Original Article

Seroprevalence and associated risk factors of *Toxoplasma gondii* infections in dogs in Egypt

Mohamed Marzok[1,2*] Hattan S. Gattan[3,6] Omar A. AlJabr[5] Mohammed H. Alruhaili[4,6] Mohamed Salem[1,7] Abdelfattah Selim[8*]

Abstract

Toxoplasma gondii is a parasite that is found all over the world and has significant importance in veterinary medicine and public health. Felids are considered a final host for the parasite and play an important role in the spreading of infection via contamination of the environment by oocysts. The study aimed to determine the seroprevalence of *T. gondii* and analyze risk factors associated with the infections in dogs. A total of 430 blood samples were collected from dogs from three governorates situated in northern Egypt and analyzed using commercial ELISA kit. The overall seroprevalence of *T. gondii* in dogs was 24%, and the Kafr ElSheikh governorate had the highest rate of seroprevalence at 29.3%. In addition, the seropositivity of *T. gondii* was higher in females (27.1%, 95 % CI: 22.28-32.42), dogs more 5 years age (32.6%, 95 % CI: 25.26-40.88), German Shepherd breed (31.5%, 95 % CI: 25.09-38.61), and in presence of rodents (31.5%, 95 % CI: 24.18-39.96). The multivariate logistic regression revealed that the sex, age, breed, and presence of rodents were potential risk factors for *T. gondii* infections in dogs. This study shows that *T. gondii* is a relevant parasite in Egypt, and more epidemiological surveys are needed to decrease the risk of human infection.

Keywords: *Toxoplasma gondii*, ELISA, Risk factors, Dogs, Egypt

[1]*Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa 31982, Saudi Arabia*
[2]*Department of Surgery, Faculty of Veterinary Medicine, Kafr El Sheikh University, Kafr El Sheikh, Egypt.*
[3]*Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia*
[4]*Department of Clinical Microbiology and Immunology, Faculty of Medicine, King AbdulAziz University, Jeddah, Saudi Arabia*
[5]*Department of Microbiology, College of Veterinary Medicine, King Faisal University, Al-Asha, P.O. Box 400, 31982, Saudi Arabia*
[6] *Special Infectious Agents Unit, King Fahad Medical Research Center, King AbdulAziz University, Jeddah, Saudi Arabia*
[7]*Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, 12613, Cairo, Egypt*
[8]*Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt*
***Correspondence**: Abdelfattah.selim@fvtm.bu.edu.eg, mmarzok@kfu.edu.sa* (A. Selim, M. Marzok)
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Introduction

Toxoplasma gondii, an intracellular obligate protozoan, is worldwide distributed and causes toxoplasmosis in all warm-blooded animals, including dogs and humans (Dubey 1987). The Centers for Disease Control (CDC) estimates that around one-third of people on the planet are infected with this parasite (Molan *et al*., 2019).

The toxoplasma life cycle is quite intricate. *T. gondii* is a protozoan that forms cysts and is present in tissue. The life cycle of *T. gondii* consists of two stages: asexual and sexual. The sexual phase occurs in the intestines of definitive hosts (felids), whereas the asexual phase occurs in the tissues of intermediate hosts (Cenci-Goga *et al*., 2011). The life cycle includes three infectious stages: bradyzoites, tachyzoites encased in tissue cysts, and sporozoites encapsulated in sporulated oocysts. *T. gondii* may be spread by different ways, including sexual transmission in a definite host and asexual transmission in intermediate hosts by meat-eating and vertical transmission (Dubey *et al*., 2020; Smith *et al*., 2021).

Human get *T. gondii* via eating undercooked or raw meat of lamb or pigs; however, environmental infection—for example, via oocysts in water, soil, or raw vegetables—may also contribute to the spread of the infection (Almeria and Dubey 2021; Kakakhel *et al*., 2021).

