



Prevalence and Risk Factors for *Toxoplasma gondii* Infection at the Cat-Human Interface in Gharbia Governorate, Nile Delta, Egypt



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THIS study aimed to estimate the prevalence of *Toxoplasma gondii* oocyst (Tgo) shedding among cats and *T. gondii* recent infection among pregnant women in Gharbia Governorate, Nile Delta, Egypt, to assess *T. gondii* infection at the cat-human interface. Copro-PCR, targeting the B1 gene, showed that 22.7% (384/1,694) of the examined cats were shedding Tgo. The prevalence rates among stray and pet cats were 27.8%, and 6.8%, respectively; stray cats were 4.08 times more likely to shed Tgo than pet cats. Among stray cats, the odds of Tgo shedding were higher in feral cats feeding on wet market garbage than in semiferal cats that received daily meals from some households in the region (odds ratio (OR) 32.4; $P < 0.001$). For pet cats, access to kitchens and outdoors were risk factors for Tg infection (OR: 4.9–12.1; $P < 0.001 - 0.046$), whereas routine checkups at vets were a protective practice (OR: 0.3; $P 0.03$). The seroprevalence of *T. gondii* recent infection (positive for anti-*T. gondii* IgM) among pregnant women was 8.8% (35/400). Only 47.9% of pet owners knew that cats could transmit zoonotic pathogens to humans, whereas 35.7% had heard about toxoplasmosis. Pet owners who did not wear gloves while removing cat excreta and those with actively infected cats were 8.4 and 18.7 times more at risk of contracting *T. gondii* infection than other pet owners ($P < 0.05$). Garbage hygienic disposal at wet markets, stray cat sheltering and health education for pet owners are compelling measures for preventing toxoplasmosis in Egypt.

Key words: *Toxoplasma gondii*; Oocyst shedding; Stray cat; Pet cat; Pregnant women.

Introduction

Toxoplasma gondii is a zoonotic, cat-borne protozoon that infects approximately one-third of the world's human population [1]. This parasite is the main cause of multiple abnormalities and complications related to humans' and animals' reproductive and nervous systems. In humans, infections during pregnancy and diseases in immune-compromised patients can be severe [2, 3]. Indeed, congenital infection can affect fetal development leading to abortion and neonatal death, or it may cause neurological effects, such as blindness, deafness, or mental retardation.

Cats play a major role in transmitting of *T. gondii* as they shed millions of oocysts that can persist for

years in the environment (e.g., in soil, water, or food) [4, 5]. A single oocyst can cause infection in intermediate host animals [6]; thus, oocysts in the environment are a major public health risk [5]. The global prevalence of *T. gondii* oocyst shedding in domestic cat feces ranged from 0.1% - 23%; the lowest prevalence rates (0.1% - 2.2%) were recorded in Europe, while the highest rates (18.8% - 23%) were recorded in Africa [7, 8].

There are several modes of *T. gondii* transmission to humans, including; ingestion of *T. gondii* tissue cysts in the undercooked meat of several animals; hand-to-mouth transfer of oocysts by contact with a sandbox or soil contaminated by sporulated oocysts; ingestion of oocyst through contaminated water, or food; and congenital transmission from mother to

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fetus when infection occurs around conception, during pregnancy, and if the mother has previous infection that reactivates [9, 10, 11].

Cats are extremely popular animals in Egypt. The ancient Egyptians were one of the first nations to domesticate cats, which they cherished as pets. Although there is no official report on the number of cats in Egypt, unofficial animal welfare organizations have declared that approximately 5 million pet cats are being raised in Egyptian households and more than 100 million cats may be strays. Given the popularity and high number of cats in Egypt, the human potential–cat interactions are high, which consequently increases the risk of cat-borne zoonotic disease transmission, especially of toxoplasmosis. Accordingly, several reports have shown that the seroprevalence among humans and cats in Egypt is high: among humans, seroprevalence is 23.8%–82% [12, 13, 14]; among cats, seroprevalence is up to 97.4% in strays [15] and up to 38.7% in pets [16]. Such high numbers of free-roaming seropositive stray cats in Egyptian villages and cities may be responsible for high rates of *T. gondii* oocyst contamination in the environment (excreted in cat feces), which in turn may be associated with high rates of infection among humans and other animals [14, 15].

Despite several reports highlighting the exceptionally high rates of seropositive cats in Egypt, data on actively infected and oocyst-shedding cats remains limited. Therefore, the current study was aimed at estimating the prevalence of *T. gondii* oocyst shedding in cats (strays and pets) and the prevalence

of recent *T. gondii* infection in pregnant women in the mid-delta region of Egypt. Cat owners were surveyed for their knowledge of toxoplasmosis and toxoplasmosis risk practices, and the risk factors associated with *T. gondii* infection in cats and owners were assessed.

Materials and Methods

Study area and design

The study was conducted in the center of the Nile Delta region, i.e., in Gharbia Governorate, which has eight districts (Figure 1). The governorate, which is the 10th largest in Egypt (1,942 km²), is located 90 km north of Cairo City. The total human population in the governorate is approximately 5 million [17].

Cats included in the present study were divided into three major categories based on the classification of [18] with some modifications: stray cats, subdivided into feral and semiferal, and pet (house) cats. Feral cats were defined as those that are homeless, usually roam freely with minimum or no social contact with humans, and typically eat from rubbish bins, garbage areas, food waste, wet markets, and shops. Semiferal cats were defined as those that also live on the street but are supplied with routine (homemade or commercial) meals from some shop owners or households; they have more social contact with humans (meal providers) than feral cats and usually stay nearby their feeding locations for most of the day. Pet cats are defined as those raised in their owners' homes that are fed on either homemade food or manufactured cat foods with limited or no access to the outside.

