., 1998; Al-Khalifa et al., 2009; El-wathig and Faye, 2013; Al-Afaleq et al., 2015). However, all the previous findings are based on parasitological (Giemsa-stained blood film (GSBF) or buffy coat) and serological methods (mainly card agglutination test (CATT) or enzyme-linked immunosorbent assay (ELISA) for *T. evansi*). The use of highly sensitive diagnostic techniques such as polymerase chain reaction (PCR) is greatly lacking in previous studies conducted in Saudi Arabia. Hence, the present study aimed to determine the actual prevalence of *T. evansi* infection and its associated risk factors in dromedary camels in Jazan region, southwestern Saudi Arabia using GSBF and PCR techniques. It is hoped that the findings of this study will be useful in facilitating the modelling of sustainable and successful control strategies of surra disease in Saudi Arabia.

Materials and Methods

Study design: A cross-sectional study was carried out on 679 camels in 11 governorates of Jazan region. Each of the camels was given a reference number and information on sex, age, breed and location was entered onto Excel data sheets. Blood samples were collected from the camels and screened for the presence of the *T. evansi* parasite using GSBF and PCR techniques. The samples were considered positive with the detection of parasites using any of these two techniques. The study protocol was reviewed and approved by the Institutional Review Board of the Sudan Academy of Science. The samples were collected from camels with the consent of the camel owners.

Study area: The present study was conducted in Jazan region, Saudi Arabia. The country lies in southwestern Asia and although, generally, it has a desert climate, the country's climate differs from one region to another due to the variety of the topography. Jazan region, the smallest region in Saudi Arabia, is located in the southwestern part of the country directly north of the border with Yemen and has 300 km of coast line along the Red Sea. It has a total area of 11,671 km² and a population of about 1.5 million, and is divided into 14 governorates.

The region is unique with regards to its landform, nature, climate and water availability. There are three distinct zones: a highland zone at an elevation of 2000–2500 m above sea level and rainfall of more than 300 mm/year; a hill zone at an elevation of 400–600 m above sea level and rainfall of less than 300 mm/year; and a coastal plain at an altitude of less than 400 m above sea level and little, if any, rain. There are three breeds of camel in the region: Awadia in the highlands; Awarkia in the hill area; and Sawahelle in the coastal area. The grazing system is open and camels depend on pasture grass.

For the present study, 11 governorates were randomly selected, namely, Jazan, Sabya, Abu Arish, Baysh, Ahad Almasarha, Alarda, Alharth, Farasan, Ar-Rrayth, Ad-Darb (Shegaig) and Samtah (Altowal) (Fig. 1).

Sample collection : The minimum sample size required for this study was calculated according to the formula provided by Thrusfield (2005) for the estimation of disease prevalence in an infinite population at a 5% level of significance, 95% confidence level and

expected prevalence of 40% (Al-Khalifa et al., 2009). The minimum number of camels required for the study was estimated at 461. This involved an additional 20% of camels to avoid missing data and to enhance the accuracy and precision of the results. Hence, 679 blood samples were randomly collected from camels and screened for the parasite. The samples were placed into labelled EDTA vacutainer tubes and then transferred to the Diagnostic Animal Laboratory at Jazan, KSA, where blood smears were prepared immediately and the remaining EDTA blood was preserved at -80°C for PCR.