Clinical toxoplasmosis in dogs is uncommon and typically associated with immunosuppression (Calero-Bernal and Gennari 2019). Some dogs were reported to have neuromuscular problems caused by *T. gondii* (Migliore *et al*., 2017; Patitucci *et al*., 1997). Furthermore, it has been reported that toxoplasmosis can cause severe and fatal respiratory distress in immunocompromised dogs (Pepper *et al*., 2019). *T. gondii* infection is widespread; however, there have been few investigations into *T. gondii* in pets. The seroprevalence rates of *T. gondii* in dogs ranged from 28% to 98.0% (El Behairy *et al*., 2013; Khaled *et al*., 1982).

The diagnosis of toxoplasmosis infection is performed by isolation of the parasite from a sample of blood or feces, serological analysis, or using polymerase chain reaction (PCR) to identify specific DNA (Khan and Noordin, 2020; Reisberg *et al*., 2013; Selim *et al*., 2019; Selim *et al*., 2013; Uddin *et al*., 2021). Serological methods such as the enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT), or other agglutination tests are often used to detect toxoplasmosis in humans and animals (Abbas *et al*., 2020; Ybañez *et al*., 2020).

Furthermore, data on *T. gondii* seroprevalence in dogs is still lacking for many Egyptian locations, and analysis of related risk factors for infection has yet to be done. Thus, the present study determined the seroprevalence of *T. gondii* in dogs from several governorates in Egypt. In addition, risk factors associated with the disease in dogs were also evaluated.

Materials and Methods

Ethical statement: This study was carried out in accordance with the regular rules and protocols established by the ethical committee of the Faculty of Veterinary Medicine at Benha University, Egypt. Furthermore, the protocol and procedures of the study were approved by the ethical committee of the Faculty of Veterinary Medicine, Benha University. Blood samples were obtained by highly experienced veterinarians after the animal owners verbally agreed to participate in the study. Throughout the investigation, the ARRIVE criteria were followed.

Study areas: The samples were collected from animals living in three governorates located in Northern Egypt from January to December 2022. These governorates include Kafr ElSheikh, Gharbia, and Giza, as shown in Figure 1.

Kafr ElSheikh Governorate is recognized for its desert climate, with an average annual temperature of 24°C and negligible rainfall throughout the year. Gharbia has a subtropical desert climate (Classification: BWh), which is located at an elevation of 8.49 meters above sea level, and the annual temperature in the city is 24°C. The summer in Giza is long, humid, hot, arid, and clear, while the winter is cold, dry, and mainly clear, and the temperature ranges between 25-45°C throughout the year.

Sample size and sampling: The following formula was used to calculate the required sample size (Thrusfield 2018).

$$n = Z^2 P(1 - P)/d^2$$

Where n is the sample size, Z is the confidence level, P is the previously reported prevalence rate (23.3%) for *T. gondii* in dogs in Egypt by Salama *et al*. (2022), and d is the accuracy. A total of 430 blood samples were collected from the cephalic veins of randomly selected dogs using vacutainer tubes. The samples were centrifuged at 3500 rpm for 10 to separate sera and kept at 20°C till serological analysis.

Questionnaire: Data of examined animals were collected during sampling from owners *via* a previously prepared questionnaire. Animal characteristics such as age (>2, 2-5, >5 years), breed (German Shepherd, Rottweiler, and Pitbull), sex (male and female), area (rural and urban), and presence of rodents in the house were all gathered.

Serological analysis: Dog serum samples were analyzed for anti-*T. gondii* antibodies using commercial ELISA kits (ID Screen® Toxoplasmosis Indirect Multi-species, IDVet, Grables, France). The kits included both positive and negative control sera, and the test was carried out in accordance with the manufacturer's guidelines. The ELISA reader (AMR 100, AllSheng, China) was used to measure the optical density (OD) of the findings at 450 nm.

