Original Article

Seroprevalence of Anti-*Toxoplasma gondii* IgG Antibody and Risk Factors among Abattoir Workers in Uyo, Southern Nigeria

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for these occupationally exposed and other populations at risk of infection.

Context: The asymptomatic nature of toxoplasmosis with its severe consequences,

especially in populations with high HIV infection rate, makes it a disease

of public health importance. Being a zoonosis, Toxoplasma gondii infection

may be high among people who work with animals. Thus, there is the need

to determine the prevalence of the infection among abattoir workers in Uyo,

where HIV prevalence rate is in double digits, which could lead to reactivation

of toxoplasmosis in those with immunosuppression. Aim: To determine the

prevalence and associated risk factors of T. gondii infection in abattoir workers in

Uyo. Settings and Design: A descriptive cross-sectional study that was conducted among persons who work with livestock and birds, in Uyo and its environs. Methodology: The study was conducted among 339 workers in abattoirs in Uyo and its environs. Data were collected using interviewer-administered questionnaire and detection of IgG antibodies to T. gondii using ELISA technique and HIV testing using the national algorithm. Data were analyzed using STATA statistical software version 20, and statistically significant level was set at P < 0.05. Results: The seroprevalence of *T. gondii* IgG antibodies among the study participants was 55.8%. At a univariate level, many factors were associated with T. gondii seropositivity; they were age, sex, level of education, ethnicity, occupation in the abattoir, type of animal exposure, always washing hands before eating in the workplace, and taking of raw/unpasteurized milk. However, after multivariate analysis, participants exposed to poultry, butchers/raw meat sellers, and those who had worked for more than 5 years in abattoirs were at a greater risk of being T. gondii IgG seropositive; odds ratio (OR) 5.46 [confidence interval (CI) 1.88, 15.86]; OR 1.89 (CI 1.14, 3.14), and OR 1.25 (CI 1.25, 6.42), respectively. Conclusion: T. gondii IgG seroprevalence is high among abattoir workers in Uyo, and there is a need for Akwa Ibom State government to establish enlightenment and prevention programs

INTRODUCTION

T oxoplasma gondii is an obligate, intracellular, parasitic protozoan and the etiologic agent of toxoplasmosis. Toxoplasmosis is a zoonotic disease that is widespread globally;^[1] about one-third of world's human population is said to be infected with *T. gondii*.^[2] However, infection rates vary significantly between and even within countries.^[3-7] Felids such as domestic cats are the only known definitive hosts, while human and other

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animals including birds are the intermediate hosts.^[1,6,8] *T. gondii* infection is said to be highest in places that have hot, humid climates with lower altitudes.^[9] The main routes of transmission are foodborne – through

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eating improperly cooked contaminated meat, unwashed contaminated fruits, vegetables, meat chopping boards, and unwashed contaminated hands – when the oocysts or tissue cyst are ingested; or congenitally where an infected pregnant woman transmits the infection to the child. However, other routes of transmission such as accidental inoculation, blood transfusion, and organ transplant have been reported.^[9]

T. gondii rarely causes disease in immunocompetent persons, but in immunodeficient persons the consequences could be grave.^[10] The asymptomatic nature of toxoplasmosis, with its high morbidity and severe complications such as congenital malformations, visual impairment, and blindness, makes it a disease of significant public health concern, thus necessitating early laboratory detection and proper management of infected persons. As a zoonosis, there is a concern that T. gondii infection may be common among people who have close contact with animals including birds. Apart from the foodborne route of infection (which may depend on their lifestyle/hygienic practices while at work), abattoir workers and meat sellers are at risk of T. gondii infection from accidental inoculation through skin abrasion or cut.^[9]

Akwa Ibom State, Nigeria, currently has a HIV seroprevalence of 10.8% and has consistently ranked among the five states with high HIV seroprevalence in the country.^[11] High HIV prevalence could lead to reactivation of toxoplasmosis in those with immunosuppression. However, the epidemiology of toxoplasmosis in Akwa Ibom State remains unknown. Thus, the research questions that this research set out to answer were as follows: what is the prevalence of anti-*T. gondii* IgG antibody seropositivity among abattoir workers in Uyo and what are the factors associated with anti-*T. gondii* IgG antibody seropositivity among abattoir workers in Uyo?

