

Article

First Seroepidemiological Study of *Toxoplasma gondii* Infection in Dromedaries (*Camelus dromedarius*) in Southern Tunisia

Afef Jeljli ^{1,2,*} , Syrine Rekik ², Boubaker Ben Smida ³, Walid Chandoul ⁴, Limam Sassi ² and Mohamed Gharbi ² 

- ¹ Department of Sciences and Pathology of Animal Reproduction, Institution of Agricultural Research and Higher Education, National School of Veterinary Medicine of Sidi Thabet, University of Manouba, Sidi Thabet 2020, Tunisia
- ² Laboratory of Parasitology, Institution of Agricultural Research and Higher Education, National School of Veterinary Medicine of Sidi Thabet, University of Manouba, Sidi Thabet 2020, Tunisia; rekiksyrene@yahoo.com (S.R.); sassilimam1960@gmail.com (L.S.); gharbim2000@yahoo.fr (M.G.)
- ³ Regional Commissary for Agricultural Development (CRDA), Tataouine 3200, Tunisia; boubaker.bensmida@gmail.com
- ⁴ Circonscription of Animal Production of Ben Guerdane, Médenine 4160, Tunisia; walid.chandoul80@gmail.com
- * Correspondence: afef.jeljli31@gmail.com; Tel.: +216-2811-5481 or +216-7155-2200

Abstract: *Toxoplasma gondii* is one of the most common zoonotic parasites worldwide, with infections in humans as well as in all mammals, including dromedaries, that affect reproductive health, leading to malformations and abortions. In the current study, we estimated, for the first time in Tunisia, the seroprevalence of *T. gondii* antibodies and its associated risk factors in dromedaries (*Camelus dromedarius*) using the enzyme-linked immunosorbent assay (ELISA) technique. A total of 248 sera samples were collected from dromedaries living in Médenine and Tataouine governorates, South Tunisia. The overall seroprevalence of *T. gondii* was estimated to be $29.8 \pm 2.9\%$ (74/248) with a significantly higher seroprevalence rate in dromedaries from Médenine ($52.5 \pm 7.9\%$; 21/40) compared to Tataouine ($25.5 \pm 3\%$; 53/208); it was also higher in dromedaries kept in intensive farming systems ($55 \pm 11.1\%$; 11/20) compared to extensive systems ($27.6 \pm 3\%$; 63/228), in non-pregnant females ($39 \pm 4\%$; 57/146) compared to pregnant ones ($16.7 \pm 3.7\%$; 17/102), and in those in contact with cats ($66.7 \pm 8.6\%$; 20/30) compared to those with no contact with cats ($24.8 \pm 2.9\%$; 54/218). The present study should be followed by others to explore the role of dromedaries in the epidemiological cycle of *T. gondii* in Southern Tunisia.

Keywords: seroepidemiology; *Toxoplasma gondii*; *Camelus dromedarius*; ELISA; Tunisia



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1. Introduction

In Tunisia, with an estimated population of 80,000 females, dromedaries (*Camelus dromedarius*) play an important socio-economic role. These animals provide milk, meat, leather, and fibres. Moreover, dromedaries are a unique pack animal species in the Sahara; they are used for eco-tourism and agricultural activities. In addition, they play a crucial socio-political role in maintaining the stability of human communities in these remote and arid regions.

The multiple roles played by these animals in contributing to the food security of the human population in southern Tunisia necessitates more attention focused on their potential to transmit zoonotic pathogens to humans through direct contact and meat and milk consumption, leading to serious disease such as brucellosis, tuberculosis, surra, toxoplasmosis, etc.

Toxoplasmosis is one of the most common zoonotic diseases; it is caused by the protozoan apicomplexan parasite *Toxoplasma gondii*, which infects all warm-blooded animal species including humans. Approximately one-third of the human population worldwide is infected by this parasite [1]. The lifecycle of *T. gondii* includes asexual multiplication in various tissues of intermediate hosts, which are warm-blooded animal species including livestock and humans, and sexual multiplications that occur only in the intestine of definitive hosts, which

are felids including domestic cats [2–4]. *T. gondii* post-natal infections in humans occur after consuming tissue cysts from raw or undercooked meat of infected animals, ingesting food or water contaminated by oocysts, or by accidentally ingesting oocysts from the environment. Transmission may also occur through tachyzoites contained in raw or unpasteurized milk. In fact, tachyzoites of *T. gondii* have been detected in the milk of several intermediate hosts (such as sheep, goats, cows, and mice), and human consumption of raw goat’s milk was documented to induce acute toxoplasmosis [5–7]. Moreover, the parasite can be transmitted vertically to the foetus through the placenta and, in several hosts, via milk containing tachyzoites from the mother to the offspring [5–9]. Tachyzoites are generally sensitive to proteolytic enzymes and are usually destroyed by gastric digestion. However, some studies reported that tachyzoites may occasionally withstand acid–pepsin solutions for a brief period, up to 2 h, and that oral ingestion of high numbers of tachyzoites may induce infection in mice and cats [10].