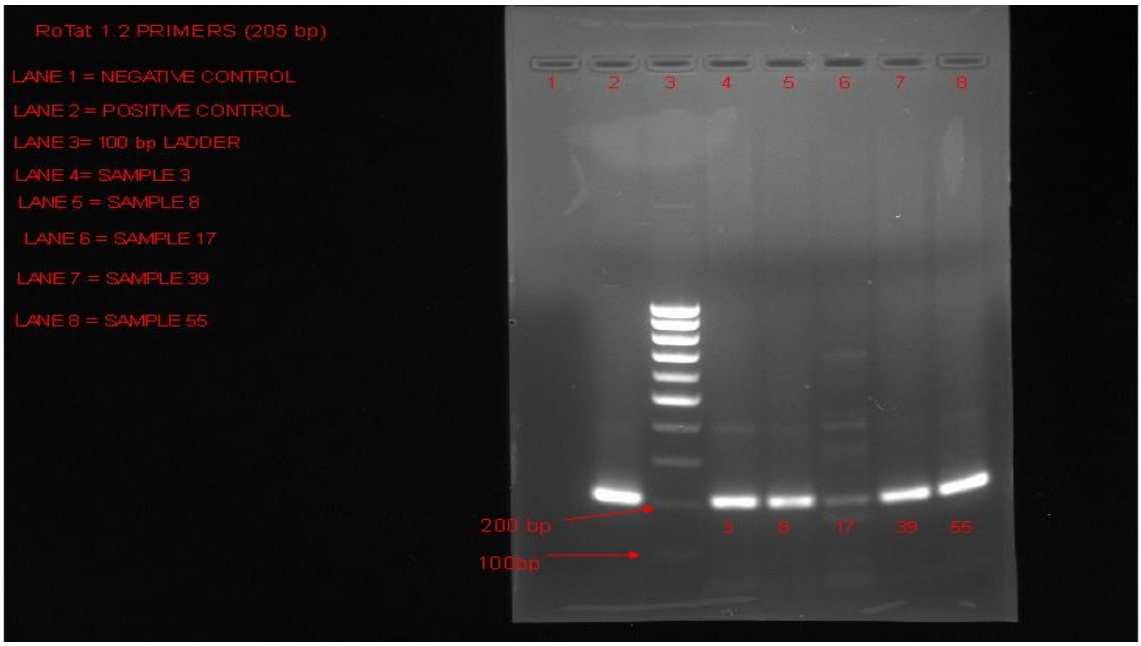

Figure 1 Features of RoTat1.2 band 205 bp with positive and negative controls also 5 samples positive

Giemsa-stained blood film: A 3–5-µl drop of blood was placed on a glass slide and a thin film was prepared, air dried and fixed in methanol for 1–5 min. The blood film was then stained with 10% diluted Giemsa stain. All the blood films were examined microscopically for the presence of *T. evansi* parasites under an oil immersion objective lens (100× magnification).

DNA extraction and molecular analysis: Genomic DNA was extracted from the blood samples using the whole blood DNA isolation kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. In brief, 200 µl of the blood sample was added to 200 µl of lysis buffer and 50 µl of proteinase-K and mixed gently and incubated for 10 min at 72°C, then mixed with 100 µl of binding buffer. Subsequently, the extracted DNA samples were purified (500 µl) and were collected after loading of the spin-column of the kit. Aliquots of DNA were stored at -20°C until used as templates for PCR.

Polymerase chain reaction amplification was done using a thermal cycler (MyCyclerTM thermal cycler, Bio-Rad Laboratories). Amplifications of the RoTat 1.2 VSG and ITS1 rDNA gene primers specific for ***T. evansi*** were carried out in a 50-µl reaction mixture of primary PCR containing 5 µl of genomic DNA, 1 µl of each primer, 25 µl of GoTaq Green Master Mix (Promega Co, USA) and 18 µl of dd H_2O Primer sequences (in 5'-3' direction) were as follow: [RoTat 1.2 VSG gene (forward primer 5'-GCGGGGTGTTTAAAGCAA TA-3' and reverse primer 3'-ATTAGTGCTGCGTGTGTTCG-3') (Claes et al., 2004), and ITS1 rDNA gene (ITS1 F: 5'-CCGGAAGTTCACCGATATTG-3' and ITS1 R: 5'-TGCTGCGTTCTTCAACGAA-3') (Salim et al., 2011)]. All the primers and PCR reagents were obtained from Promega Corporation (Promega Co, USA). The amplification thermal conditions started with a single initial denaturation cycle at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min, and a final extension step at 72°C for 5 min.

The PCR products were then analysed using agarose-gel electrophoresis. Ten microlitres of each amplicon were loaded into a 1.5% agarose gel, run in a TAE buffer containing ethidium bromide (0.5 µg/ml) and visualized under ultraviolet light. The RoTat 1.2 VSG gene primer gave a product of approximately 205 bp while the ITS1 rDNA gene primer gave a product of approximately 480 bp.

Data analysis: The statistical data analysis was performed using SPSS version 20 (IBM, SPSS). For the descriptive analysis, proportion was used to present the prevalence of infection and its distribution among groups. Association between the prevalence and the potential risk factors was examined by Pearson's Chi-square (χ^2) test. Moreover, a binary logistic regression model was used to examine the interaction of age and breed. The adjusted odds ratio (AOR) and 95% confidence interval (CI) were computed. A *P* value of < 0.05 was considered to be statistically significant.