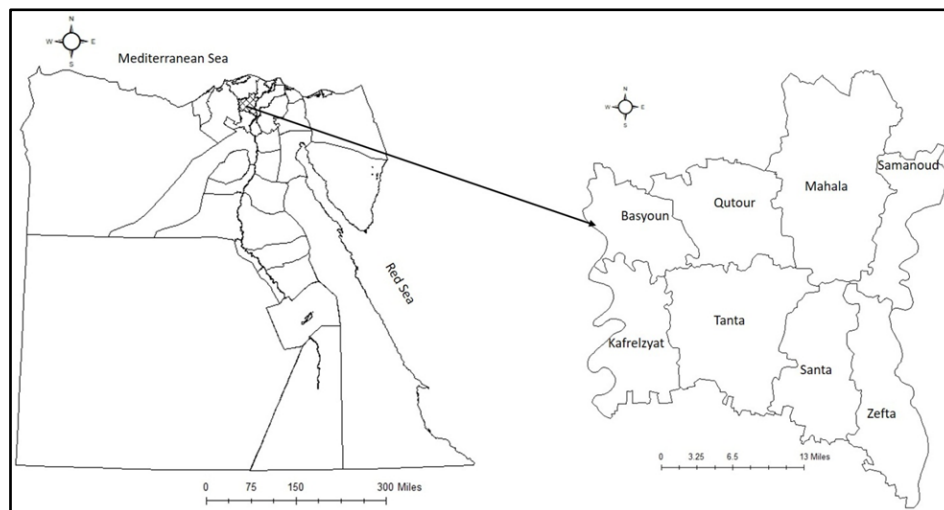


Fig. 1. Map of Egypt showing the study area; Gharbia governorate

Sample size estimation

Sample size estimation in cats

A cross-sectional study was conducted in the eight districts of Gharbia Governorate (Tanta, Zefta, Mahala, Santa, Qoutour, Kafrelzyat, Samanoud and Basyoun) (Figure 1) to estimate the prevalence of

active infection with *T. gondii* among the cat population. The minimum sample size was calculated for an expected prevalence of 50% (as there was no prior information on the prevalence of cats in this region), 95% confidence intervals (CIs), and an accepted error of 5% using Win Episcope 2.0 (Ignacio de Blas. Facultad de Veterinaria,

Universidad de Zaragoza ©2006) for each cat category; pet, feral, or semiferal cats. The required samples were estimated at 385 cats for each category (Total = 1155 cats). The samples size was inflated to 1,694 cats: 640 feral, 640 semiferal, and 414 pet cats. Cat samples were distributed equally among the eight districts, and they were collected in the period from June to August, 2018.

Sample size estimation in pregnant women

The target population was pregnant women attending private laboratories for a routine toxoplasmosis examination during pregnancy. The sample size was estimated at 385 pregnant women according to an expected 50% exposure rate, 95% CI, and an accepted error of 5% using Win Episcope 2.0 (Ignacio de Blas. Facultad de Veterinaria, Universidad de Zaragoza ©2006). This sample number was increased to 400 and divided equally between the eight districts (50 per district). Samples were collected from June to August, 2018.

*Detection of *T. gondii* oocyst shedding in cats' feces*

Toxoplasma gondii oocyst shedding in cats' feces was diagnosed by microscopic detection of *T. gondii* oocysts and molecular detection of *T. gondii* DNA in cat feces samples using Copro-microscopy and Copro-PCR tests, respectively.

Collection of cat fecal samples

Cat fecal samples were collected from different localities in Gharbia Governorate from June to August 2018. Wet markets were chosen for the collection of feral cat samples, as these locations are the typical feeding and defecation sites of the cats. Semiferal cats were chosen according to the location of their meal providers (i.e., shops or households). Pet cat fecal samples were collected from pet shops and volunteer households. For fecal sample collection, stray cats were observed depositing and burying their feces in separate holes in sandy spots. The deposited fecal samples were then collected in polyethylene bags, sealed and labeled. Fecal samples of pet cats were directly collected from sandboxes at their owners' houses. Pet owners were instructed to clean the sandbox and to use fresh sand before sampling (under the supervision of one of the authors), to observe the tested cat, and to collect the fresh fecal sample once the cat leaves the sandbox. All samples were transferred to the laboratory of the Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, for analysis.

Copro-microscopy test

The *T. gondii* oocysts were recovered from cat fecal samples using the sugar flotation technique

previously described by [19]. Briefly, approximately 2 g of cat feces was mixed with 8 mL of Sheather's sugar flotation solution (specific gravity: 1.27). The mixture was filtered through gauze into 15-mL tubes and centrifuged at 1,000g for 10 min. A drop from the top of the sugar float was examined at 100× and 400× magnifications, and evidence of *T. gondii*-like oocysts (9–12 µm in size) was recorded.

Copro-PCR test

Oocyst DNA extraction: In total, 2 mL of the float in the previous step was mixed with 8 mL of distilled water, and the mixture was centrifuged at 2,000g for 10 min. The supernatant was discarded, and the final purified oocyst pellet was mixed with 200 µL of ASL buffer from a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The oocyst walls were disrupted by 5 cycles of freezing (–80°C)/thawing (65°C), and then another 1.2 mL of ASL buffer was added before DNA was extracted according to kit instructions with one modification, i.e., digestion by proteinase K was completed at 60°C for 1 h [19]. Finally, 100 µL of DNA elution was stored at –20°C.

