



## Sero-Prevalence and Risk Factors of *Toxoplasma Gondii* among Blood Donors in Menoufia Governorate, Egypt

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### ABSTRACT

**Background:** *Toxoplasma gondii* is one of the parasitic protozoa with worldwide distribution that affects warm-blooded lives. Toxoplasmosis transmission does not occur from one-to-another, with the exception for transplacental route, blood transfusion and organ transplantation. **Objectives:** To measure the occurrence rate of *Toxoplasma gondii* infection among healthy blood donors and highlight the associated risk factors among this important group. **Method:** Cross-sectional research was conducted in Menoufia University hospital blood transfusion center. Six hundred and thirty (630) blood donors were recruited in this study. They filled a self-administered questionnaire including personal data and suspected risk factors as well as routine investigation for blood donors and sero-logical tests for *Toxoplasma gondii* (IgG and IgM) were performed. **Results:** IgG sero-prevalence was 11.1%, IgM was 5.9%, both IgG and IgM positivity was 0.3% with total toxoplasmosis sero-prevalence 17%. Poor knowledge about toxoplasmosis transmission, dealing with cat, agricultural environment, eating ready to eat or undercooked meat, improper hand washing practices and AIDS infection were significantly associated with toxoplasmosis infection. Multivariable logistic regression analysis revealed that gender (AOR, 26.21; 95% CI: 7.77-88.45), poor knowledge (AOR, 15.39; 95% CI: 6.55-36.16), eating ready to eat meat (AOR, 50; 95% CI: 30-215), agricultural environment (AOR, 6.33; 95% CI: 3.07 – 13.05) and decreased frequency of hand washing (AOR, 30.66; 95% CI: 8.73-107.64) were independent risks for toxoplasmosis. **Conclusion:** *Toxoplasma gondii* positivity is prevalent among healthy blood donors. This finding underscores the importance of screening of toxoplasmosis among them and favours health education to improve the public knowledge about toxoplasmosis for proper prevention of infection.

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### INTRODUCTION

*Toxoplasma gondii* is one of the parasitic protozoa with worldwide occurrence that affects warm-blooded lives. About one third (33%) of the world population is recorded to be infected with it.<sup>1</sup> The global sero-prevalence has a great variation between nations (10–80%) and even in the same nation.<sup>2</sup> *Toxoplasma gondii* transmission starts usually by swallowing of cysts present in ready to eat infected

meat, also ingestion of adult oocysts from cat feces in food or water. Human to human transmission doesn't occur except in two routes: vertically from mother to fetus causing devastating outcome. The other route of transmission is through organ transplantation and blood donation, which encouraged researchers to check effect of *Toxoplasma gondii* infection on blood safety.<sup>3</sup>

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Clinical picture of Toxoplasmosis varies from severe manifestations and complications in babies and individuals with immunocompromised state to subclinical and self-limited in immunocompetent individuals.<sup>1</sup> To diagnose a case of toxoplasmosis we cannot depend only on clinical state but also confirmation by parasitological and sero-logical investigations are essential.<sup>4</sup> Many serum-based tests which find out specialized antibodies (IgG and IgM) in the serum of the patient against *Toxoplasma gondii* can be used effectively, one of those assays, ELISA which shows a high level of sensitivity and specificity;<sup>5</sup> IgM denotes for recent infection while IgG for old infection they may be present both if recent on top of old infection has occurred, taking in consideration that these antibodies might not be discovered in the recent infection, because they are not produced till after some weeks of parasitaemia. This is essential in some conditions like immunosuppressed individuals whose titre of these antibodies might not be detectable.<sup>6</sup>

The difference in the spread of *Toxoplasma gondii* immunoglobulins declared in previous laboratory investigations of blood donors<sup>7</sup> describes the obvious geographic impact on the different patterns of spread between countries. In Egypt, it was reported that sero-positivity of *Toxoplasma gondii* among the public ranged between 30 to 60%,<sup>8</sup> but those focused little on blood donors.

A previous study conducted on blood donors in the University Hospitals of Ain Shams, Egypt in 1986 detected that IgG sero-positivity was 19.5%.<sup>9</sup> A higher prevalence of sero-positivity for IgG of toxoplasma, 59.6%, was declared by another study implemented in Mansoura, Egypt using test ELISA test.<sup>10</sup> From this point we were inspired to implement this work to ensure the importance of screening *Toxoplasma gondii* among blood donors in Menoufia, Egypt, using ELISA test. The objective of the current study was to measure the occurrence rate of *Toxoplasma gondii* infection among healthy blood donors and highlight the associated risk factors among this important group to ensure blood safety.

