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Serological survey and associated risk factors' analysis of Trypanosomiasis in camels from Southern Tunisia

Kalthoum Sana [a,*], Lachtar Monia [a], Ben Salem Ameni [a], Hajlaoui Haikel [a], Ben Slimane Imed [a], Chendoul Walid [b], Hechmi Bouabdella [c], Bel Haj Mohamed Bassem [a], Dabbek Hafedh [d], Bennaceur Samed [d], Ourabi Makram [e], Ben Houcine Atef [e], Bouajila Mohsen [g], Khelifi Taib [f], Jamii Ammar [b], Seghaier Chedia [a], Jemli Mohamed Habib [h]

[a] National Center of Zoosanitary Vigilance, Ministère de l'agriculture et des ressources hydrauliques, Tunisia
[b] Commissariat régional au développement agricole de Médenine, Tunisia
[c] Commissariat régional au développement agricole de Gabes, Tunisia
[d] Commissariat régional au développement agricole de Kebili, Tunisia
[e] Commissariat régional au développement agricole de Tozeur, Tunisia
[f] Commissariat régional au développement agricole de Gafsa, Tunisia
[g] Commissariat régional au développement agricole de Tataouine, Tunisia
[h] Service de Parasitologie, École Nationale de Médecine Vétérinaire de Sidi Thabet, Université de la Manouba, Tunisia

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ABSTRACT

Surra (*Trypanosoma evansi* infection) is one of the main causes of dromedary (*Camelus dromedarius*) abortion, besides generating severe economic losses in herds. A sero-epidemiological survey was carried out between December 2018 and December 2019 in Southern Tunisia to estimate the seroprevalence of *Trypanosoma evansi* infection in camels and to determine its possible associated risk factors. Two-stage sampling was conducted to select breeders and camels targeted in our study. A total of 1205 blood samples were collected from 277 randomly selected farms belonging to six governorates of southern Tunisia. Sera were tested with the card agglutination test for *Trypanosoma evansi* (CATT/*T. evansi*) to detect the presence of anti-*Trypanosoma. evansi* antibodies. The overall individual and herd seroprevalence were 30.8% (95%CI 27.9–33.1%), 64.9% (95%CI 61.7–73), respectively. The seroprevalence of *T. evansi* infection both at the animal (26.2% (95%CI 21.4–30.9%) and herd level (84.4 (95%CI 76.3–92.5)) was higher in Kebili than in other governorates ($P = 0.003$). At the animal level, the infection rate with *T. evansi* was significantly associated to the age group among camels ($P = 0.0008$), production system ($P = 0.006$), bioclimatic stage ($P = 0.02$), and herd size ($P = 0.04$) in the univariable analysis. Multivariable logistic regression indicated that only age group and herd size were potential risk factors associated with *Trypanosoma evansi* infection. However, no significant variation of the seroprevalence of *T. evansi* with the sex of camels, farm type, and previous trypanocidal treatment were detected ($P > 0.05$). The findings of this study are crucial for this disease surveillance and control. Further investigations on the efficacy of the treatment against surra are needed to explain the persistence of the disease in the south of Tunisia.

* Corresponding author.
E-mail address: Sana.Kalthoum@iresa.agrinet.tn (K. Sana).