Therefore, this study aimed to determine the prevalence of *T. gondii* infection among HIV-positive and HIV-negative abattoir workers in Uyo and its environs. The result of this study will provide epidemiologic information for the prevention and control of toxoplasmosis, particularly among the occupationally exposed persons, and thus awareness will be created among those working in abattoirs irrespective of their HIV status; in addition, there will be stimulation of further research into the dynamics of the disease.

Methodology

Study area

The study was conducted in three large abattoirs and animal markets located in Uyo metropolis and environs

in Akwa Ibom State, Nigeria. Akwa Ibom State is located in the South-South geopolitical zone of Nigeria. It has a 2017 estimated population of 5.6 million people spread across 31 local government areas (LGAs) with an annual growth rate of 3.4%.^[12] It is ranked the ninth most populated state in Nigeria and lies between latitude 40.32' and 50.53' N and longitude 70.25' and 80.25' E of the equator. Uyo is the state capital, located along latitude 5° 01' 59" N and longitude 7° 55' 35" E with a projected population of about 447,187 and is one of the fastest growing cities in Nigeria, economically and population wise. It is also a commercial nerve center of Akwa Ibom State and can be accessed by inhabitants of many surrounding LGAs and the neighboring Cross River State for economic and social activities. Subsistence agriculture accounts for 55% of the total workforce of the inhabitants.

Study design and population

This was a descriptive cross-sectional community study where consented participants were tested for the presence of anti-*T. gondii* IgG antibody and anti-HIV antibody, and questionnaires were administered to collect information on sociodemography, occupation and related issues, and potential risk factors among others. The study was conducted between May 2016 and July 2017. The sample size was calculated using StatCalc in Epi Info version 7.2.1.0. Using a prevalence of 26.7%,^[13] from a similar study, Uyo LGAs' 2017 estimated population of 447,187, and a 5% error margin, a minimum sample size of 301 was obtained.

The study population included 339 male and female abattoir workers of all age groups present at the abattoir at the time of visit. They comprised livestock (cattle, goat, sheep) and chicken farmers/traders, butchers, and meat sellers in all the main/large public abattoirs in Uyo and environs including Ntak Inyang, Mbak Itam, and Akpan Andem livestock markets. Persons of all ages, ethnic groups, and educational status who actively participated in abattoir operations were included in the study. Meat buyers - wholesale and retailers - who are middlemen at the abattoirs also included in the were study. Livestock farmers/traders were those who raised livestock for sale at the abattoir/livestock markets. Butchers were those responsible for slaughtering and dressing the animals, while meat sellers were those who sold raw meat.

Ethical considerations

Ethical clearance was sought and obtained from the Ethical Committee of the Akwa Ibom State Ministry of Health. Verbal and written permission was obtained from the leaders of the respective livestock sellers and abattoir workers' association. Written informed consent was obtained from all the participants before commencement of the study.

Sampling

The study recruited all eligible persons who consented (by signing or thumbprinting) to participate, serially as they came for the study. Eligible persons were abattoir workers such as animal farmers (sheep, goat, cattle, pigs, birds); those who slaughtered and butchered the animals; persons who killed, defeathered and/or cut into pieces and prepared the birds for sale; and meat sellers (middle men) who came to the abattoir to buy meat in bulk for re-sale at other outlets.

Data collection

Data collection tool was a questionnaire that was pre-tested at a large abattoir in Abak metropolis, Abak LGA of Akwa Ibom State; thereafter corrections were made accordingly. Evaluation of the pre-tested questionnaire led to the adjustment of the data collection instrument. The instrument was validated by the most senior member of the team. Before commencement of the study, a pilot study was conducted in one of the abattoirs in Uyo, after leaders of the abattoir associations had been consulted and informed of the purpose and significance of the study.

Group pretest counseling was done for all eligible participants who consented to take part in the study. Those who tested HIV-positive were referred to the University of Uyo Teaching Hospital for posttest counseling, treatment, and follow-up, according to the test and treat strategy.

Sociodemographic information of each of the participants was obtained at the time of sample collection through interviewer-administered questionnaires by well-trained research assistants. Information on potential risk factors associated with toxoplasmosis were also collected in the formatted questionnaire. Some of these include owning at least one cat or presence of stray cats at home, keeping of other pets (dogs, goat, pig, sheep, cow, horse, donkey, birds) at home, source of drinking water (pipe borne well, borehole, stream, sachet, bottled), hand washing habit, eating of undercooked meat, and having contact with soils during gardening within the past 3 months before this study.