## METHODS

Cross-sectional study was conducted in Menoufia University hospital blood transfusion center. Data were collected from the first of August till first of October 2023

Six hundred and thirty blood donors had been included in this survey. Inclusion criteria were set

according to criteria for eligibility for blood donation which included; age between 18 and 60 years, normal pulse, normal blood pressure and normal temperature. Exclusion criteria were also set according to contraindications for blood donation, e.g., known infection with hepatitis viruses or HIV, drug abuse... etc.

Sample size estimation: According to Yilmaz et al., 2021 who revealed 2.3% toxoplasmosis IgM sero-positivity, at alpha error 0.05 and power of the study 95% confidence interval,<sup>11</sup> and from total annual number of blood donors in Menoufia university hospital blood transfusion center, 11255, by using Epi Info 7 software, the estimated sample size was 630 healthy blood donors.

Participants were recruited using convenience sampling

Data collection tool: Study Participants were asked to fill a self-administered questionnaire which included, (1) sociodemographic data, (2) knowledge about toxoplasmosis was assessed using (12 questions scale) including questions about mode of transmission, risk factors and protective measures. Each point was given 1 for right reply and zero for wrong and I don't know ones, the scores were gathered to divide the participants into "good knowledge" whose knowledge score was 60% or more (score of 8 or more) and "poor knowledge" that scored less, (3) behaviors related to toxoplasmosis transmission as dealing with cats, soil or contact with rodents as well as protective behavior like hand washing.

Blood Sampling: Five ml of venous blood obtained from the cubital vein were collected from each participant in the study and transferred into a plain tube, then centrifuged at 300 rpm for ten min to split up the serum, labeled and kept at -20 °C for subsequent biochemical analysis.

Sero-logical testing: the following infections were routinely tested; immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus surface antigen (HBs Ag), and syphilis. Additionally, the immunoassay enzyme approach was used to measure the titre of serum antibodies against *Toxoplasma gondii*. To accomplish this, commercial kits were used to measure serum titre of IgG and IgM antibodies Cat no. 201-12-1847, 201-12-1845 Shanghai Sunred Biological Technology Co., Ltd using the manufacturer's recommended procedures. The cases were considered positive for toxoplasmosis when IgG, IgM or both sero-positivity was detected.

Table 1: Sero-prevalence of *Toxoplasma gondii* among the studied blood donors (N = 630)

	Number	%	95% CI
Toxoplasmosis sero-prevalence			
IgM	39	6.2	4.1 - 8.2
IgG	70	11.1	8.9 - 13.7
IgM alone	37	5.9	4.0 - 7.8
IgG alone	68	10.8	8.4 - 13.0
Both IgM and IgG	2	0.3	0.0 - 0.8
Negative	523	83.0	80.0 - 86.0
Toxoplasmosis overall positivity			
Positive	107	17.0	14.1 - 19.8
Negative	523	83.0	80.0 - 86.0

Statistical analysis: SPSS (statistical software for social science; SPSS Inc., Chicago, IL, USA) version 23.0 was used to analyze data on an IBM compatible computer. Qualitative variables were expressed as number and percentage and Chi-square analysis for them was provided. Student t test was used to compare two independent groups have quantitative data. Predictors were identified using binary logistic regression analysis. Significant variables on univariate analysis (suggested risk factors) were analyzed by binary logistic regression to identify independent predictors for toxoplasmosis infection. Significant level was determined at P value < 0.05.

## RESULTS

Of the overall 630 blood donors; the total *Toxoplasma gondii* sero-positivity prevalence was 17.0%, 11.1% were positive for the anti-*Toxoplasma gondii* IgG antibodies with mean of quantitative level 4.79, 6.2% were positive for the IgM antibodies with mean of quantitative level and only 2 (0.3%) were positive for both antibodies (IgG and IgM) (table 1). The sociodemographic data showed that the mean age of studied individuals was 31.8 ranged from 17.0 to 66.0 years old, 78.7% of them were below forty, 96.7% of them were male, 63.3% of them had a high standard of education and 57.8% of them were from rural dwellers. Regarding the behavioral characteristics of the studied group; 9.2% of them were dealing with cats, 19.0% of them were consuming ready to eat or processed meat, 16.7% had activity in agriculture. Previous blood transfusion was presented in 5.65% of them. Regarding their current virology profile 2.7%, 1.7% and 1.9% were positive for HBV, HCV and AIDS respectively, while there were no any positive cases of syphilis (Table 1).