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1. Introduction

Trypanosoma is a genus of hemoflagellate protozoa affecting plants, mammals, and other animal species (Toma et al., 1999). Trypanosomes are unicellular organisms belonging to phylum *Sarcomastigophora*, the order of *Kinetoplastidae*, the family of *Trypanosomatidae (*Shah et al., *2004;* Toma et al., *1999)*. Trypanosomiasis in human was known as "the sleeping sickness", caused by *Trypanosoma brucei*. However, for the veterinary concern, many species and subspecies of *Trypanosoma* (*T. congolense, T. vivax, T. brucei brucei and T. brucei evansi*) can affect hosts like domestic livestock (Benaissa et al., 2020; Giordani et al., 2016). Of these species, *Trypanosoma evansi*, agent of "surra", has the widest host range and geographical distribution. This genus is vector-borne, mechanically transmitted through the bite of infected tabanid flies (Desquesnes et al., 2013). The transmission of this protozoa can also be vertical, horizontal, per-oral, and iatrogenic (Desquesnes et al., 2013). *Trypanosoma evansi*, a salivarian trypanosome, is widely distributed in Africa, Asia, and Southern America (Aregawi et al., 2019). Infection with *Trypanosoma evansi* was reported for the first time in 1880 in horses and camels in the Indian subcontinent (Mahmoud and Gray, 1980).

Trypanosomiasis affects a large number of domestic and wild mammals, but historically, the main host was the camel (Desquesnes et al., 2013), and the highest prevalence was found in this species (Aregawi et al., 2019). furthermore, trypanosomiasis is a severe cameline disease leading to important economic losses (reduced market value of exported animals, decreased milk yield, and lessening animal body condition score and abortions), hence it is considered as a major constraint for the development of camel breeding, especially under its chronic form (Abera et al., 2015; El-Bahnasawy et al., 2014; Salah et al., 2015). Indeed, it has been demonstrated that this pathology is one of the main causes of infectious abortions in camels (*C. dromedarius*) in Africa (Boushaki et al., 2019).

Chronic and acute forms of trypanosomiasis were both observed in camels; and the severity of symptoms varies with hosts and the geographical areas, ranging from the unapparent to the lethal forms (Jaiswal et al., 2015). The most common clinical expression in camels includes progressive emaciation, severe anemia, reproductive problems, and eventually death. All the age groups can be infected, and the disease can persist during several years in the diseased organisms. Different parasitological techniques, such as blood examination by light microscopy, were developed to detect the parasite in the blood of infected animals, and immunological techniques were tuned to circumvent the limitations of the parasitological techniques (Molyneux, 1975).

T. evansi is widespread, and high seroprevalence was in Somalia (68,7%), Sudan (52,2%), and Southeastern Algeria (49,5%) (Babeker and Hassab Elrasoul, 2014; Benaissa et al., 2020; Kadle et al., 2019), with a signaled presence in all African countries, and a continued spreading to the Middle East and South-East Asia (Desquesnes et al., 2013). In Tunisia, despite the large population of camels and the reported outbreaks of trypanosomiasis, the disease is poorly documented and is underestimated. Few studies on the

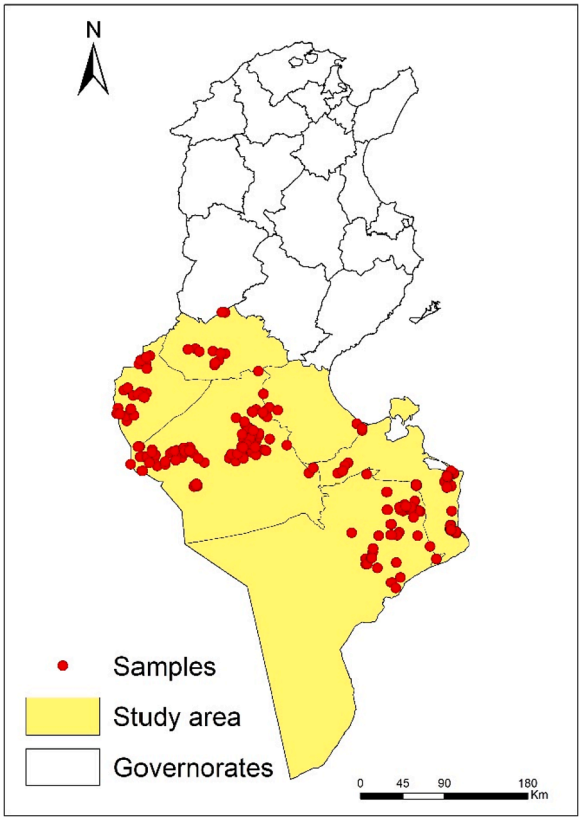

Fig. 1. Map of Tunisia showing the six investigated governorates included in the study and sampled herd locations.