Nested PCR: *T. gondii* was detected using nested PCR using 4 primers (two outer and two inner) targeting the B1 gene [20]. The nested PCR cycling conditions were conducted as previously described [21]. The first PCR mix contained 5 µL of DNA template (~50 ng), 12.5 µL of EmeraldAmp MAX PCR Master Mix (Takara Bio, Kusatsu, Japan), 1 µL (20 pmol) of forward primer (TOXO1: 5'-GGAAGTGCATCCGTTTCATGAG-3'; position 694 to 714), 1 µL (20 pmol) of reverse primer (TOXO2: 5'-TCTTTAAAGCGTTCGTGGTC-3'; position 887 to 868), and water to a final reaction volume of 25 µL. PCR cycling was conducted in an Applied Biosystem 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA) with initial denaturation at 94°C for 7 min followed by 35 cycles of 94°C for 1 min, 60°C for 30 s, and 72°C for 30 s, and then a final extension at 72°C for 10 min. Subsequently, 1 µL of first PCR product was used as a template for the second PCR reaction using the same cycling conditions and the two inner primers. The primers used for the second PCR reaction were the reverse primer (TOXO3: 5'-GGCGACCAATCTGCGAATACACC-3'; position 853 to 831) and the forward primer (TOXO4: 5'-TGCATAGGTTGCAGTCACTG-3'; position 757 to 776). For each PCR reaction, the DNA of *T. gondii* (RH strain) was used as positive control and sterile distilled water was used as negative control. The second PCR products (96 bp) were electrophoresed in 2% agarose gel and then visualized with an Alpha Imager (Alpha Innotech, San Leandro, CA, USA) (Figure 2).

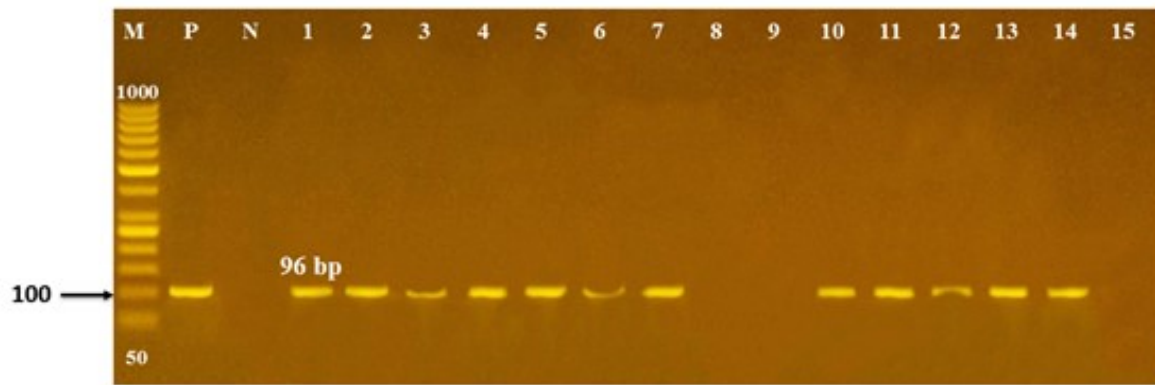


Fig. 2. The result of second PCR reaction in nested PCR for B1 gene used for confirmation of *Toxoplasma gondii* in cat fecal samples.

M: Marker. P: Positive control. N: Negative control. Lanes 1-9: stray cat fecal samples. Lanes 10-15: Pet cat fecal samples. Positive samples: Lanes 1-7, and 10-14 (96 bp target PCR product). Negative samples: Lanes 8, 9, and 15.

Detection of recent T. gondii infection in pregnant women

Collection of sera samples from pregnant women

Blood samples (5 mL per case) were collected from pregnant women by medical staff after the women provided informed consent. The collected samples were centrifuged at 3,000 rpm for 5 min to separate the sera, which were then stored at -20°C until use.

Serological test

Serological confirmation of recent *T. gondii* infection among pregnant women was achieved by detecting *T. gondii* IgM antibodies using SERION ELISA classic (Virion\Serion, Würzburg, Germany). Sera were considered positive at >350 U/mL; using this positive value results in a specificity of 98.4% and a sensitivity of 97.7% (data supplied by the kit manufacturer). Furthermore, all IgM-positive sera were tested for IgG avidity (Virion/Serion). Cases with an IgG avidity index of $<40\%$ were considered recent *T. gondii* infection.

Epidemiological investigation

Prevalence estimation

The apparent prevalence of PCR confirmed *T. gondii* oocyst shedding among examined cats was estimated as follows: apparent prevalence = (number of positive animals / number of examined animals) \times 100. Significant differences in the prevalence of *T. gondii* oocyst shedding (at $P < 0.05$) between stray and pet cats and between feral and semiferal cats were identified using univariate logistic regression analysis.

Knowledge and practices of pet owners

Data on the knowledge and practices of pet cat owners toward toxoplasmosis were collected using a

prepiloted questionnaire completed during pet cat sample collection. The questionnaire contained closed questions on the knowledge of toxoplasmosis and its health outcomes in humans. Data on practices that could prevent or increase the risk of infection to cats or their owners were collected through questions on hygienic measurements undertaken to avoid infection or disease transmission, the nature of foodstuffs presented to cats, and the potential access of cats to infection sources. In total, 140 respondents were interviewed.