Age and sex showed substantial difference between both groups (p value 0.001 and <0.001). Concerning

behavioral data; dealing with cat, eating ready to eat or undercooked meat, agricultural environment and improper hand washing had a significant association with infection (p value; <0.001, 0.001, 0.001 and 0.032, respectively) as well as poor knowledge about toxoplasmosis was also a significant risk factors for infection (p value <0.001, Table 2).

Multivariate analysis revealed that sex, eating ready to eat meat, working in agriculture, improper hand washing and poor knowledge about toxoplasmosis were independent predictors for toxoplasma infection (p value; <0.001, Table 3).

## DISCUSSION

Blood transfusions are one of the possible *Toxoplasma gondii* transmission routes. Due to the immunocompromised nature of transfusion-dependent patients, infectious donors may cause chorioretinitis, brain infection, and myocarditis among other severe clinical consequences. Aside from acute toxoplasmosis, the prognosis could be fatal due to the possibility of cysts reactivating in cancer patients, HIV patients, and organ transplant recipients. The requirement for multiple transfusions increases the risk of infection even more. Therefore, controlling toxoplasmosis in donors and patients is necessary for this reason. Environmental conditions and hygienic behaviors may influence toxoplasmosis transmission among blood donors. Identifying the danger elements of infection in blood donors, being a part of general population, is therefore critical. In Egypt, the sero-epidemiology of the infection caused by *Toxoplasma gondii* in blood donors has received little attention. The present research revealed that the prevalence of *Toxoplasma gondii* sero-positivity among studied blood donors was high at 17% compared to similar studies conducted in Mexico,<sup>12</sup> Taiwan,<sup>13</sup> and China<sup>14</sup> which was 13.5%, 9.3%, and 4.83% respectively. Nonetheless, this study's prevalence was lesser than that of Tunisia (44.4%),<sup>15</sup> and West Algeria (47.7%).<sup>16</sup> A systematic review conducted in Egypt by Taman and Alhusseiny showed that the *Toxoplasma* IgG sero-prevalence in healthy blood donors varied from 33.7% to 67.4%, whereas in the general population, it was between 3% and 42.5%.<sup>17</sup> The study's donor population's infection frequency was 11.1% and 6.2% by ELISA IgG and IgM respectively. This was consistent with the finding of Sarkari et al<sup>18</sup> who detected that the overall infection sero-prevalence was 19.3%, 1.6% were positive concerning IgG and IgM, 12.3% were only positive

Table 2: Toxoplasma gondii sero-positivity in relation to sociodemographic criteria among the studied blood donors

	Toxoplasma gondii sero-positivity			Unadjusted Odds ratio 95% CI	P-value
	Total N - 630	Sero-positive N = 107	Sero-negative N = 523		
<b>Sociodemographic data</b>					
Age (years)					
Mean±SD	31.85±10.08	30.09±9.64	32.84±10.62	0.76 (0.09 - 2.33)	0.01
Range	17 - 66	17 - 52	17 - 66		
Age groups					
< 40	496 (78.7)	89 (83.2)	407 (77.8)	0.710 (0.411 - 1.226)	0.217
≥ 40	134 (21.3)	18 (16.8)	116 (22.2)		
Sex					
Male	609 (96.7)	96 (89.7)	513 (98.1)	1	<0.001
Female	21 (3.3)	11 (10.3)	10 (1.9)	5.88 (2.43- 14.22)	
Education					
Low level	231 (36.7)	39 (36.4)	192 (36.7)	0.989 (0.642 - 1.52)	0.959
High level	399 (63.3)	68 (63.6)	331 (63.3)	1	
Residence					
Rural	364 (57.8)	60 (56.1)	304 (58.1)	1.09 (0.72 - 1.65)	0.695
Urban	266 (42.2)	47 (43.7)	219 (41.9)	1	
Blood transfusion	35 (5.6)	9 (8.4)	26 (5.0)	1.76 (0.80- 3.86)	0.157
Poor knowledge about toxoplasma transmission	568 (90.2)	84 (78.5)	484 (92.5)	3.39 (1.93 - 5.98)	<0.001
<b>Behavioral data</b>					
Dealing with cat	58 (9.2)	29 (27.1)	29 (5.5)	6.33 (3.59-11.17)	<0.001
Contact with rodents	230(37.5)	36 (33.6)	194 (37.1)	0.86 (0.55-1.33)	0.50
Agriculture (contact with soil)	105 (16.7)	42 (39.9)	63 (12.0)	4.72 (2.95-7.54)	<0.001
Eating ready to eat or under cooked meat	120 (19.0)	71 (66.4)	49 (9.4)	19.8 (11.60- 31.37)	<0.001
Contact with Fresh milk	227 (36.00)	37 (34.6)	190 (36.3)	0.93 (0.60- 1.43)	0.730
Contact with Fruits and vegetables	232 (36.80)	35 (32.7)	197 (37.7)	0.80 (0.52-1.25)	0.330
Improper hand washing practice	402 (63.8)	78 (72.9)	324 (62.0)	1.65 (1.04-2.62)	0.030
Blood groups					
A	219 (34.7)	46 (42.9)	173 (33.1)		0.792
B	151 (23.9)	27 (25.2)	124 (23.7)		
O	111 (17.6)	6 (5.6)	105 (20.1)		
AB	149 (23.6)	26 (2)	121 (23.1)		
<b>Comorbidities</b>					
HBV	17 (2.7)	5 (4.7)	12 (2.3)	2.09 (0.72-6.05)	0.186
HCV	11 (1.7)	4 (3.7)	7 (1.3)	2.86 (0.82-9.96)	0.099
AIDS	12 (1.9)	6 (5.6)	6 (1.1)	5.12 (1.62-16.19)	0.008
Syphilis	0 (0.0)	0 (0.0)	0 (0.0)	-	-

