



## Toxoplasma gondii Prevalence and Risk Factors among Female Students at Al-Yemenia University, Sana'a City, Yemen

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Received 29 September 2024; accepted in final form 4 November 2024

### Abstract

**Background:** Toxoplasmosis is one of the most common human infections worldwide. It is caused by the intracellular protozoan *Toxoplasma gondii*. Therefore, this study aimed to determine the prevalence of *Toxoplasma gondii* infection among female students at Al-Yemenia University in Yemen. **Methods:** Two hundred female students were selected for this study and screened for *Toxoplasma gondii* antibodies using an immunochromatographic technique (ICT). Demographic, socioeconomic, risk factors, and behavioral data were collected using a pre-tested questionnaire via face-to-face interviews. **Results:** The seropositivity rate for *T. gondii* was (6.0%). The majority were from age groups of 18–20 and 21–24 years (41.7%), who lived in the urban area (83.3%), unmarried individuals (83.8%), and middle-income participants (66.7%), with a non-significant difference ( $P < 0.05$ ). Female students who washed vegetables before eating had a high risk of *T. gondii* infection (OR = 4.810), followed by raising cats in the house (OR = 3.812), and had a history of blood transfusion (OR = 1.618). **Conclusion:** This study reported low seroprevalence among female students at Al-Yemenia University, Yemen. Environmental sanitation, personal hygiene, and health education are all required for promotion to prevent *Toxoplasma gondii* infection among female students.

**Keywords:** Cats, Risk factors, Prevalence, Toxoplasmosis, *Toxoplasma gondii*, Yemen.

**المخلص:** الخلفية والهدف: داء المقوسات هو أحد أكثر أنواع العدوى شيوعاً بين البشر في جميع أنحاء العالم ويسببه كائن أولي داخل الخلايا يسمى بالمقوسات الغوندية لذلك، هدفت هذه الدراسة إلى تحديد معدل انتشار عدوي المقوسات الغوندية بين طالبات الجامعة اليمنية، اليمن. الطرق: تم اختيار إجمالي 200 طالبة لهذه الدراسة وتم الفحص بحثاً عن أجسام مضادة لتوكسوبلازما جوندي باستخدام تقنية الكروماتوغرافيا المناعية. البيانات الديموغرافية والاجتماعية والاقتصادية وعوامل الخطورة والسلوكية باستخدام استبيان تم اختياره مسبقاً من خلال مقابله وجها لوجه. النتائج: وجد بأن معدل الانتشار المصلي للمقوسات الغوندية (6.0%)، كانت الأغلبية من الفئات العمرية 18-20 و 21-24 سنة (41.7%)، والطالبات المقيمات في المناطق الحضرية (83.3%)، والطالبات غير المتزوجات (83.8%) والطالبات ذات الدخل المتوسط (66.7%)، مع وجود فرق فروقات غير معنوية ( $P < 0.05$ ). كان الخطر المرتفع للإصابة بداء المقوسات الغوندية لدى الطالبات اللاتي يغسلن الخضروات قبل الأكل (OR = 4.81)، يليهن تربية القطط في المنزل (OR = 3.812)، وتاريخ نقل الدم (OR = 1.618) الاستنتاج: لوحظ في هذه الدراسة بأن معدل انتشار أجسام المضادة للمقوسات الغوندية منخفض بين الطالبات في الجامعة اليمنية، اليمن. ان تعزيز النظافة البيئية، والنظافة الشخصية، والتثقيف الصحي كلها متطلبات مطلوبة لتجنب الإصابة بداء المقوسات الغوندية بين الطالبات.

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## Introduction

*Toxoplasma gondii* (*T. gondii*) is one of the most important parasites of medical importance [1-2]. It is found in almost all warm-blooded vertebrates, including humans, and classifies humans globally as an intermediate host. At the same time, the final hosts are cats, rodents (*Ctenodactylus gundi*), and others [3]. Approximately 30% of the world's population is infected with *Toxoplasma gondii* [4], and it is acquired mainly through food and water contaminated with parasite eggs released from infected cats when eaten raw or undercooked meat containing cysts of these residues [3].

Toxoplasmosis is ranked fourth among more than 24 pathogens transmitted by ingested food, according to the classification of the Food and Agriculture Organization and the World Health Organization [5]. Many diseases, such as blindness, mental retardation, epilepsy, and death, are transmitted as a result of infection with *T. gondii* to the fetus. Problems increase with infected patients, those with weak immunity, and those suffering from chorioretinitis.[6].

Primary infection with *T. gondii* in pregnant women may lead to the development of congenital toxoplasmosis [7]. Active infection may lead to severe complications in the fetus or newborn infant, including brain or eye damage at birth or death of the fetus and spontaneous abortion [8]. The risk for toxoplasmosis increases with age, lower socioeconomic status, low education level, contact with cats, and poor hygiene practices [9].

Detection of IgM antibodies or both IgM and IgG antibodies indicates an acute infection. Negative results may suggest either a very recent infection or the absence of infection [10]. However, positive IgM results may be due to unreliable commercial test kits or because IgM can remain detectable in serum after an acute infection has ended [11]. To increase the sensitivity and specificity of the serological evolution, additional confirmatory assays such as seroconversion and IgG avidity testing should be done [12].