The Toxoplasmosis kit uses a peroxidase-conjugated anti-multi-species secondary antibody to identify particular immunoglobulin G (IgG) antibodies against the P30 *T. gondii* protein. The following formula was used to determine the percentage of sample (S) to positive (P) ratio (S/P%):

$$S/P\% = \text{OD sample - OD negative control/ OD positive control - OD negative control} \times 100$$

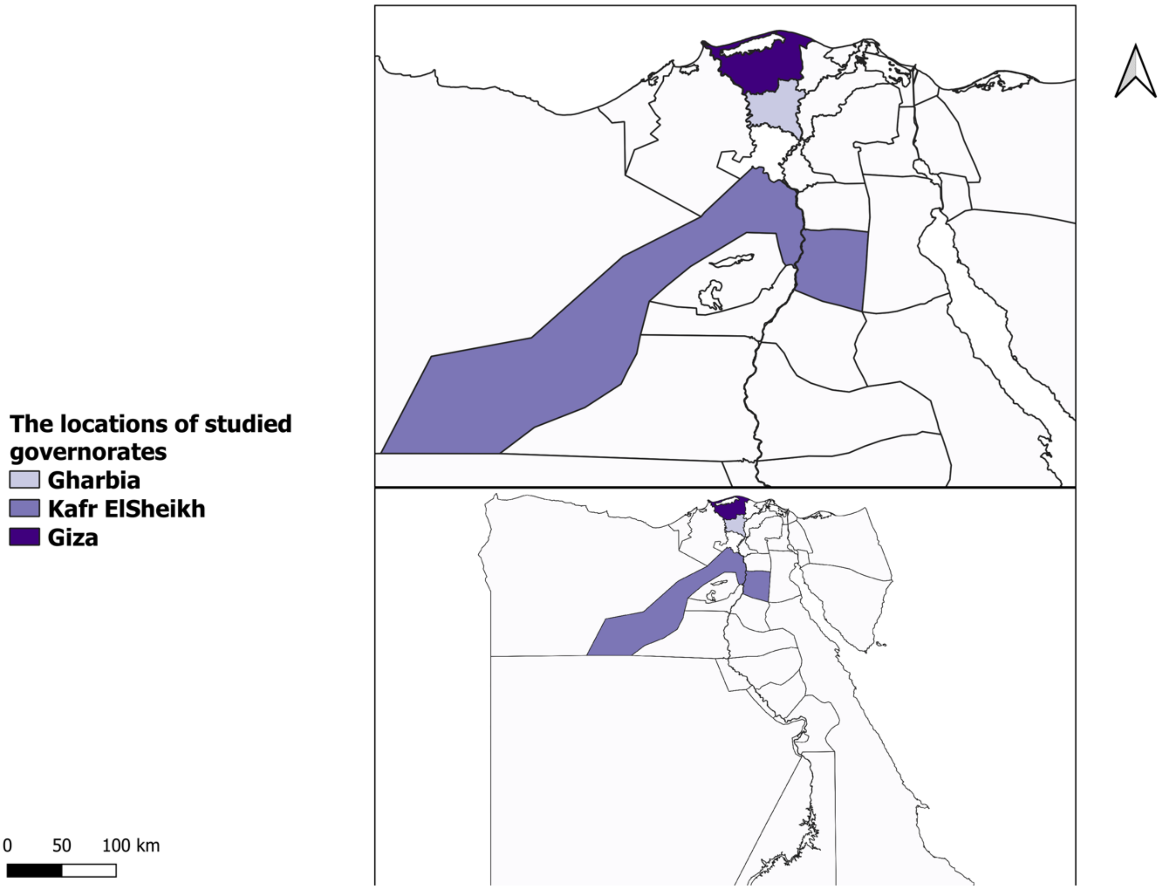

Figure 1 The locations of studied governorates (MAP generated using QGiS software)