# = t test,  $\chi^2$ ; chi square test of significance, CI = Confidence interval.

for IgG, and 5.47% were only for IgM. On the other hand, El-Sayed et al reported that 33.67% and 3.33% had positive ELISA IgG and IgM respectively,<sup>19</sup> which is high compared to that reported in the current study. Sero-prevalence differences could be

attributed to differences in blood donor behaviour, sampling time, sample size, environmental conditions, and sero-logical methods used.

A multivariate analysis that included the sociodemographic, clinical and behavioral factors of

Table 3: Multivariable regression analysis for independent risk factors for Toxoplasma gondii infection among blood donors

	B	Wald test	P value	AOR (95% CI)
Age	0.075	14.82	<0.001	1.08 (1.04-1.12)
Gender	3.27	27.69	<0.001	26.21 (7.77-88.45)
Poor knowledge	2.75	37.53	<0.001	15.58 (6.47-37.51)
Dealing with cat	0.086	0.034	0.853	1.09(0.44 - 2.69)
Eating ready to eat or undercooked meat	3.55	29.38	<0.001	35.66 (19.02-99.15)
Agricultural environment	2.01	26.82	<0.001	7.42 (3.48 - 15.85)
Improper Hand washing practice	3.65	30.37	<0.001	38.64 (10.53-114.72)

CI = Confidence interval, AOR; adjusted odds ratio

participants showed that female sex was a significant predictor for Toxoplasma gondii seropositivity, this could be as a result of raising and dealing with cats is higher among female than males. This was not in line with Sarkari et al. study, which found that gender was not related to toxoplasma sero-positivity and that it was difficult to make a connection between gender and seroprevalence because over eighty percent of people donating blood were males<sup>18</sup> as present in the current study. This result was in contrast to the study of Stopić et al, which revealed that male blood donors have a higher probability than female blood donors to be infected and the increased risk of Toxoplasma infection in men has been attributed to either soil contact or poor hand hygiene and thus a gender difference may appear in populations with high soil exposure.<sup>20</sup>

Taking in undercooked meat was discovered to be a substantial threat factor for Toxoplasma gondii infection in the studied donors. According to Sadooghian et al. study, there is a statistically significant link between eating undercooked meat and getting the infection.<sup>21</sup>It is in accordance with the outcomes of Nadia et al, who discovered that using meat, whether cooked or raw, as well as the meat-tasting habit while preparing food, are statistically important risk variables related to toxoplasmosis sero-positivity.<sup>22</sup> This is most likely due to the presence of tissue cysts of the Toxoplasma gondii parasite in undercooked or uncooked meat. In contrast to the present findings Lupu et al. discovered no significant link between eating ready to eat or meat in its raw state and the hazard of developing toxoplasma antibodies. Lupus et al. explained their findings by the presence of enhanced standards of cleanliness in meat processing facilities and on animal farms, combined with the use of contemporary technologies to improve sanitation conditions, and increasing the use of frozen meat

and industrially processed meat products.<sup>23</sup> This disparity between the different studies could be outlined by the participants' food habits in each study. The analytical methods used in these studies could also explain it.