Sample collection

Seven milliliters of venous blood samples was aseptically collected from consented participants by qualified medical personnel using a vacutainer blood collection device and shared into plain and EDTA tubes. The blood samples were kept in ice-packed cooler and transported to the laboratory. Serum was separated after centrifugation of the plain blood samples at 3000 rpm for 10 min and stored in 2-mL vials at -20° C until required for testing.

Sample analyses

All universal precautions were strictly followed when carrying out laboratory analyses, which included assay for *T. gondii* IgG antibody by ELISA technique and rapid HIV testing using the national testing algorithm.

Serological testing of T. gondii IgG antibody

The determination of the presence of anti-*T. gondii* IgG-specific antibody in serum of participants was carried out using the Enzyme Linked Immunosorbent Assay kit (Chemux Bioscience, Inc, CA, USA) according to the manufacturer's manual. The optical density was measured in a spectrophotometer using a single wavelength of 450 nm. Sensitivity, specificity, and accuracy of the test kit were 94%, 100%, and 98%, respectively. Toxo IgG index of \geq 1.00 or >8 IU/mL as prescribed by World Health Organization is regarded as positive reaction. Samples with equivocal reaction having Toxo IgG index of 0.91–0.99 were retested. All universal precautions were strictly adhered to while carrying out laboratory analyses.

Serological testing of HIV antibody

HIV testing was carried out according to the national serial algorithm using three rapid HIV Enzyme Immunosorbent Assay (EIA) kits – Determine HIV 1/2 (Abott, Japan), Unigold (Trinity Biotech, Ireland), and Stat-Pak HIV 1/2 (Chembio, USA). Determine was used for first-line testing followed by Unigold, while Statpak was used as a tie-breaker for samples with inconclusive results. All test protocols and interpretation of results were done according to the respective kit's manual.

Statistical analysis

Data collected in the questionnaires and *T. gondii* and HIV serological test results were analyzed using STATA statistical software version 12 (special edition) (StataCorp, College Station, Texas, USA). Chi-square test was used to test for association between categorical variables. Dependent variables with a P value <0.05 at the bivariate level were used at the multivariate level of analysis.

RESULTS

A total of 339 occupationally exposed people were recruited for the study. They were 15–78 years of age, with a mean age of 34.8 ± 11.6 years. They were mostly 21–30 years (36.6%), males (83.5%), in marriage relationship (61.4%), have had secondary education (54.6%), and earned less than 20,000 naira per month (46.6%) [Table 1].

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Table 1: Sociodemographic characteristics and <i>Toxoplasma gondii</i> IgG seropositivity of abattoir workers in Uyo, Nigeria, 2017				
Characteristics	Total (<i>n</i> =339)	Toxoplasma IgG seropositivity (<i>n</i> =189), <i>n</i> (%)	Statistical indices	
Age (years)				
≤20	21 (6.2)	13 (6.9)	$P \leq 0.0001^+$	
21-30	124 (36.6)	49 (25.9)	$\chi^2 = 5.8358$	
31-40	105 (31.0)	64 (33.9)	<i><i></i></i>	
41-50	57 (16.8)	39 (20.6)		
51 and above	32 (9.4)	24 (12.7)		
Age range (years)	15-78	15-78		
Mean age±SD	34.8±11.6	36.9±12.0	P=0.0003+	
Sex				
Male	283 (83.5)	151 (79.9)	P=0.046 ⁺	
Female	56 (16.5)	38 (20.1)	$\chi^2 = 3.9845$	
Marital status				
Single	131 (38.6)	72 (38.1)	P=0.816	
Married	208 (61.4)	117 (61.9)	$\chi^2 = 3.9845$	
Level of education				
No formal education	39 (11.5)	14 (7.4)	P=0.045+	
Primary	75 (22.1)	40 (21.2)	$\gamma^2 = 8.0558$	
Secondary	185 (54.6)	111 (58.7)		
Tertiary	40 (11.8)	24 (12.7)		
Ethnicity				
Ibibio/Annang	193 (56.9)	143 (75.7)	P=0.0001	
Hausa Fulani	124 (35.6)	36 (19.1)	$\gamma^2 = 65.1508$	
Others**	22 (6.5)	10 (5.3)	<i>y</i> t	
Monthly income				
<20,000	154 (46.6)	88 (46.6)	P=0.568	
20,000-49,999	104 (30.7)	59 (31.2)	$\chi^2 = 2.0207$	
50,000-99,999	44 (13.0)	27 (14.3)		
100,000 and above	33 (9.7)	15 (7.9)		