seroprevalence of trypanosomiasis in the camel population were conducted (Azzabi, 1993; Gallo et al., 1989). The most recent study investigated *Trypanosoma* spp. in camels, sheep and horses, reported that 22% of the sampled camels were positive (Selmi et al., 2019). Still, a thorough understanding of the epidemiology of surra is required for a better control and survey of this disease.

Our current study aimed to investigate the seroprevalence of *T. evansi* infection in camels in southern Tunisia and to identify its associated potential risk factors. The result of this study will be useful for the surveillance and the control of *T. evansi* infection.

2. Materials and methods

2.1. Description of the study area

This study was conducted in six (Gafsa, Tataouine, Kebili, Tozeur, Medenine, and Gabes) out of 24 governorates in Tunisia (Fig. 1). These six governorates are located in the southern part of the country, and have a desert climate, with extremely hot summers, warm winters, and a very low annual rainfall amount. They are characterised by a distinct geographical location with a 480 km coastal zone, bordering Libya to the East and Algeria to the West. These governorates are considered the main camel rearing area in the country, where the one-humped dromedary is the most productive livestock species. According to the South Development Office (http://www.ods.nat.tn/fr/, n.d.), the camel population of the six governorates is about 40,868 camels, which represents the majority of the dromedary livestock in Tunisia. The governorate of Kebili records the highest number of dromedaries (32.6% of the national herd), followed by the governorates of Medenine (27.3%) and Tataouine (23.9%).

2.2. Study design and sample size determination

A cross-sectional study was conducted between December 2018 and December 2019 in the above-mentioned governorates, with a two-stage sampling process: the primary sampling units being breeders and the secondary sampling units being the animals within the investigated herds. The minimum number of specimens required for this study was calculated according to the method of Thoma Bernard (Toma et al., 1999), with a 5% level of significance, 95% confidence level, and the expected prevalence of 25% (Azzabi, 1993). Thus, the minimum number of camels required for the present study was estimated at 1153. Besides, to prevent missing data and to enhance the precision of the results, additional samples ($n = 52$) were considered, representing more than 4% of the required samples.

The list of breeders was provided by the Regional Veterinary Services, and data included the name of breeder, his phone number, and the number of camels he owns. The selection of the breeders was carried out using a table of random numbers generated by an Excel spreadsheet. Systematic sampling was used to select animals in the field.

2.3. Questionnaire design and data collection

A detailed and structured questionnaire was elaborated and pre-tested in ten herds. The first part of the questionnaire combined information regarding the breeders (name, phone number, age, gender, level of education, herd size, production system, bioclimatic stage, and type of farm), and the sampled animals (age, sex, breed, previous trypanocidal treatment). The second part of the questionnaire focused on the possible risk factors associated with the *T. evansi* infection. Herds and individual animal data were obtained from the breeders, using a face-to-face interview. The geographical coordinates of each visited location were recorded using the Global Positioning System (GPS) device (Trimble Juno B) and were used to generate a map of the sampled herds.

2.4. Specimen collection and laboratory analysis

We collected 10 ml of jugular vein blood from each randomly sampled camel, in labelled tubes with predefined codes and reserved at 4 °C. The sera were extracted then stored at −20 °C until use. Card Agglutination Test CATT/*Tryapanosoma evansi* (Institute for Tropical Medicine, Antwerp, Belgium) was performed following the manufacturer's instructions (Bajyana Songa and Hamers, 1988). The animals are considered positive if agglutination occurs within eight minutes. The positive animals were then scored into three groups according to the intensity of the infection (+), (++), and (+++). For each plate, negative and positive controls were added.