Risk factors for T. gondii oocyst shedding in pet cats

The association between the pet owners' practices and their cat being shedding *T. gondii* oocysts was estimated using a multivariate logistic regression model. The initial step consisted of a univariate logistic regression for the detection of the association between some cat owners' high-risk practices and *T. gondii* oocyst shedding in their cats. The assumed risk practices included the number of cats per household, allowing the cat to enter the kitchen or bedrooms, allowing the cat to go outside, and routine veterinary checkups. Feeding raw meat (chicken, other meats, or fish) was not common among the interviewed cat owners; however, the majority had fed their cats a meal containing offal, tripe, or meat trimmings as follows: meat raw meal (trimmings consisting of small pieces of fat with portions of meat), chicken raw meal (offal, tripe, feet, and/or neck), and fish raw meal (gills, gut content, and/or whole small fish). Feeding with any of these raw meals was also included as a risk practice in the univariate analysis. The level of significant association was set at $P < 0.2$. All behaviors with $P < 0.2$ were included in the final multivariate model. Subsequently, the collinear association between these behaviors was identified by calculating the Phi correlation coefficient. If two or more behaviors with significant Phi correlation coefficients existed, the

factor considered “most biological” was retained in the final model. The final multivariate model was built with the variables that passed the last two steps, and only variables with $P < 0.05$ were retained using manual stepwise selection. All two-way interactions between risky behaviors in the model were assessed. Confounding factors associated with changes in the logit of other factors were identified and removed from the model.

Risk factors for T. gondii infection among pregnant women who own pet cats

In total, 95 participants (22 seropositive and 73 seronegative) were included in this analysis with inclusion criteria including participants who approved participation, those who were serologically tested for recent *T. gondii* infection in the study, and those who had owned a cat at home for at least 6 months. A multivariate model similar to the cats' logistic regression model described above was built to identify the association between seropositive cases of recent *T. gondii* infection in pet owners and their practiced risky behaviors recorded earlier. The behaviors examined included whether the cats had access to kitchens or bedrooms, and if the owners wore gloves during the removal of pet fecal matter.

Statistical analysis

All analyses were performed via SPSS statistics software version 21.0 (IBM SPSS Inc., Armonk, NY, USA) and SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). A choropleth map of Egypt showing the administrative boundaries of the governorates and the study area (Gharbia Governorate) was created using Quantum GIS (Quantum GIS Development Team 2017). The prevalence of *T. gondii* oocyst shedding among

different cat categories in different districts of the governorate was shown on the created map.

Ethical Approval

The study proposal and procedures were approved by the institutional Animal welfare, Hygiene and Zoonoses committee at Kafrelsheikh University, Egypt (KFS-2018/4-3). For pregnant women samples, the test purpose and procedures were explained for all participant and they gave informed consents before samples collection. The medical staff of private laboratories collected all sera samples from participants.

Results

Prevalence of T. gondii oocyst shedding in cats

Of the 1,694 cat fecal samples collected and examined, *T. gondii* oocysts were identified in 171 samples by Copro-microscopy (10.1%) and in 384 samples by Copro-PCR (22.7%) (Table 1). A total of 26 positive fecal samples by Copro-microscopy were negative in Copro-PCR (Table 1). The prevalence of PCR confirmed *T. gondii* oocyst shedding among stray cats was 27.8% (356/1,280), which included 52.3% (335/640) feral and 3.3% (21/640) semiferal cats (Table 1). The odds of *T. gondii* oocyst are found in feral cats in wet markets were 32.4 times greater than in semiferal cats (95% CI: 20.4–51.4; $P < 0.001$). The prevalence of PCR confirmed *T. gondii* oocyst shedding among pet cats was 6.8% (28/414), and the odds of shedding *T. gondii* oocysts were higher in stray cats than in pet cats (OR: 5.3; 95% CI: 3.6–7.9; $P < 0.001$). The prevalence of *T. gondii* oocyst shedding among cats in different districts among examined cat categories is shown in Figure 3.

TABLE 1. Prevalence of *Toxoplasma gondii* oocyst shedding in fecal samples of pet and stray cats in Gharbia government, Egypt.

Cat types	Sample No.	Oocyst detection		**Prevalence		Univariate model			
		Copro-Microscopy	*Copro-PCR	Pos.	%	OR	95% CI	P_{value}	
Stray	Feral	640	335	21	335	52.3	32.4	20.4 – 51.4	<0.001
	Semiferal	640	10	3	21	3.3	-	-	-
Stray _{Total}	1280	164	356	24	356	27.8	5.3	3.6 – 7.9	<0.001
Pet	414	7	28	2	28	6.8	-	-	-
Total	1694	171	384	26	384	22.7			

*Nested PCR: All samples that were positive in 1st step of nested PCR were also positive in 2nd step;

**Prevalence was estimated based on positive samples by Copro-PCR; OR: Odd ratio; CI: Confidence interval.

Prevalence of recent T. gondii infection in pregnant women

The overall seroprevalence of recent *T. gondii* infection among pregnant women in the study region

was 8.8% (35/400). The seroprevalence of recent *T. gondii* infection per district is shown in Figure 3.