Table 1 Seroprevalence of *T. gondii* in dogs in relation to different factors

Variable	Total examined animal	No of positive	No of negative	% of positive	95%CI	Statistic
Locality						
Kafr ElSheikh	150	44	106	29.3	22.63-37.06	$\chi2$=5.248 df=2 *P*=0.073
Gharbia	140	25	115	17.9	12.4-25.04	
Giza	140	34	106	24.3	17.94-32.02	
Sex						
Male	138	24	114	17.4	11.97-24.57	$\chi2$=4.804 df=1 *P*=0.028*
Female	292	79	213	27.1	22.28-32.42	
Age			0			
<2	110	16	94	14.5	9.16-22.33	$\chi2$=10.927 df=2 *P*=0.004*
2-5	185	43	142	23.2	17.74-29.83	
>5	135	44	91	32.6	25.26-40.88	
Breed						
German Shepherd	178	56	122	31.5	25.09-38.61	$\chi2$=11.073 df=2 *P*=0.004*
Rottweiler	132	29	103	22.0	15.75-29.77	
Pitbull	120	18	102	15.0	9.7-22.47	
Area						
rural	180	30	150	16.7	11.93-22.8	$\chi2$=9.025 df=1 *P*=0.003*
Urban	250	73	177	29.2	23.91-35.12	
Presence of rodent						
Yes	130	41	89	31.5	24.18-39.96	$\chi2$=5.885 df=1 *P*=0.015*
No	300	62	238	20.7	16.47-25.61	
Total	430	103	327	24.0	20.16-28.2	

*Results considered significant if the *P* value is less than 0.05

Table 2 Multivariate analysis for risk factors associated with *T. gondii* infection in dogs

Variables	B	S.E.	OR	95% CI for OR	*P* value
Sex					
Female	0.549	0.270	1.73	1.02-2.94	0.042
Age					
2-5	0.434	0.332	1.54	0.81-2.96	0.192
>5	0.844	0.339	2.33	1.19-4.52	0.013
Breed					
German Shepherd	0.751	0.314	2.12	1.15-3.92	0.017
Rottweiler	0.291	0.342	1.34	0.68-2.61	0.394
Area					
Urban	0.694	0.251	2.00	1.22-3.27	0.006
Presence of rodent					
Yes	0.497	0.249	1.64	1.01-2.68	0.046

B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval

According to the manufacturer, samples with S/P% values more than 50% were regarded as positive, those between 40 and 50% were regarded as dubious, and measures less than or equal to 40% were regarded as negative.

Statistical analysis: Using the EpiInfo software version 6.0, the prevalence was calculated as the ratio of positive findings to the total number of dogs investigated, with an accurate binomial 95% confidence interval. The correlation between seroprevalence of antibodies against *T. gondii* in dogs and related risk factors was assessed using logistic regression in two steps: univariate and multivariate analysis. The variables that showed $P < 0.25$ in the univariate analysis according to the Chi-square test were chosen and subjected to multivariate analysis (Selim *et al.*, 2022; Selim *et al.*, 2021c; Selim *et al.*, 2021d). The results are considered significant if the *P* value is less than 0.05. The statistical analysis was performed using SPSS ver 24 (IBM, USA).

Result

In the three governorates under study, the overall seroprevalence of *T. gondii* in dogs was 24%, with no significant variation between the governorates. The seroprevalence was higher in Kafer ElSheikh (29.3%) than in Giza (24.3%) and Gharbia (17.9%), Table 1.

The results of the univariate analysis showed a significant association (P<0.05) between seroprevalence of *T. gondii* and sex, age, breed, area, and presence of rodents. Females (27.1%, 95 % CI: 22.28-32.42) and German Shepherd breed (31.5 %, 95 % CI: 25.09-38.61) had a significantly higher seroprevalence of *T. gondii*. Furthermore, dogs raised in urban areas or in close contact with rodents had a higher *T. gondii* seroprevalence of 29.2% and 31.5%, respectively.

The results of the multivariate logistic regression model revealed that the following factors were found to be associated with an increased risk of *T. gondii* infection in dogs: female (OR=1.73, 95 % CI: 1.02-2.94), dogs older than 5 years (OR=2.33, 95 % CI: 1.19-4.52), German Shepherd breed (OR=2.12, 95 % CI: 1.15-3.92), raising dogs in an urban area (OR=2, 95 % CI: 1.22-3.27), and contact with rodents (OR=1.64, 95 % CI: 1.01-2.68), Table 2.