Toxoplasmosis can cause serious conditions in individuals of all age groups, especially immunosuppressed and neonate persons [11]. The veterinary importance of *T. gondii* was recognised when it was discovered as the cause of sheep abortion storms in 1957 in Australia [9]. Since then, it has been shown that toxoplasmosis can affect various species, leading to clinical signs such as fever, diarrhoea, and abortion in livestock, consequently having significant economic impacts on domestic livestock.

Several studies conducted in various regions of Tunisia reported a high *T. gondii* seroprevalence in domestic animals; it reached 22.5% in sheep [12], 31.2% (24/77) in goats [13], 24.3% in chickens [12], 17.7% (28/158) in horses [14], and 34.9% (15/43) in wild rodents [15]. However, despite the importance of dromedaries in Tunisia, there is no study on their infection by *T. gondii*. We report herein a seroepidemiological study of *T. gondii* infection in dromedaries reared in southern Tunisia in order to estimate the prevalence of this parasite and identify the associated risk factors.

2. Materials and Methods

2.1. Study Region

The present study was conducted between February and September 2021 in the governorates of Médenine and Tataouine, southern Tunisia. The governorate of Médenine covers an area of 9167 km², representing 5.6% of the total surface of Tunisia. The governorate of Tataouine is the largest governorate of Tunisia with an area of 38,889 km², representing 23.8% of the total surface of Tunisia. These two governorates have the highest numbers of dromedaries in Tunisia, 16,927 and 12,293, respectively (Figure 1, Table 1).

Table 1. Geographic and demographic characteristics of the two studied governorates.

	Governorate	
	Médenine	Tataouine
Surface area in km ² (% surface of Tunisia)	9167 (5.6%)	38,889 (23.77%)
Köppen classification	BWh	BWh
Bioclimatic zone	Sahara	Sahara
Mean altitude (m)	103	237
Mean annual precipitation (mm)	131	116
Range of mean annual temperature (°C)	11.4–28.7	10.5–28.6
Mean temperature in winter (°C)	12.1	11.2
Mean temperature in summer (°C)	27.7	27.7
Human population (% Tunisian population)	522,000 (4.4%)	152,500 (1.29%)
Annual meat production in tons (% Tunisian production)		
Sheep	2500	1800
Goats	1955	400
Camels	1800	230
Cows	1000	30



Figure 1. A map of Tunisia and the two governorates where the present study was conducted.

2.2. Sample Size Determination

To determinate the approximate sample size, we used the formula by Thrusfield [16].

$$N = 1.962 \times P_{exp} (1 - P_{exp}) / d^2$$

where

N: the required sample size;

P_{exp}: the expected prevalence;

d: the desired absolute precision fixed to 5%.

As there are no Tunisian studies on *T. gondii* infection in dromedaries, the expected *T. gondii* seroprevalence was based on studies performed in a neighbour country, Southeast Algeria (15%) [17]. Using this formula, the number of required samples was 246 and we collected 248 serum samples.

2.3. Animal and Sample Collection

Approximately 5 mL of blood was collected from the jugular vein of 248 dromedaries using identified sterile dry Vacutainer[®] tubes. Blood samples were centrifuged at 1200 rpm for 20 min to collect sera and then transferred in iceboxes to the laboratory in order to be stored in Eppendorf tubes at −20 °C until analysed.

For the realisation of this study, we used an enzyme-linked immunosorbent assay (ELISA) commercial kit (ID Screen[®] Toxoplasmosis Indirect ELISA Multi-species, ID VET. Innovative Diagnostics. Montpellier, France) recommended for multi-species, which utilises native P30 (SAG1) antigens and anti-multi-species conjugates as secondary antibodies, enabling the detection of anti-*Toxoplasma gondii* IgG antibodies indicating a chronic *T. gondii* infection. The experimental protocol was conducted according to the manufacturer's instructions. Initially, we added the samples and the controls (negative and positive sera) to a 96-well plate which was coated with antigens. Then, the plate was incubated for 30 min at 21 °C (+/−5 °C). Subsequently, the wells were washed three times and conjugate was

added. The plate was then incubated for thirty minutes at 21 °C (+/−5 °C) and washed another time; then, we added the enzyme substrate. After fifteen minutes of incubation at 21 °C (+/−5 °C), we added the stop solution. The optical density (OD) of the microtiter plate was measured using an ELISA reader (Multiskan™ FC, Thermo Fisher Scientific, Waltham, MA) at 450 nm longwave.