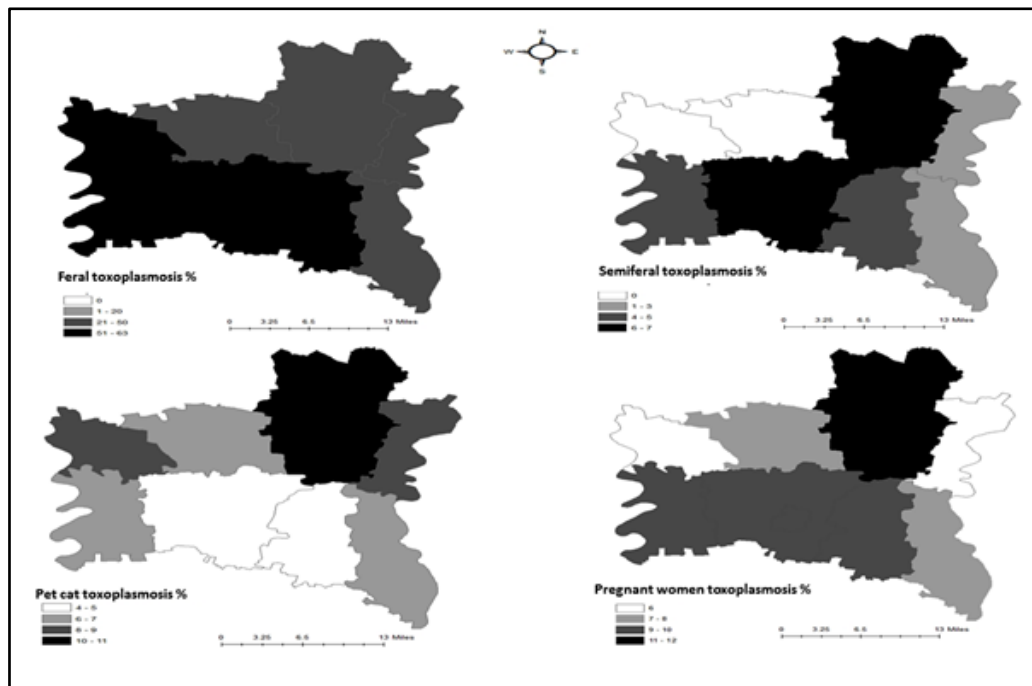


Fig. 3. The prevalence of *T. gondii* oocyst shedding among feral, semiferal and pet cat and among humans in different districts of Gharbia governorate, Egypt.

Knowledge and practices of pet owners regarding toxoplasmosis

Results from the completed questionnaires of 140 pet owners surveyed about their knowledge and practices related to toxoplasmosis are summarized in Table 2. Only 35.7% (50/140) of pet owners had heard about toxoplasmosis, whereas only 3.6% (5/140) declared that it affects pregnant women. Other respondents claimed that cats can transmit mange only (19.3%, 27/140), rabies only (12.1%,

17/140), or both mange and rabies (1.4%, 2/140) to humans. The majority of pet owners (60.7%–91.4%) presented meals including raw meat, chicken, or fish to their cats. Approximately 30% of pet owners did not wear protective gloves during cat feces removal. Cats belonging to 67.1% and 66.4% of respondents have free access to kitchens and bedrooms, respectively. Additionally, cats had free access to the outdoors in 34.4% of homes. Overall, 75 owners (53.6%) declared that they regularly visited veterinarians to check their cat's health.

TABLE 2. Knowledge and practices of cat owners towards toxoplasmosis at Gharbia governorate, Egypt

Topics	Yes (%)	No (%)
Feeding cats raw meat meal	85 (60.7)	55 (39.3)
Feeding cats raw chicken meal	107 (76.3)	33 (23.7)
Feeding cats raw fish meal	128 (91.4)	12 (8.6)
Cat use litter box for defecation	140 (100)	0 (0)
Wear gloves during cat feces removing	97 (69.7)	43 (30.7)
Cat access to kitchens	94 (67.1)	46 (32.9)
Cat access to bedrooms	93 (66.4)	47 (33.6)
Cat access to outdoor	48 (34.3)	92 (65.7)
Regular visit to the veterinarian for checkup	75 (53.6)	65 (46.4)
Can the cat transmit disease to human	67 (47.9)	73 (52.1)
Awareness of toxoplasmosis as a cat zoonotic disease	50 (35.7)	90 (64.3)

Risk factors identification

Risk factors for T. gondii oocyst shedding in pet cats

Table 3 shows the results of univariate logistic regression analyzing the association between cats being infected and shedding *T. gondii* oocysts and cat

owners' high-risk practices. Feeding cats with raw meat or fish meals and the number of cats per household were not significantly associated with *T. gondii* oocyst shedding ($P > 0.05$). Feeding cats raw chicken meals (OR: 0.2; $P < 0.01$) and regular visits to the vet (OR: 0.3; $P < 0.03$) were associated with

lower odds of *T. gondii* oocyst shedding. Allowing cats to go outside the home (OR: 5.9; $P < 0.001$) and giving them access to kitchens (OR: 13.2; $P < 0.02$) were significantly associated with a higher risk of contracting *T. gondii* infection. The later four variables were incorporated into the final multivariable logistic regression model, despite the collinearity found between some variables. Results of the multivariate logistic regression showed that the

risk of *T. gondii* oocyst shedding among cats that access the outdoors was 12.1 times greater than that among cats without outdoor access (95% CI: 3.7–39.3; $P < 0.001$). Additionally, cats with access to kitchens were 4.9 times more likely to shed oocysts than other cats (95% CI: 1.03–24.7; $P = 0.046$). Feeding raw chicken meals and a lack of regular visits to the vet were not significant variables in the multivariate regression model.