Results and Discussion

Prevalence of T. evansi infection: Out of 679 blood samples examined using GSBF, 151 (22.2%, CI: 19.3–25.5%) samples were found to be positive as shown by

the presence of morphological features of *T. evansi*. On the other hand, 210 (30.9%, CI: 27.6–34.5%) samples were found to be positive for *T. evansi* using both PCR amplifications (RoTat1.2 VSG gene primer and ITS1 rDNA gene primer). The distribution of *T. evansi* infection according by governorate is shown in Figure 2. The highest prevalence of infection was found in Ad-Darb (Shegaig) (55.6%) followed by Farasan (44.4%) and Alarda (42.1%) while Samtah (Altowal) had the lowest prevalence rate (10.6%). These differences were statistically significant ($P < 0.001$).

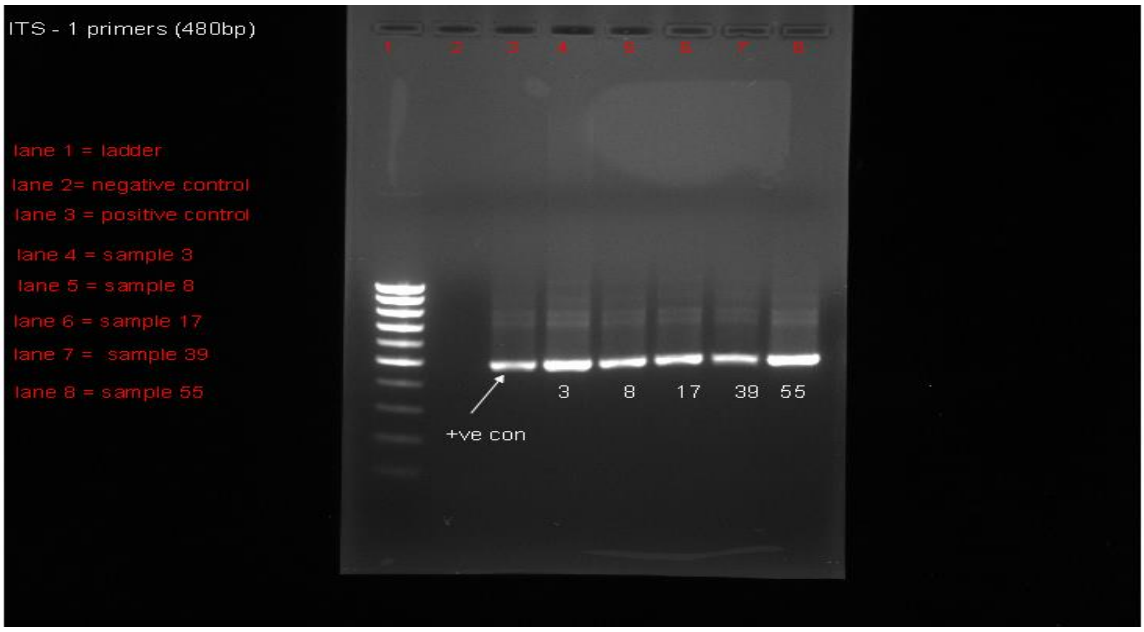

Figure 2 Features of ITS-1 band 480 bp with positive and negative controls 5 samples positive

Association of Trypanosoma evansi infection with specific risk factors: The results showed a significant association between prevalence and location ($\chi^2 = 5.31$, $P = 0.023$). The highest infection rate (36.4%, 84/231) was found in the northern part of the Jazan region while the southern governorates had the lowest rate (26.8%, 68/254), regardless of the sex, breed or age of the camels. The likelihood of camels in the northern part of Jazan being infected with *T. evansi* was 1.5 times higher than for camels in the southern part of the region. As regards breed, the Awadia had the highest infection rate (40.4%) compared to the Awarkia (27.8%) and Sawahelle (30.7%) breeds. However, these differences were statistically insignificant ($\chi^2 = 2.771$, $P = 0.250$). Moreover, there was no significant association between disease prevalence and the age or sex of the camels ($P > 0.05$). The obtained results are summarized in Table 1.