The test was validated if $OD_{PC} > 0.350$ and $OD_{PC}/OD_{NC} > 3$, and the results were interpreted as follows: $S/P\% = (OD_{sample} - OD_{NC}) / (OD_{PC} - OD_{NC}) \times 100$. A serum sample was considered positive if $S/P\% \geq 50\%$, where

NC: negative control;

PC: positive control;

S: sample;

P: positive control.

2.4. Data Collection

During blood sampling, an interview containing open- and closed-ended questions was performed on the dromedaries' owners to gather information about (i) their socio-demographic characteristics; (ii) the rearing system of the animals, the herd size, and the presence of cats in the dromedaries' flocks; and (iii) information about the sampled dromedaries: breed, age, pregnancy, and history of abortion.

2.5. Statistical Analyses

Odds ratios (ORs) and 95% confidence intervals of the ORs (IC 95%OR) of different risk factors (locality, age, husbandry system, contact with cats, reproductive status and history of abortion) were estimated using Epi Info version 7 (CDC). The same software was used to study the differences in seroprevalences in different categories at the 5% threshold.

3. Results

Among the 248 tested sera, anti-*T. gondii* antibodies were detected in 74 animals ($29.8 \pm 2.9\%$). Seroprevalence was significantly higher in Médenine ($52.5 \pm 7.9\%$; 21/40) compared to Tataouine ($25.5 \pm 3\%$; 53/208). Moreover, the seroprevalence was significantly higher in dromedaries raised in intensive farming systems ($55 \pm 11.1\%$; 11/20) compared to extensive systems ($27.6 \pm 3\%$; 63/228), in non-pregnant females ($39 \pm 4\%$; 57/146) compared to pregnant ones ($16.7 \pm 3.7\%$; 17/102), and in those in contact with cats ($66.7 \pm 8.6\%$) compared to those with no contact with cats ($24.8 \pm 2.9\%$; 54/218). However, there was no statistically significant difference according to the age groups and history of abortion (Table 2).

Table 2. Association between *Toxoplasma gondii* seroprevalence in dromedaries and different parameters.

Factor		Positive/Examined	% ±SE *	OR [95% CI] #
Locality	Médenine	21/40	52.5 ± 7.9	3.232 [1.614; 6.474] #
	Tataouine	53/208	25.5 ± 3	
Age group (years)	<5	12/45	26.7 ± 6.6	0.827 [0.401; 1.708]
	≥5	62/203	30.5 ± 3.2	
Husbandry system	Extensive	63/228	27.6 ± 3	0.312 [0.124; 0.789] #
	Intensive	11/20	55 ± 11.1	
Contact with cats	Yes	20/30	66.7 ± 8.6	6.074 [2.678; 13.778] #
	No	54/218	24.8 ± 2.9	
Reproductive status	Pregnant	17/102	16.7 ± 3.7	0.312 [0.168; 0.579] #
	Non-pregnant	57/146	39 ± 4	
History of abortion	Yes	3/4	N.A.	N.A.
	No	71/244	29.1 ± 2.9	

* S.E.: Standard Error; # Statistically significant.

4. Discussion

To the best of our knowledge, the present study is the first serological survey that estimated the seroprevalence of *T. gondii* infection and the associated risk factors in dromedary populations in southern Tunisia, which was estimated as $29.8 \pm 2.9\%$ (74/248).

This prevalence is higher than those reported in other countries such as South Iran and Southeast Algeria with similar seroprevalence rates (15%) [17,18]. Lower seroprevalence was reported in Iran (1.87%) [19] and 13.1% in Saudi Arabia [20]. The overall animal- and herd-level seroprevalence of toxoplasmosis in dromedaries from Ethiopia was 8.33% and 37.5%, respectively [21]. In Pakistan, study conducted in 2019 showed a seroprevalence rate higher than that obtained in our study (40.1%) [22], lower than those found in Ethiopia (40.49%) [23] and in Egypt (46.9%) [24]. The results of the current study were comparable to the study carried out in Saudi Arabia (34.2%) [25]. Differences mentioned in rates reported across countries may be attributed to various factors such as the density of domestic cats and/or wild felids, livestock management practises (density of animals, stress factors, etc.), soil type and climate conditions, serum dilutions, serological test limits, and sensitivity variations [24–32]. The high *T. gondii* seroprevalence rate obtained in the present study suggests that the wide spread of *T. gondii* in southern Tunisia is consequently associated with a high zoonotic risk. There is no information about human toxoplasmosis in the two areas where our study was performed. Moreover, the rate revealed in the current study could be attributed to numerous factors among these elements, with the age of dromedaries being related to an absence of regular culling programmes, as well as the migration of dromedaries to other areas to find feed and water resources, local changes in land ownership, and increasing farming, which requires the presence of cats for rodent control [4–23]. Some dromedary diseases like surra (*Trypanosoma evansi* infection) contribute to the decrease in animal resistance to diverse infections [23], and in this context, a recent study conducted in southern Tunisia revealed a high seroprevalence of anti-*T. evansi* antibodies estimated as 62.54% [26]. The seroprevalence rate of anti-*T. gondii* antibodies was higher in Médenine ($52.5 \pm 7.9\%$) compared to Tataouine ($25.5 \pm 3\%$). This seroprevalence difference may be due to the sample size and the climatic variations. In fact, the governorate of Médenine, due to its geographical location, experiences two different climates. In the south, it faces a dry and hot Saharan climate, while in the east, it encounters a relatively humid and temperate upper arid climate [27]. Oocysts shed by the definitive hosts require environmental conditions to sporulate and become infectious, mainly oxygen, humidity, and high temperature [5–28]. The current study showed a statistically significant difference in seropositivity for *T. gondii* based on exposure to domestic cats ($66.7 \pm 8.6\%$; OR = 6.074 95% CI [2.678; 13.778]).