TABLE 3. Results of a univariate and multivariate models for the association between selected potential risk factors and positive status of cat shedding *T. gondii* oocysts

Variable	C.	Number		Univariate model			Multivariate model		
		Pos.	Neg.	OR	95% CI	P	OR	95% CI	P
Number of cats per household	1	15	63	2.1	0.6 – 7.7	0.3			
	2	4	29	1.2	0.2 – 5.8	0.8	EX		
	>2	3	26	-	-	-			
Feeding raw meat meal	Yes	12	73	0.5	0.3 – 1.9	0.7			
	No	10	45	-	-	-	EX		
Feeding raw chicken meal	Yes	10	97	0.2	0.1 – 0.5	0.01			
	No	12	21	-	-	-	NS		
Feeding raw fish meal	Yes	22	106	5.3	0.3 – 92.5	0.3			
	No	0	12	-	-	-	EX		
Regular visit to the veterinarian	Yes	7	68	0.3	0.1 – 0.9	0.03			
	No	15	50	-	-	-	NS		
Cat access to kitchen	Yes	20	74	5.9	1.3 – 26.8	0.02	4.9	1.03 – 24.7	0.046
	No	2	44	-	-	-	-	-	-
Cat access to outdoor	Yes	18	30	13.2	4.1– 42.1	<0.001	12.1	3.7 – 39.3	<0.001
	No	4	88	-	-	-	-	-	-

EX: Excluded in Multivariate model ($P > 0.2$ in univariate model); NS: Not significant at $P \leq 0.05$ in Multivariate model.

Risk factors for recent T. gondii infection in pregnant women who owned pet cats

Results of univariate logistic regression among humans showed that allowing cats' access to bedrooms was not a significant risk factor for human toxoplasmosis at $P < 0.05$ (Table 4). The risk of toxoplasmosis among pet owners who allowed cats to enter the kitchens was 26 times more than others (OR 95% CI: 1.55 – 456.36) and this was significant at $P < 0.02$. Pet owners who do not wear gloves during removal of cat fecal matter are at risk 11.2 times of getting toxoplasmosis more than others (95% CI: 3.6 – 34.9, $P < 0.001$). Having cat shedding *Toxoplasma* oocysts at home increases the odds of getting toxoplasmosis among pet owners by 24.9 times compared to others (95% CI: 6.7 – 93.1, $P <$

0.001) (Table 4). All of these tested variables were incorporated into the final multivariate model. The variable allowing cats to enter the kitchens was removed from the final model as it was a confounder to wearing gloves variable which was kept in the final model. Also, the variable allowing cats to enter the bedrooms was not significant at $P < 0.05$ and was removed from the final model. Pet owners who do not wear gloves during removal of cat fecal matter and those owners who have cat shedding *Toxoplasma* oocysts at home were at risk of getting *T. gondii* infection 8.4 and 18.7 times, respectively the risk among others who wear gloves and have no active infected cats with toxoplasmosis (95% CI: 2.3 – 31.1, $P < 0.001$ and 4.3 – 80.6, $P < 0.001$, respectively).

TABLE 4. Results of a univariate and multivariate logistic regression model for the association between selected potential risk factors and individual seropositive status for recent *T. gondii* infection in pet owners.

Variable	C.	Number		Univariate model			Multivariate model		
		Pos.	Neg.	OR	95% CI	P	OR	95% CI	P
Don't wear gloves during disposal of cat feces	Yes	17	17	11.2	3.6 – 34.9	<0.001	8.4	2.3 – 31.1	0.02
	No	5	56	-	-	-	-	-	-
Allowing cat access to Kitchen	Yes	22	46	26.6	1.55–456.4	0.02		C	
	No	0	27	-	-	-			
Allowing cat access to Bedroom	Yes	17	46	2	0.7 – 6.02	0.2		NS	
	No	5	27	-	-	-			
Having cat shedding <i>Toxoplasma</i> oocyst at home	Yes	13	4	24.9	6.7 – 93.1	<0.001	18.7	4.3 – 80.6	<0.001
	No	9	69	-	-	-	-	-	-

C: Excluded in Multivariate model as it was confounder to wear gloves variable; NS: Not significant at $P \leq 0.05$ in Multivariate model.

Discussion

The prevalence of *T. gondii* oocyst shedding in stray cats was 27.8%. This was comparable with other reports (19.4% - 21.4%) from Ethiopia [22], and Nigeria [23]; however, the prevalence in the current study was much higher than that previously reported (6% - 8.5%) from the USA [24] and Malaysia [25]. Among stray cats, feral cats in wet markets showed the highest rate of *T. gondii* oocyst shedding in the present study (52.3%). Wet markets in Egypt are sites at which feral cats commonly gather as they provide plentiful food from the rubbish of butchers, poulterers, or fish shops. The food available for feral cats in these markets may be contaminated with *T. gondii* tissue cysts because it usually includes remnants of beef carcasses, chickens, and fish. This could explain the high rate of infection among feral cats around wet markets. Additionally, infected cats shed millions of oocysts over 1-3 weeks and usually defecate in loose soil or sandy places near their food sources [4, 5]. Therefore, these infected cats likely caused high rates of contamination in the wet market environment; hence, the infection cycle would continue by affecting any new cats entering the contaminated zone. This may also explain the high and consistent rate of infection among feral cats. Similarly, studies in several countries have reported high rates of environmental (especially soil) contamination with *T. gondii* oocysts in places where stray cats are gathered and fed [5]. Notably, possible contamination of feral cat samples by the sand/soil of wet markets may have contributed to the high prevalence of *T. gondii* oocysts in feral cats. This possible samples contamination may be of minimum effect as we recorded both positive and negative cat samples in each sampling site and we tried to collect samples with minimum sand/soil amount. However, we can't

rule out the possible contamination of soil especially in potentially highly contaminated environments such as in wet markets. Future studies should consider testing samples of sand/soil near the collection spots to assess both the extent of environmental contamination by *T. gondii* oocysts in such high risk places and the contribution of soil contamination in prevalence estimation of *T. gondii* oocyst among feral cats.