2.5. Data analysis

Data were entered into Microsoft Access and analyzed with R software version 3.4 (R Core Team, 2017). Maps were generated with ArcGIS version 10.4 (Environmental Systems Research Institute (ESRI), 2021). The overall prevalence was calculated at the individual level, by dividing the total number of the positive samples by the total number of tested samples and 95% confidence intervals were given for the calculations. At the herd level, seroprevalence was determined by dividing the number of positive herds by the total number of sampled herds.

The Chi-square test and Fisher test were performed to compare the seroprevalence of *Tryapanosoma evansi* infection among the governorates, age classes, with a significance level α set at $P < 0.05$. Associations between the seropositivity to *Trypanosoma evansi* and its potential risk factors were first screened in a univariable analysis using the Chi-square test and significance level α was set at $P < 0.05$. Then, multivariable analysis using Logistic regression model (LRM) was performed using R software version 3.4 (Jaiswal et al., 2015) with variables showing statistical significance in the univariable analysis ($P < 0.05$). Odds ratios (OR) with 95% confidence intervals (CIs 95%) were calculated. In the logistic regression model, the seroprevalence of *Trypanosoma evansi* infection was

considered as the dependent variable, and the significant risk factors as independent variables and the level of significance was set at $P < 0.05$.

3. Results

3.1. Trypanosomiasis seroprevalence

A total of 1205 camels reared in 277 herds were examined to detect the presence of *T. evansi* antibodies using CATT/*T. evansi*. The overall animal-level seroprevalence rate of *T. evansi* infection was 30.8% (368/1205) (95% CI 27.9–33.1%), with 22.2% (95% CI 3–41.4%) in Gabes, 28.6% (95% CI 15.9–41.2%) in Gafsa, 37.5% (95% CI 32.6–42.3%) in Kebili, 26.2% (95% CI 21.4–30.9%) in Medenine, 25.4% (95% CI 20.5–30.2%) in Tataouine and 36% (95% CI 27.1–44.9%) in Tozeur. The difference in seroprevalence between governorates was statistically significant (chi-squared = 17.841, $P = 0.003$), and the highest value was recorded in the governorate of Kebili (Southwest of Tunisia). At the herd level, the overall herd-level seroprevalence rate of *T. evansi* infection was 64.9% (and the highest value was recorded also in the governorate of Kebili with 74.7% (95% CI 65.583.8%). The lowest value of seropositivity to *T. evansi* was reported in the governorate of Medenine (57.5% (95% CI 46.1–68.8%) (Table 1).

3.2. Association with risk factors

The association of potential risk factors (age, sex, production system, bioclimatic stage, farm type, previous trypanocidal treatment, and herd size (number of heads)) are summarised in Table 2. Of the seven analyzed variables, only four factors (age, production system, bioclimatic stage, and herd size) were significantly associated with the *T. evansi* infection. Univariable analysis showed a significant difference between age group (Chi-squared = 10.611, $P = 0.001$). The age-wise comparison revealed that the highest infection rate of surra was recorded in young camels (under 4 years) compared to the older camels (older than 4 years). Similarly, the seropositivity for *T. evansi* varied significantly with the herd size ($P = 0.04$) and the bioclimatic stage ($P = 0.03$). A significant difference in the seroprevalence between the intensive, extensive, and semi-extensive types of herds was observed, suggesting that the production system should be considered as a risk factor for the *T. evansi* infection ($P = 0.006$). However, according to the sex of camels, farm type, and any previous trypanocidal treatment, no significant differences were detected ($P > 0.05$), and the univariate analysis did not show their role in the disease occurrence (Table 2).

Multivariate analysis identified age as a risk factor for the seroprevalence of *T. evansi* and young camels were nearly two times more likely to be surra seropositive than older camels (OR = 1.46, CI = 1.07–1.98). However, herd size was found to be a protective factor, and camels raised in large herds (more than 10 animals) were found to be more affected than camels reared in small herds (1–10 animals) ((OR = 0.68, CI = 0.47–0.98) (Table 3).