SD=Standard deviation. **Other ethnic groups like Igbos and Yorubas, *Statistically significant

Table 2: Plausible occupation-related factors associated with <i>Toxoplasma gondii</i> IgG seropositivity among abattoir workers in Uvo, Nigeria, 2017				
Variables	Total (<i>n</i> =339)	<i>Toxoplasma gondii</i> seropositivity (<i>n</i> =189), <i>n</i> (%)	Statistical indices	
Working duration				
<1 year	40 (11.8)	16 (8.5)	P=0.074	
1-5 years	113 (33.3)	62 (32.8)	$\chi^2 = 5.8358$	
Above 5 years	186 (54.9)	111 (58.7)	<i>,</i> ,	
Types of animal exposure				
Livestock (cattle, goat, sheep)	309 (91.2)	166 (87.8)	P=0.016 ⁺	
Poultry	30 (8.9)	23 (12.2)	$\chi^2 = 5.2209$	
Occupation				
Livestock seller	165 (48.7)	77 (43.0)	$P=0.028^+$	
Butchers/meat seller	174 (51.3)	102 (57.0)	df=1	
			$\chi^2 = 4.8561$	
Always wash hand before eating at				
workplace				
Yes	304 (89.7)	164 (86.8)	P=0.049+	
No	35 (10.3)	25 (13.2)	$\chi^2 = 3.8880$	

+Statistically significant

The seroprevalence of anti-*Toxoplasmosis gondii* IgG among the participants was 55.8% (189 of 339), mostly 31–40 years

of age (33.9%), males (79.9%), with secondary education (58.7%), and of Ibibio/Annang ethnic group (75.7%).

Uyo, Nigeria, 2017				
Characteristics	Total (<i>n</i> =339)	Toxoplasma gondii seropositivity (n=189), n (%)	Statistical indices	
Ownership of pet				
Yes	129 (38.1)	65 (34.4)	P=0.119	
No	210 (62.0)	124 (65.6)	χ ² =2.4294	
Number of people in the house hold				
<6	227 (67.0)	96 (64.0)	P=0.302	
6 and above	112 (33.0)	54 (36.0)	$\chi^2 = 1.0667$	
Drinking/eating raw egg				
Yes	107 (31.6)	58 (30.7)	P=0.697	
No	232 (68.4)	131 (69.3)	$\chi^2 = 0.1516$	
Taking of raw/unpasteurized milk				
Yes	151 (44.5)	61 (32.3)	$P=0.0001^+$	
No	188 (55.5)	128 (67.7)	χ ² =26.0203	
+Statistically significant				

 Table 3: Plausible domestic factors associated with *Toxoplasma gondii* IgG seropositivity among abattoir workers in Uvo. Nigeria. 2017

*Statistically significant

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Table 4: Multivariate logistic regression of some sociodemographic factors and other factors associated with *Toxoplasma gondii* IgG seropositivity among abattoir workers in Uvo, Nigeria, 2017

Variables	OR	95% CI	P
Age (years)			
<20	Reference		
21-30	0.39	0.13-1.12	0.081
31-40	0.96	0.32-2.85	0.938
41-50	1.38	0.42-4.50	0.597
51 and above	1.96	0.49-7.78	0.339
Sex			
Male	Reference		
Female	1.21	0.60-2.45	0.599
Level of education			
No formal education	Reference		
Primary	1.51	0.61-3.72	0.375
Secondary	2.26	0.98-5.21	0.055
Tertiary	2.08	0.75-5.80	0.161
Occupation			
Livestock seller	Reference		
Butchers/meat sellers	1.89	1.14-3.14	0.014^{+}
Working duration (years)			
<1	Reference		
1-5	1.74	0.83-4.36	0.131
Above 5	1.25	1.25-6.42	0.013^{+}
Type of animal exposure			
Livestock	Reference		
Poultry	5.46	1.88-15.86	0.002^{+}
Taking raw/unpasteurized			
milk			
Yes	Reference		
No	1.95	1.95-3.31	0.014^{+}
Always washing of			
hand when eating at the			
workplace			
Yes	Reference		
No	0.99	0.99-1.01	0.862

noted according to the sociodemographic variables of the participants revealed that the differences in age groups, sex, ethnicity and educational level were significant [Table 1].