This high rate of infection among cats in wet markets and consequent high load of environmental contamination are major risk factors for public health. Acquiring infection from oocysts in contaminated environments (e.g., from the soil, water, or raw vegetables) has previously been reported [4, 5]. People who work in these markets, live nearby, or regularly purchase food from them are therefore at higher risk of *T. gondii* infection; hence, they should receive continuous education on the burden of this public health threat and how to avoid infection using hand hygiene and food sanitation (Lilly and Wortham, 2013).

The odds of shedding *T. gondii* oocysts among feral stray cats were approximately 30 times higher than among semiferal stray cats ($P < 0.001$). Although semiferal cats are free-roaming, they were provided with daily meals by some households in the study area. Therefore, they usually socialized with and stayed near their meal providers. The meals of semiferal cats consisted mainly of homemade cooked food or commercial cat foods, which are expected to contain few, if any, *T. gondii* infectious agents. The difference in meals could explain the very low prevalence of *T. gondii* oocyst shedding (3.3%) in semiferal cats compared with feral cats that feed in wet markets. Consistent with our findings, lower seroprevalence rates for *T. gondii* were recorded in

stray cats roaming a set of condominiums in Barra da Tijuca district, Brazil, when they were provided daily with manufactured foods and clean drinking water [26]. Additionally, provision of such meals may discourage stray cats from seeking other high-risk food sources, e.g., live rodents or garbage. In summary, caring for stray cats by providing safe meals and/or shelter (in which they could also receive monitoring from vets) is not only good for cat welfare but also important for public safety as it helps to reduce the *T. gondii* oocyst burden in the environment.

The prevalence of *T. gondii* oocyst shedding among pet cats was 6.8%, which was higher than that reported in previous studies from the USA (1.3%) and Europe (0.6%) by [1] and [27], respectively. However, a much higher prevalence (41%) was recorded in Malaysia [28]. The relatively high prevalence of oocysts shedding among pets in the present study area may be attributable to the high-risk practices of cat owners, e.g., allowing cats to access the outdoors and kitchens. Both of these practices significantly increased the odds of *T. gondii* oocyst shedding among pet cats in a multivariate regression model. As previously reported, such practices expose pet cats to additional sources of infection via contaminated environments, hunting of rodents outdoors, or exposure to raw food and trash in kitchens [1, 4, 29]. Despite these risks, odds for *T. gondii* oocyst shedding in stray cats in the current study were significantly higher than pet cats. This may be due to some of the protective practices implemented by pet owners including the use of litter boxes (100% in the present study) with frequent removal of cat feces, which would lower the chance of cat exposure to sporulated oocysts. Furthermore, regular veterinary checkups by 53.6% of pet owners were shown to be a protective practice against *T. gondii* oocyst shedding in univariate analysis.

A recent global trend has emerged for feeding pet cats on raw meat and animal by-products in the belief that these items provide health benefits because they are rich in vitamins, minerals, and amino acids, such as taurine, which are required to maintain the health of cats [30]. However, it is still debatable whether these benefits supersede the risks of infection to pets and/or household members via these raw materials. All pet owners in the present study fed their cats with at least one type of raw animal meal (e.g., beef, chicken, or fish); however, regression analysis showed that raw meals were not significant risk factors for *T. gondii* oocyst shedding in pet cats. These findings were comparable with those from a study in Mexico [31], which found no association between raw meat meals and *T. gondii* infection in cats. In contrast, another study reported that feeding raw meat was a risk factor for *T. gondii* infection in cats [29]. Our finding could be related to *T. gondii* tissue cysts having a high affinity for nervous tissue

and skeletal muscle [32], whereas the raw meals provided for the pets studied here consisted mainly of fat, tendons, gut content, viscera, and tripe, which are expected to have less or no concentration of tissue cysts.

The seroprevalence of recent *T. gondii* infection among pregnant women in the study region was 8.8%. This was comparable with a report of 6.7% from Thailand [33], but higher than in other studies (2.8%–5.7%) from Egypt [12] and Iran [34].

The present results showed that a lack of knowledge exists among high numbers of pet owners (64.3%) about toxoplasmosis and its health consequences. Such a lack of knowledge was previously reported to be a risk factor among infected pregnant women [12]. Removal of cat fecal matter without wearing gloves increased the risk of *T. gondii* infection among pet owners 8.4-fold. Exposure to litter box material on bare hands may result in hand to mouth transmission of infectious sporulated oocysts, which explains the increased risk of infection among this group of respondents. In agreement, bare-hand exposure to soil or other environments contaminated with *T. gondii* oocysts has been reported as a risk factor for *T. gondii* infection among humans in previous studies [4, 12]. Additionally, persons who handle the contents of cat litter boxes without gloves may also spread the oocysts to ready-to-eat food via their contaminated hands posing a risk of infection transmission to himself and other family members. Allowing cats to access the kitchen may contaminate the kitchen environment, including food, with their contaminated fur or shedding hairs. Indeed, the role of contaminated fur in the transmission of *T. gondii* oocysts has previously been reported [35, 36] and may explain our findings.