4. Discussion

T. evansi is a severe cameline disease, commonly associated with considerable economic losses in Tunisia, but it is undocumented. According to our knowledge, our study is the first to estimate the seroprevalence of *T. evansi* infection in a large number of camels in Tunisia, and to determine the potential risk factors associated with surra. Only two studies were conducted in Tunisia on the seroprevalence of *T. evansi* in camels using (Azzabi, 1993; Gallo et al., 1989).

This study was carried out only in the southern governorates (Gafsa, Tataouine, Kebili, Tozeur, Medenine, and Gabes), given that the camel population is concentrated (Kamoun and Jemmali, 2014; Moslam and Megdiche, 1989). To evaluate the seroprevalence of *T. evansi* infection in dromedary, the Card Agglutination Trypanosoma Test (CATT) was favored. It is considered to be one of the best serological methods to detect *T. evansi* antibodies in camels, due to its higher specificity compared to the other serological techniques, in addition to the fact that is easy to perform (Gutierrez et al., 2000; Verloo et al., 2000). Previous studies demonstrated that the sensitivity of the CATT/*T. evansi* ranged between 86 and 100% and the specificity varied from 96 to 98% (Gutierrez et al., 2000; Verloo et al., 2000).

The current study confirms that *T. evansi* is present in dromedaries in the six investigated governorates in Tunisia, and the overall

Table 1
Seroprevalence rate of Trypanosomiasis in camels according to governorates.

Governorates	At the animal level				At the herd level			
	Number of tested camles	Positive samples	Prevalence (%)	95% CI	Number of tested herds	Positive herds	Prevalence (%)	95% CI
Gabes	18	4	22.2	3–41.4	5	3	60	17–100
Gafsa	49	14	28.6	15.9–41.2	10	7	70	41.5–98.4
Kebili	384	144	37.5	32.6–42.3	87	65	74.7	65.5–83.8
Medenine	328	86	26.2	21.4–30.9	73	42	57.5	46.1–68.8
Tataouine	315	80	25.4	20.5–30.2	76	44	57.8	46.7–68.9
Tozeur	111	40	36	27.1–44.9	26	19	73	56–90.1
TOTAL	1205	368	30.5	27.9–33.1	277	180	64.9	59.3–70.6

Table 2
Univariable analysis of potential risk factors associated with the seropositivity of *T. evansi* infection in camels in Tunisia

Variable	Category	Negative samples	Positive samples	Total	Positive percentage (%)	*P*-value
Age	Adult (>4 years)	675	265	940	28.1	0.001*
	Young (≤4 years)	162	103	265	38.8	
Sex	Female	827	363	1190	30.5	0.8
	Male	5	10	15	66.6	
Production system	Extensive	575	281	865	32.4	0.006*
	Intensive	3	3	9	33.3	
	Semi-extensive	259	84	343	24.4	
Bioclimatic stage	Arid	291	104	395	26.3	0.03*
	Desert	546	264	810	32.5	
Farm type	Meat	152	66	218	30.2	0.8
	Dairy	96	46	142	32.3	
	Mixed	589	256	845	30.2	
Previous trypanocidal treatment	Yes	23	9	32	28.1	0.7
	No	814	359	1173	30.6	
Herd size	[1−10]	182	59	241	24.4	0.04*
	[11–50]	307	156	463	33.6	
	[>50]	348	153	501	30.5	

Variables with statistical significance in the univariable analysis ($P < 0.05$).

Table 3
Multivariable logistic regression analysis for risk factors associated with the seropositivity of *T. evansi* infection in camels in Tunisia.