Contact with soil was a threat factor for a significant upsurge in the sero-prevalence of anti-Toxoplasma gondii IgG antibodies among the studied donors. A study carried out in Egypt by Ibrahim et al<sup>24</sup> concluded that exposition to soil was an important hazards factor for expectant mothers. In line with this finding, Achaw et al<sup>25</sup> found that farming (gardening and agriculture) was highly linked to the sero-prevalence of anti-Toxoplasma gondii IgG antibody. The reason for this may be due to the cat feces containing Toxoplasma gondii oocysts may contaminate the soil, as the oocysts can survive in the soil for up to 18 months under favorable temperatures. This was different from the outcomes of Al-Daoudy et al<sup>26</sup> showed that there was no statistically significant differences regarding soil contact.

Although dealing with cats was found to have a significant relation to the Toxoplasma gondii infection in the current research, it was not realized to be a predictor of developing the infection. This conclusion was in accordance with the studies performed by Alvarado-Esquivel et al,<sup>12</sup> Stopić et al<sup>20</sup> and Al-Daoudy et al,<sup>26</sup> but in disagreement with Ibrahim et al,<sup>24</sup> Egyptian study conducted among pregnant women. The present results can be interpreted as a higher risk of Toxoplasma gondii infection by eating tissue cysts of undercooked meat than by ingestion of oocysts shed by cats in the blood donors studied.

Hand contamination is a significant factor in the spread of germs and pathogenic parasites. Hand hygiene prior to ingesting food has been recognized as a Toxoplasma gondii sero-positivity protective factor. Toxoplasma gondii infection can thus be

obtained through the swallowing of oocysts; multiple studies have before documented an equivalent finding demonstrating that the possibility of *Toxoplasma* exposure increases by failing to wash hands before eating.<sup>15</sup>

According to the current study, the majority of blood donors were unaware of Toxoplasmosis. This was consistent with Yi-Qing et al. finding that only 16.8% of participants were aware of the disease.<sup>27</sup> The level of toxoplasmosis knowledge, according to the current findings, is an additional threat factor for *Toxoplasma gondii* infection. According to Sint et al,<sup>28</sup> the level of knowledge and sero-positivity among the studied group were related. According to the findings of Cédric et al, sero-prevalence was higher in expectant mothers who are unaware of the disease. However, no correlation was discovered between knowledge level and toxoplasmosis sero-positivity.<sup>22</sup> The current situation could be attributed to a lack of access to media and a lack of focus on the infection, in addition to a shortage of knowledge on the part of health authorities.

The current study showed that eating raw vegetables did not increase the likelihood of *Toxoplasma gondii* sero-positivity. It was in line with the conclusions of Hung et al. study in Taiwan.<sup>29</sup> However, it contradicts the findings of Wam et al.<sup>30</sup> in Cameroon, who found that eating raw vegetables boosted the possibility of picking up *Toxoplasma gondii*. In addition, Paul et al.<sup>31</sup> study found that consuming uncooked vegetables was linked to a reduced chance of *Toxoplasma gondii* sero-positivity. The availability of healthy tap water, which reduces the likelihood of infection with *Toxoplasma gondii*, and participants' understanding of hygiene principles, could explain Paul et al. findings.

The study was done on blood donors who contribute to the provision of a very vital service; blood transfusion, and as much as we know it is the initial research done on blood donors in Menoufia governorate. Like all cross-sectional studies, it has some weak points. The studied risk factors cannot be generalized on the general population as it was conducted only for a certain age group who are eligible for blood transfusion.

#### Recommendations

It is recommended to screen blood donor prior to transfusion to prevent transfusion-transmitted toxoplasmosis. Educational interventions to raise knowledge level about the disease transmission

methods, proper meat handling, the importance of hand washing, the advantages of consistent use of safeguarding gloves, and proper hand washing after working in agriculture or gardening.

#### CONCLUSIONS

*Toxoplasma gondii* infection was incredibly common among blood donors. Lack of knowledge, and several behavioral characteristics, such as eating ready to eat meat, agricultural environment, and improper hand washing practice were all threat factors for *Toxoplasma gondii* infection. To stop and take control of *Toxoplasma gondii* infection, it is critical to put into practice-integrated strategies along with effective management approaches.

#### Ethical Consideration

The study obtained all required approvals from the Institutional Review Board of faculty of medicine (Menoufia University), under the number (8/2023COM). All included subjects were instructed about the target of this work. Written consent was obtained to get samples of serum.

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