Owning a cat shedding Toxoplasma oocysts showed the highest odds for Toxoplasmosis among pet owners in this study (OR 24.9 – 26.6, $P < 0.001$). In line with this finding, some studies reported contact with cats as a risk factor for Human Toxoplasmosis [33, 34].

Conclusion

The present study revealed a high prevalence rate for active shedding of *T. gondii* oocysts from cats in Gharbia, Egypt, especially from feral cats in wet markets. This highlights the high levels of environmental contamination by the protozoon in these markets and the increased risk it imposes on public health. In addition, providing safe food to semiferal cats remarkably decreases the odds of their infection. However, the inadequate knowledge and high-risk practices of pet owners contribute to the spread of infection to their pet cats, themselves, or other household members. Effective prevention of *T.*

gondii infection among humans and cats will require additional effort to improve sanitary measures at wet markets, to shelter or domesticate stray cats, and to increase public awareness campaigns about toxoplasmosis, especially for pet owners.

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Data Availability Statement

On request, the authors will provide all necessary data.

Author contributions

W.E., I.M. Y.H. Were responsible for Writing manuscript and statistical analysis, while collection of samples and field work and laboratory work were the responsibility of W.E. I.M, R.A.M. and W.F.E. All authors participate in the idea of the articles and revision of the manuscript.

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Conflict of interest

Authors declared that there is no conflict of interest

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مدى انتشار عدوى التوكسوبلازما جوندي وعوامل خطرها عند التفاعل بين القطط والإنسان في الغربية، دلتا النيل، مصر

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التوكسوبلازما هي واحدة من أكثر الأمراض المنقولة من القطط شيوعاً في جميع أنحاء العالم. هدفت الدراسة الحالية إلى تقدير انتشار إفرزات طفيليات التوكسوبلازما جوندي بين القطط والعدوى الحديثة بالتوكسوبلازما جوندي بين النساء الحوامل في محافظة الغربية بدلتا النيل في مصر، وذلك لتقييم معرفة وممارسات أصحاب الحيوانات الأليفة فيما يتعلق بالتوكسوبلازما، وأخيراً، لتحديد العوامل المرتبطة بالعدوى بالتوكسوبلازما جوندي بين القطط الأليفة وأصحابها. أظهرت نتائج الفحص بواسطة فحص البراز باستخدام تقنية البوليميراز المتسلسلة (Copro-PCR) والمستهدفة لجين B1 أن 22.7% (1,694/384) من القطط المفحوصة كانت تفرز طفيليات التوكسوبلازما جوندي. كانت معدلات الانتشار بين القطط الضالة والقطط الأليفة على التوالي 27.8% و 6.8%؛ وعلاوة على ذلك، كانت القطط الضالة عرضة بنسبة 5.3 مرات أكثر لإفرز طفيليات التوكسوبلازما جوندي من القطط الأليفة. ($P < 0.001$) بالنسبة للقطط الضالة، كانت فرص إفرز طفيليات التوكسوبلازما جوندي أعلى في قطط الشوارع التي تتغذى على القمامة في الأسواق الرطبة مقارنة بالقطط شبه الضالة أو غير الأليفة التي تتلقى وجبات يومية من بعض الأسر في المنطقة (نسبة الخطر 32.4 (OR)؛ $P < 0.001$). بالنسبة للقطط الأليفة، كان الوصول إلى المطابخ والخارج عوامل خطر للإصابة بالتوكسوبلازما جوندي (النسبة المئوية للخطر: 12.1-4.9؛ $P < 0.001 - 0.046$)، في حين كانت الفحوصات الروتينية لدى الأطباء البيطريين ممارسة وقائية (النسبة المئوية للخطر: 0.3؛ $P = 0.03$). بلغت نسبة الإصابة بطفيليات التوكسوبلازما جوندي (إيجابية لمضادات الأجسام الموجهة ضد التوكسوبلازما جوندي IgM) بين النساء الحوامل 8.8% (400/35). كانت نسبة المعرفة بين المالكين للحيوانات الأليفة حول إمكانية انتقال الأمراض المشتركة مع الحيوانات من القطط إلى البشر تبلغ 47.9% فقط، في حين سمع 35.7% عن التوكسوبلازما. كانت نسبة الأشخاص الذين لا يرتدون قفازات أثناء إزالة مخلفات القطط والذين يمتلكون قطة مصابة نشطة هي 8.4 و 18.7 مرة، على التوالي، أكثر عرضة للإصابة بالتوكسوبلازما جوندي مقارنة بباقي أصحاب الحيوانات الأليفة ($P < 0.05$). كانت هذه من أوائل الدراسات التي تقدر انتشار إفرزات طفيليات التوكسوبلازما جوندي بين القطط في الغربية، منطقة دلتا النيل في مصر. التلخص الصحي من القمامة في الأسواق الرطبة، وإيواء القطط الضالة، وتثقيف أصحاب الحيوانات الأليفة هي إجراءات قاطعة للوقاية من التوكسوبلازما في مصر.

الكلمات المفتاحية: التوكسوبلازما، تساقط البويضة، قطة ضالة، قطة أليفة، النساء الحوامل، مصر.