Variable	Category	OR 95%C.I.		P-value
Age	Young /Adult	1.46	1.07–1.98	0.02
Bioclimatic stage	Desert/Arid	1.03	0.72–1.47	0.88
Production system	Extensive	1		
	Intensive	2.63	0.47–14.7	0.25
	Semi-extensive	0.74	0.51–1.05	0.09
Herd size (number of heads)	[>50]	1		
	[11–50]	1.06	0.78–1.42	0. 72
	[1–10]	0.68	0.47–0.98	0.04

Variables associated with the seropositivity of trypanosoma evansi in the final model.

animal-level seroprevalence was found to be 30.8% by CATT/*T. evansi*, which is higher than reported by the previous studies in which the seroprevalence ranged between 18% and 22% (Gallo et al., 1989). Our result is highly consistent with other studies conducted in south Algeria (Boushaki et al., 2019), and Chad (Delafosse and Doutoum, 2004), where the seroprevalence of *T. evansi* was estimated at 32.4% and 30.5%, respectively.

On the other hand, the animal level seroprevalence of *T.evansi* in Tunisia was higher than those reported in previous studies carried on Morocco (14.1%), Mauritania (Trarza, Gorgol, Adrar, Hodh El Chargui and Nouakchott) (16.2%), and Ethiopia (between 13.7% and 24.1%) (Atarhouch et al., 2003; Birhanu et al., 2016; Dia et al., 1997; Fikru et al., 2015). High seroprevalence of *T.evansi* was recorded also in Nigeria (44.09%), Kenya (45.9%), and Egypt (82%) which is in contrast with our result (Kyari et al., 2021; Njiru et al., 2004; Zayed et al., 2010).

The observed seroprevalence of *T. evansi* in dromedaries in southern Tunisia may however overestimate the real situation, since the CATT does not differentiate between current and past infections in animals, given that the majority of the sampled animals in this study were adult (older than 4 years) (Nantulya, 1990; Tehseen et al., 2015).

The seroprevalence of *T. evansi* varied significantly among governorates, and the governorate of Kebili seems to be the most affected with an infection rate of 37.5%. The high density of dromedaries and the presence of cross-border herd movement with Algeria could explain the high seroprevalence in this governorate (Benaissa et al., 2020; Bouslikhane, 2015). Indeed, it was demonstrated that the seroprevalence of *T.evansi* in southern Algeria, an area bordering the governorate of Kebili, was assessed at 45% (Benaissa et al., 2020). Moreover, the presence of an important number of watering points (Centre national de veille zoosanitaire 2017), wetlands, and saline lakes in Kebili in comparison to the other governorates, is likely to enhance the spread of trypanosomiasis, through the development and proliferation of tabanids, implicated vectors in the transmission of *T. evansi (*Abera et al., *2015;* Ben*, 2019;* Rafu et al., *2021)*. There is no doubt that the difference between the seroprevalences of *T.evansi* among governorates is related to the differences in the geo-climatic features of these areas, the age of tested animals, and the environmental conditions of their rearing.

At the herd level, the overall seroprevalence rate of *T. evansi* infection was high (64.9%) in our study which revealed the wide spread of surra in the south of Tunisia which could be related to vector abundance in the study area. The result obtained by Benaissa et al. was different from our finding indicating high seroprevalence rates of surra in camels in Algeria at the herd level (Benaissa et al., 2020). The governorate of kebili has the high seroprevalence rate of *T.evansi* infection at the herd level. This governorate seems to have the suitable environmental conditions for the tabanids facilitating the transmission of surra.

Seven risk factors were investigated in this study, using the univariable and multivarible analysis. The multivariable logistic

regression indicated that the age group and the herd size were the only potential risk factors associated with the seroprevalence of *T. evansi* infection, since no significant variation of the seroprevalence of *T. evansi* with sex, farm type, common grazing, common watering point, and previous trypanocidal treatment was revealed. Similar to this finding, various other studies showed no effect of the animals' sex on their seropositivity for *T. evansi* infection (Benaissa et al., 2020; Giro and Jilo, 2020; Khosravi et al., 2015).