This could be explained by the fact that cats are definitive hosts; they shed oocysts in their faeces that contaminate the soil, water, and livestock's food [29].

In Tunisia, there are five wild felid species (*Felis (silvestris) libyca*, *Felis margarita*, *Caracal (Felis) caracal*, *Leptailurus (Felis) serval* and *Acinonyx jubatus*) that could play a role in toxoplasmosis epidemiology; unfortunately, to the best of our knowledge, there is no study about their ecology (geographic distribution and approximate biomass) [30]. These species could be studied in order to identify the role of each of them in the epidemiology of toxoplasmosis in Saharan ecosystems. There was a statistically significant association between seropositivity regarding *T. gondii* and husbandry systems (intensive and extensive systems). Despite the fact that intensive livestock farming is generally associated with a low risk of *T. gondii* infection compared to extensive farming systems due to the high level of confinement and biosecurity, some practises specific to dromedary intensive systems increase this risk, such as supplement food being stored openly or placed in areas where it may attract birds, rodents, and cats. Additionally, that risk is accentuated when supplements are contaminated by oocysts shed by cats [29]. Indeed, intensive systems put cats that are present in dromedary owners' houses in close contact with dromedaries.

The association of seropositivity in livestock with intensive production systems does not offer a clear indication of how dromedaries become infected despite the role of production systems in increasing the risk of *T. gondii* infection [29].

In southern Tunisia, dromedaries are mainly raised under an extensive pastoral (either nomadic or transhumant) system for meat and secondary milk production. In this system, dromedaries' herdsman move with their dromedaries between March and October in search of water and grazing lands deep in the Sahara. Although, in Tunisia, profound changes are occurring in management systems with the emergence of some intensive systems following the Tunisian national programme to develop and promote dromedary production. In this intensive system, animals are not put in specific independent farms but rather in areas juxtaposed to the dromedary owners' houses, where cats are abundant; they are frequently supplemented with hay and concentrate that are bought several months before and stored near the dromedaries' sheds.

Three out of four dromedaries have a history of abortion. Studies on the incidence and aetiology of abortion in Camelidae are scarce [31]. *T. gondii* was reported as an abortive agent in a Bactrian camel in Greece [32]. *T. gondii* is a less important abortive agent compared to *Neospora caninum* in New World Camelids as shown in Peruvian alpacas and llamas [33].

A statistically significant difference in seropositivity was observed according to the reproductive status. Indeed, non-pregnant females showed higher seroprevalence (39 ± 4 ; 57/146) than pregnant females ($16.7 \pm 3.7\%$; 17/102). This could be explained by the presence of high-risk factors of abortion (including *T. gondii* infection) in these females, leading them to be non-pregnant.

There was a non-significant difference in seroprevalence in dromedaries younger than 5 years ($26.7 \pm 6.6\%$; 12/45) when compared to dromedaries aged more than 5 years ($30.5 \pm 3.2\%$; 62/203). This finding might be due to the small sample size of young animals, and further studies are needed to confirm this trend.

5. Conclusions

The present study revealed a high seroprevalence of *T. gondii* in dromedaries living in Médenine and Tataouine governorates in southern Tunisia. This finding highlighted the possibility of transmission of *T. gondii* to human populations, which become infected by ingesting undercooked meat and milk of infected animals, the latter frequently consumed raw in this region due to the belief that raw camel milk treats several diseases including human diabetes mellitus and cancer. For this reason, a specific control programme must be established by educating animal owners about the zoonotic risks related to consuming crude milk. Further One Health studies are to be conducted to establish the epidemiological cycle of *T. gondii* in Saharan ecosystems and the role of wild felids in this cycle.

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