Further analysis on the interaction of breed and age on disease prevalence was undertaken using binary logistic regression. A significant difference was observed between Awadia camels that were less than 5 years old and Awarkia camels that were more than 15 years old ($\chi^2 = 6.31$, $P = 0.012$). The likelihood of infection among camels of the Awadia breed that were less than 5 years old was 3.6 times more likely than that for camels of the Awarkia breed that were more than 15 years old (AOR = 3.6, 95% CI: 1.32–9.56). The results are presented in Table 2.

To our knowledge, the present study is the first to provide information on the actual prevalence of *T. evansi* infection (surra) in the Jazan region of Saudi Arabia using a combination of conventional and PCR techniques. A total of 679 camels from 11 governorates of Jazan were screened for the presence of *T. evansi* parasites using GSBF and PCR techniques. Our findings showed that 30.9% (210/679) of the camels were positive for *T. evansi* by both PCR primers, each of which targets different genes; a higher proportion than that identified by the GSBF method which, in this study, revealed a prevalence of 22.2% (151/679). This infection prevalence (by GSBF and PCR) is much higher than that reported by earlier studies among camels in the Jazan region that used the GSBF method (13.2%) (Hussein et al., 1991), as well as in the Al-Qasim region that used the GSBF method (5.5%) and the passive haemagglutination test (19.7%) (Omer et al., 1998). The high prevalence recorded in the present study could be due to some environmental factors that may favour the presence of tabanid vectors because the characteristics of the Jazan region, including its climatic conditions including rainfall, presence of pools, valleys and animal hosts, are strongly conducive to trypanosome vector biting flies. Previous studies in different regions of Saudi Arabia have observed that in areas with a high incidence of *T. evansi* infection camels are heavily infested with tabanid flies (Hussein et al., 1991; Al-Khalifa et al., 2009).

However, the prevalence reported by the present study is lower than that reported by previous studies that were conducted in different regions across Saudi Arabia. For instance, a previous study conducted in 1990 and 1991, which screened camels, sheep, goats and cattle in six regions in Saudi Arabia for blood parasites using GSBF and wet blood film techniques, found that ***T. evansi*** is the predominant parasite with a prevalence range of 5% to 40%, exclusively detected in camels, with the highest prevalence in Jazan (40%) followed by the Eastern (38%) and Riyadh (21.8%) regions (Al-Khalifa et al., 2009). Similarly, a previous

survey of four breeds of clinically healthy and suspected camels in the Al-Jouf region in the northern part of Saudi Arabia found a prevalence rate of 43.8% by using the CATT/*T. evansi* test (El-wathig and Faye, 2013). Also, a recent study on *T. evansi* infection among camels in different regions of Saudi Arabia found a prevalence of 0.8% based on the buffy coat technique but this increased to 39.4% when the CATT/*T. evansi* method was utilized (Al-Afaleq et al., 2015).

Table 1 Univariate analysis for the association of *T. evansi* infection with selected potential risk factors.

Risk factors	No. examined	*T. evansi* infection		Chi-square	*P*-value
		Positive (%)	Negative (%)		
Location					
North	231	84 (36.4)	147 (63.6)	5.31	0.023[a]
Center	194	58 (29.9)	136 (70.1)		
South	254	68 (26.8)	186 (73.2)		
Age (years)					
< 5	208	70 (33.7)	138 (66.3)	1.419	0.492
5 – 15	132	42 (31.8)	90 (68.2)		
> 15	339	98 (28.9)	241 (71.1)		
Sex					
Male	280	87 (31.1)	193 (68.9)	0.005	0.946
Female	399	123 (30.8)	276 (69.2)		
Breed					
Awadia	52	21 (40.4)	31 (59.6)	2.771	0.250
Sawahella	501	154 (30.7)	347 (69.3)		
Awarkia	126	35 (27.8)	91 (72.2)		

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

Table 2 Binary logistic regression of the interaction of *T. evansi* infection with breed and age of camels.