It is interesting to note that the infection rate with *T. evansi* was found to be higher in females than in males in some studies (Dia et al., 1997; Shah et al., 2004; Sobhy et al., 2017), while the contrary was reported by some other researchers (Bogale et al., 2012; Ndoutamia et al., 1999).

The infection rate with *T. evansi* decreased with camel age. This result agrees with the surveys of Lemecha (Lemecha et al., 2008), and Delafosse (Delafosse and Doutoum, 2004) that revealed the increase of the infection with *T. evansi* in young camels (Delafosse and Doutoum, 2004; Lemecha et al., 2008). However, our finding differs from other studies where old camels were slightly more likely to be infected with *T. evansi* (Atarhouch et al., 2003; Bogale et al., 2012; Gerem et al., 2020). In contrast to our study, other researchers report no significant difference in the prevalence between age groups (Benaissa et al., 2020; Boushaki et al., 2019; Pacholek et al., 2000).

Besides, the herd size-wise comparison showed that there is a significant association between the herd size groups and the occurrence of the disease. In our study, a high infection rate with *T. evansi* was registered in herds counting more than 11 camels, and this result is in line with the findings of other studies over the world (Benaissa et al., 2020; Bhutto et al., 2010). This association could be explained by the increased contact between infected and non-infected animals, compared to small herds.

In our study, more infected herds and animals were found in the desert stage compared to those from the arid stage. This difference could be likely to be an indication of a difference in the ecology of these areas.

Finally, the seroprevalence to *T evansi* is highly associated with the production system in our study. The extensive system seems to shelter the most important number of animals infected with *T. evansi,* in comparison to the others production systems. Our finding agrees with the result of a survey conducted in Sudan, reporting that the nomadic dromedaries are afflicted with the highest seroprevalence, in comparison with the other management systems (Elamin et al., 1998). Important transhumance movements of dromedaries between different areas and countries in extensive management systems explain most likely the wide distribution of *T. evansi* infection (Benaissa et al., 2020).

5. Conclusion

This study showed a high rate of seropositivity of *T. evansi* infection in dromedaries in southern Tunisia and identified its potential associated risk factors. Age group and herd size were highly correlated to the occurrence of this disease. These results are crucial to establish an efficient surveillance system against this pathology handicapping its full expansion. Notwithstanding, further investigations using other diagnosis techniques and the exploration of the ecology of the vector and its distribution are decisive to control trypanosomiasis and to curtail its generated economic losses. The role of the camel's mobility in the spread of disease and the efficacy of the treatment against surra must be explored to explain the persistence of the disease in the south of Tunisia.

Author contributions

Sana Kalthoum, Seghaier Chedia: Conception of the study.

Monia Lachtar, Ben Salem Ameni, BassemBel Haj Mohamed, Haikel Hajlaoui, Ben Slimane Imed, Chandoul Walid, Hechmi Bouabdallah, Dabbek Hafedh, Bennaceur Samed, Ourabi Makram, Ben Houcine Atef, Khelifi Taib: Data Collection and validation:

Mohamed Habib Jemli: Laboratory analysis.

Sana Kalthoum, MoniaLachtar, Ben Salem Ameni: Writing – original draft.

Sana Kalthoum: Statistical analyses.

Sana Kalthoum, Jamii Ammar and Mohamed Habib Jemli, Seghaier Chedia: Review.

All authors have approved the final version of the paper.

Ethical disclosures

The authors declare that the study was conducted according to the national guidelines without causing damage to the animals and respecting their welfare (Number: CEEA-ENMV 36/21). The authors declare also that data related to breeders was not published. The study was approved by the Ethics Committee in Animal Experimentation of the Sidi Thabet national school of veterinary medicine in Tunisia.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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"Posthumous tribute - This article is dedicated to the memory of our colleague and friend, Bouajila Mohsen, who left us far too early."

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