Discussion

The prevalence study of *T. gondii* in felids is always very necessary to reduce oocyst adulteration in the environment and to transmit infections to humans (Marzok *et al.*, 2023; Selim *et al.*, 2023a; Selim *et al.*, 2023b). Serological detection is a reliable and accurate approach to identifying *T. gondii* infection in dogs because clinical could not be a specific way to identify the infection. *T. gondii* infection may be diagnosed by examining various tissues; however, this requires the animals to be dead (Ali *et al.*, 2003; Bjö̈rkman *et al.*, 1994; Dubey *et al.*, 2020). In the present study, a serosurvey for *T. gondii* infection in dogs was conducted to determine the seroprevalence of antibodies against *T. gondii* and to assess the associated risk factors.

The seroprevalence in this study was non significantly varied between studied governorates, and the overall seroprevalence of *T. gondii* in dogs was 24%, which comes in agreement with the previously reported rate of 23.3% in Egypt by Salama *et al.* (2022). The seroprevalence rate of this study was higher than that reported in Iran (8.94%) (Hosseininejad and Hosseini 2011) and Taiwan (7.9%) (Lin 1998). In other studies, the higher seroprevalence for *T. gondii* in dogs was observed, it was 45.1% in Brazil (Azevedo *et al.*, 2005), 30% in Sweden (Bjö̈rkman *et al.*, 1994), 51.3% in Turkey (Oncel *et al.*, 2007), 67% in Senegal (Ahmad *et al.*, 2001), 39% in Lahore (Shahzad *et al.*, 2006) and 50% in Vietnam (Dubey *et al.*, 2007).

It is possible to attribute this variation in seroprevalence between countries to environmental and geographical factors, serological tests used, the diversity of cultures and behaviors, socioeconomic conditions, and sanitary conditions, dog populations, as well as parasite intrinsic factors like genetic diversity (Liu *et al.*, 2012; Marques *et al.*, 2019; Rodrigues *et al.*, 2016; Selim and Abdelhady 2020; Selim *et al.*, 2021a; Selim *et al.*, 2021e).

Contrary to the findings of Azevedo *et al.* (2005), sex was significantly associated with *T. gondii* seropositivity in dogs, and it was higher in females than males. The current results agreed with the findings of Arunvipas *et al.* (2013) but differed from those of other studies (Ali *et al.*, 2003; Cañón-Franco *et al.*, 2004). In contrast, Salama *et al.* (2022) found that males, especially the German Shepherd breed, had higher seropositivity for *T. gondii* when compared to females and other breeds.

The high seroprevalence seen in dogs older than five years may be explained by the increased chance of parasite interaction with age (Ahmad *et al.,* 2014). Elderly dogs showed higher seroprevalence, which comes in agreement with previously reported findings by Wu *et al.* (2011) and Pena *et al.* (2006). Adult dogs are more likely to be exposed to *T. gondii* oocysts due to their larger wandering behavior and potential interaction with contaminated surroundings, waste, and leftover meals (Selim *et al.,* 2021b; Watanabe *et al.,* 2020).

The current study revealed that the breed was substantially associated with *T. gondii* seropositivity, and the German Shepherd is the most vulnerable breed, in contrast to the findings of Raimundo *et al.* (2015), Yan *et al.* (2012), and Azevedo *et al.* (2005).

Based on present findings, *T. gondii* seropositivity increased significantly among urban dogs than rural ones, which comes in accordance with Saldanha-Elias *et al.* (2019) and Watanabe *et al.* (2020). The risk of *T. gondii* may be increased by various urban-specific characteristics such as a high cat population, human activity resulting in more garbage cans on the street, and exposure to undercooked meat. (Fábrega *et al.,* 2020).

Notably and consistent with our results, prior research revealed a positive association between toxoplasmosis and the presence of rodents (Silva *et al.,* 2010). This might be explained by the fact that rodents are nocturnal creatures that may not have been seen despite being present in the home and drawing in cats.

In conclusion, *T. gondii* infection exists in dogs, according to the assessment of serological data and their association with the examined epidemiological factors. The results of this study revealed that sex, age, breed, area, and presence of rodents are significant risks for *T. gondii* infection. This study also highlights the necessity of looking at dogs' involvement in toxoplasmosis cases as part of public health monitoring initiatives due to the shared sources of infection between people and dogs.

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