An analysis of T. gondii seropositive proportions

An assessment of the occupationally related factors showed that the type of occupation (livestock seller or butcher/raw meat seller), type of animal exposure (livestock or poultry), and always washing hands before eating at the workplace were significantly associated with *T. gondii* IgG seropositivity [Table 2].

A further analysis of domestic factors and *T. gondii* seropositivity showed that 34.4% of the study participants owned or kept pets; most (64%) had a family size of less than six people in their households. About a third (32.3%) took raw (unpasteurized) milk. However, only consumption of raw/unpasteurized milk had a significant association *T. gondii* seropositivity (P < 0.05) [Table 3].

А multivariate logistic regression of the sociodemographic, occupation-related, and domestic variables that were significantly associated with T. gondii seropositivity revealed that occupation, working duration (duration of working in the abattoir), type of animal exposure, and taking of raw/unpasteurized milk remained statistically significant, showing an odds ratio (OR) of 1.89 [95% confidence interval (CI) 1.14, 3.14, P = 0.014] for butchers/raw meat sellers compared with livestock sellers; OR 1.25 (95% CI 1.25, 6.42, P = 0.013) for those who worked in the abattoir for > 5 years versus those who worked for 1 year; OR 5.46 (95% CI 1.88, 15.86, P = 0.002) for exposure to poultry versus exposure to livestock; and OR 1.95 (95% CI 1.95, 3.31, P = 0.014) for not drinking raw/unpasteurized compared with those who

reported that they drank raw/unpasteurized [Table 4]. Participants who deal with poultry had a 5-fold higher risk of being *T. gondii* seropositive, when compared with participants who deal with livestock. In addition, those who had spent more than 5 years in their occupation (slaughtering, butchering, or selling animals) were 1.25 times more likely to be *T. gondii*—positive compared with those who spent less than 1 year, while not taking unpasteurized milk had almost a 2-fold risk when compared with taking it.

DISCUSSION

An overall T. gondii IgG seropositive rate of 55.8% was observed among our participants, who were persons who work in abattoirs. Although the prevalence falls within the global estimated range of 30%-65%,^[14] with even up to 90% said to occur in some countries.^[1] vet it is much higher than the Nigerian average of 32% obtained from an analysis of pooled reports across geopolitical zones in the decade 2005-2015.^[7] A similar study that was conducted among abattoir workers in Sokoto and butchers in Iran reported prevalence of 26.7% and 41.8%, respectively.^[13,15] While studies in Abuja and Maiduguri, Nigeria, recorded 31.5% and 48.9%, respectively.^[16,17] A similar study that was conducted in Malaysia reported an overall T. gondii seroprevalence of 19.9%.^[18] Even among HIV-infected individuals, the highest prevalence of T. gondii reported within the past decade was 54%, in Lagos, Nigeria.^[19] There is no doubt that some earlier studies conducted before 2005 observed higher prevalence rates, for instance, a 75.4% prevalence of T. gondii antibodies was observed among pregnant women in Oyo, Nigeria.^[20] It is possible that the variation in prevalence could be due to the difference in study populations (such as pregnant women and HIV-infected persons), the sample size, and laboratory method used (e.g., dye test, indirect hemagglutination test, ELISA). It could also be that since toxoplasmosis is a zoonotic disease, our study population has a higher risk of infection when compared with the general population. To the best of our knowledge, this is the first reported Nigerian study on the prevalence of T. gondii antibodies among people who work with animals, in the South-southern part of the Country.