Interaction	β	SE-β	AOR (95% CI)	*P-value*
Awarkia with > 15 years	0.000	-	1.00	-
Awadia with < 5 years	1.268	0.505	3.55 (1.32 – 9.56)	0.012[a]
Sawahella with < 5 years	0.088	0.206	1.09 (0.73 – 1.64)	0.668
Awadia with 5 – 15 years	-0.070	0.686	0.93 (0.24 – 3.56)	0.919
Sawahella with 5 – 15 years	0.382	0.245	1.47 (0.91 – 2.37)	0.119

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

This obvious variation in results could be attributed to the diagnostic methods used to screen blood samples. Generally, GSBF is commonly used in a range of different types of survey despite its relatively low sensitivity (Monzón et al., 1990). Wet blood film and buffy coat methods showed better sensitivity compared to GSBF (Monzón et al., 1990). However, a higher positive prevalence has been reported using serological methods, which can be as much as double that of GSBF results (Tehseen et al., 2015). This difference could be due to the inability of serological techniques to differentiate between current and past infection, particularly in endemic areas, which leads to diminished accuracy (Fikru et al., 2015). Many previous studies have supported the use of molecular techniques in epidemiological studies on livestock in order to obtain more accurate and reliable results (Njiru et al., 2004; Ramírez-Iglesias et al., 2011; Elhaig et al., 2013). Interestingly, the only previous study in Saudi Arabia that utilized the PCR method to investigate the epidemiology of camel trypanosomosis, specifically in Al-Jouf region, found a prevalence of 25%, using blood samples collected in 2011 (El-wathig et al., 2016).

In comparison with the findings of previous studies conducted in neighbouring countries, a higher prevalence rate of *T. evansi* infection was reported in Jordan (51%) using mouse inoculation (Al-Rawashdeh et al, 2000), in Oman (44%) using GSBF (Srivastava et al, 1984), in Sudan (35.6%) using PCR (Salim et al., 2011) and in Egypt (46%) using PCR (Elhaig et al., 2013). Interestingly, a recent study conducted in Sudan found that *T. evansi* is the first leading cause of surra in camels and horses with a prevalence of 59% (41/70) followed by *T. vivax* with a prevalence of 31% (59/189) (Mossaad et al., 2017). Again, this variation could be explained by the different environmental and climatic factors that favour the fly vectors in different countries, the presence of reservoir animals with susceptible hosts in the same pastures and the diagnostic methods

used for the screening of blood samples as well as the presence of active surveillance and control programmes.

The present study showed significant differences in surra prevalence between location regardless of the camel sex, breed or age group. A higher infection rate was found in the northern governorates, which decreased gradually the further south the governorate. The different terrains in the Jazan region, camel herd size and density of animal population may have contributed to this variation in surra prevalence. However, the distribution pattern of *T. evansi* inside an infected governorate is related not only to the movements of infected carriers or healthy hosts, but also to the pathogenicity of the prevailing stain, the vector–host range and contact frequency, as well as the frequency of the usage trypanocides (Desquesnes *et al*., 2013). In addition, it has been found that camel herded under a nomadic pastoralist system have a higher exposure to *T. evansi* infection than camels under an extensive system management (Najira, 2003).

In the present study, the examined camels consisted of three breeds: Awadia, Awarkia and Sawahelle. The infection rates within the breeds were insignificant although it was relatively high in Awadia breed. Nevertheless, a binary logistic regression revealed a significant difference between breeds; camels of the Awadia breed aged below 5 years were 3.6 times more likely to harbour *T. evansi* when compared to camels of the Awarkia breed aged more than 15 years. This difference in susceptibility between breeds could be attributed to genetic variation, physiological and nutritional status, camel herd size, time of exposure to the parasite and/or surrounding environment favouring disease transmission (Wernery and Kaaden, 2002). Within endemic areas, all camel breeds regardless of sex exhibit a range of susceptibilities to infection (Bourn *et al*., 2001).

The present study revealed that *T. evansi* infection (surra) is prevalent among camels in the Jazan region, southwestern Saudi Arabia, particularly in the northern and central parts of the region. Although the information obtained during the course of this investigation is not extensive, it does shed light on and drawn attention to the extent of the disease burden in the region, which is at a level that could clearly pose major problems and serious economic loss due to reduced livestock productivity. Hence, these findings call for the development and implementation of an integrated control programme against surra disease in endemic areas. Such a programme could include extensive and intensive vector surveillance to identify vector species and potential reservoir hosts in the region. In addition, further studies need to be undertaken on the clinical pattern and economic impact of surra disease as a matter of urgency.

Conflict of interest: The authors declare no conflicts of interest.

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