At univariate level, *T. gondii* seropositive rate increased with age and peaked in the age group 31–40 years (33.9%), after which it showed a decline, and the difference in the different age groups was shown to be significant. However, multivariate logistic regression did not show age to be a predictor of *T. gondii* seropositivity, meaning that there could have been other confounding variables. This is similar to

the picture that was observed in Sokoto among abattoir workers^[13] and even in Kano, among HIV-seropositive pregnant women,^[21] where there was no significant association between T. gondii seropositivity and age. In contrast, the report of a study of indigenes of Maiduguri, a systematic review of the studies in the Iranian general population, a study of people who had close contact with animals in Malaysia, and even among Military personnel in Czech Republic,^[18,22-24] all indicated positive linear trend of seropositive T. gondii prevalence with age. Even in Germany, a nationwide survey among adults 18 years of age and above showed a clear increasing T. gondii seroprevalence with increase in age.^[25] However, the fact that the study populations differ makes it difficult to draw conclusions between the association between T. gondii seropositivity and age.

Although the difference in T. gondii seropositive proportions of the males in this study was significantly higher than that of the females, the multiple logistic regression did not establish any significant relationship. This is similar to the findings of the Malaysian study,^[18] which observed a significant association between sex and T. gondii seropositivity at a univariate level, but none after multivariate analysis. This finding corroborates the report of other studies.^[23,26] In addition, a comparison of three subgroups (pregnant women, immunocompetent, and immunocompromised persons) in Nigeria revealed that T. gondii seropositivity was not sex-related.^[16] The fact that most human toxoplasmosis studies in Nigeria were conducted among pregnant women further undermines the basis for sex and even age comparisons.

In this study, none of the sociodemographic variables that showed a significant association with T. gondii seropositivity at the univariate level was a predictor after multivariate logistic regression. Most of the studies conducted in Nigeria did not analyze the data using multivariate logistic regression. At multivariate level, the confounding effect of some of the sociodemographic variables was controlled for, thus leaving the true picture of predictors of T. gondii seropositivity. Besides, apart from the abattoir workers in Sokoto study, most of the other Nigeria studies targeted pregnant women;[17,20,21] such studies are therefore gender- and even age-limited, thus eroding the basis for comparison of results. But among abattoir workers, it is reported that handling of infected meat is the main driver of T. gondii infection (through eating and drinking during evisceration and flaving).^[14] Some butchers and meat sellers who are mostly Ibibio/Annang eat pork and dog meat^[27] (which is an abomination to the Hausas/Fulanis); these may explain why butchers and meat sellers had a

higher risk of *T. gondii* seropositivity when compared with livestock sellers.

Those who reported that they had worked in the abattoir for more than 5 years showed a higher risk of *T. gondii* seropositivity when compared with those who worked for less than 1 year. A similar observation was made in the Malaysian study of people who had close contact with animals^[18] and can be explained by the fact that the longer the duration in the occupation, the higher the exposure to *T. gondii*.

T. gondii seropositivity was more than five times higher in participants who worked with poultry, when compared with those who worked with livestock (OR 5.46). While it is been established that virtually all warm-blooded animals including chicken and birds can be infected with T. gondii, ^[28] it is difficult to conclude that poultry exposure poses a greater risk of T. gondii infection compared with livestock, since no other study reviewed investigated this association. However, a review of the prevalence of T. gondii in animal studies in Nigeria in the past decade showed a high seroprevalence among chicken, 66% against the maximum of 36% observed among livestock,^[7] which was linked to chicken's relatively increased contact with contaminated soil.^[26] Another possible reason for our observation could be a cohort effect, since poultry workers were almost homogeneously involved in flaving and evisceration of the birds, while livestock workers were a mixture of farmers, sellers of whole animals, butchers, and meat sellers.

CONCLUSION

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This study shows a high seroprevalence of *T. gondii* IgG antibodies in abattoir workers in Uyo. Factors associated with *T. gondii* seropositivity were being a butcher/raw meat seller when compared with livestock seller, working in the abattoir for more than 5 years, and working with birds as against other livestock such as cows, sheep, and goat. There is a need for Akwa Ibom State government to establish enlightenment and prevention programs for these occupationally exposed and other populations at risk of *T. gondii* infection.

The study was limited by assuming that all the responses by the participants concerning their sociodemographic characteristics and habits that could affect *T. gondii* infection were all correct. In addition, being a cross-sectional study, the incidence of toxoplasmosis in the study population could not be determined, and actual risk of the variables considered could not be determined. The study was limited in scope since only IgG antibodies to *T. gondii* (which depict latent/chronic infection) were used to assess seroprevalence.

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Conflicts of interest

There are no conflicts of interest.

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