In Yemen, the published reports on *T. gondii* infections are entirely serologically-based surveys that indicate varied seroprevalence across the country. The overall seroprevalence of *T. gondii* infection has been reported to range from 14-65%, with seroprevalence of acute *Toxoplasma*-specific IgM antibodies ranging from 1.5 to 14% [13–16].

Yemen is one of the emerging nations lacking policies and plans in place to manage or stop the spread of harmful microbes among their populace [17–21]. Information on the frequency of *Toxoplasma gondii* infection among female students in Yemeni universities is scarce. Thus, the goal of the current investigation was to ascertain the incidence of *Toxoplasma gondii* infection among female students at Al-Yemenia University, Yemen.

## Materials and Methods

### Study area and period

This is a cross-sectional study that was conducted from January 2023 to October 2023 and performed on women of childbearing age (between 18 and 28 years) from Al-Yemenia University, Sana'a Governorate, Yemen.

### Data collection

Data was collected using a structured questionnaire, which was given to each healthy participant who was subjected to the current study. The questionnaire was composed of questions related to demographic clinical data and some other information, including age, marital status, and socioeconomic status on toxoplasmosis. Also, information on potential risk factors.

### Sample size

The sample size was estimated based on the WHO's practical manual for sample size determination in health studies [22]. Sample size estimated according to the prevalence of *T. gondii* in Dhamar governorate of Yemen (12.9%). The sample size was calculated by using the following formula:

$$N = \frac{(Z)^2 \times p \times (1-P)}{(d)^2}$$

Where:

$N$  = sample size required,  $Z$  = confidence level at 95% (95%;  $Z = 1.96$ ),  $P$  = expected prevalence or proportion ( $p = 12.9\%$ ) according to the prevalence of *T. gondii* in Dhamar [23], and  $d$  = relative precision (margin of error at 5%) ( $d = 5\%$ ). Therefore, the minimum sample size was 173 patients. The sample size was increased ( $13.5\% = 27$ ) to increase the power and validity of the study. Therefore, the final sample size was set to 200.

### Ethical statement

The ethical statement of the study was approved by the Research Ethics Review Committee of Al-Yemenia University, Faculty of Medical Sciences, Yemen. The college deans granted permission to start data collection. Before collecting specimens, the purpose of the study was explained to the managers of female students who agreed to be part of this work. Further, participation was voluntary, and participants completed a consent form provided by the investigators.

### Sample collection

Aseptically, 5 ml of venous blood was collected from 200 healthy female students, aged. The blood specimens were then labeled and transferred into a sterile anticoagulant-free bottle and allowed to clot, and the serum was used to detect the *Toxoplasma gondii* antibodies.

### Laboratory assay

The rapid test technique was used to assess the *Toxoplasma gondii* antibodies. Each serum specimen was examined for anti-*T. gondii* IgG and IgM antibodies put the cassette onto the desk with the sample window up, took 80–100  $\mu$ l of serum with a pipettor vertically to each sample hole of the test device, added one drop of buffer, and read the test results immediately within 15–20 minutes [24].

### Data analysis

The obtained data were analyzed by the computer software program, the Statistical Package for Social Sciences (SPSS), version 21. Categorical variables were reported as frequencies and percentages in tables and figures. Moreover, confidence interval (95% CI) and odds ratio (OR) were used to assess the association between dependent and independent variables. Additionally, all probability values were considered statistically significant at  $P$ -values  $<0.05$ .

## Results

### Socio-demographic parameters of study subjects

A total of two hundred female students were enrolled in the current study. The highest proportion of specimens were collected from participants aged 21–24 years old (55.5%), who live in the urban areas (91.5%), unmarried individuals (82.0%), their father's employment (75%), their mothers are working as housewives (81.0%), their fathers had a university certificate (36.0%), their mothers had a basic certificate (30.5%), and their families had a middle income (87.5%), as listed in Table 1.

**Table 1.** Socio-demographic parameters of study subjects

Variables	Categories	No.	%
Age groups (Years)	18-20	69	34.5
	21-24	111	55.5
	25-28	20	10.0
Residence	Rural	17	8.5
	Urban	183	91.5
Marital status	Single	164	82.0
	Married	36	18.0
Father occupation	Working	150	75.0
	Not Working	50	25.0
Mother Occupation	Employee	38	19.0
	Housewife	162	81.0
Father education	Illiterate	10	5.0
	Basic (Read and write)	25	12.5
	Primary	41	20.5
	Secondary	52	26.0

	University	72	36.0
<b>Mother education</b>	Illiterate	36	18.0
	Basic (Read and write)	61	30.5
	Primary	52	26.0
	Secondary	33	16.5
	University	18	9.0
<b>Income status</b>	Low	10	5.0
	Middle	175	87.5
	High	15	7.5

**Prevalence of anti-Toxoplasma gondii IgG and IgM antibodies**

According to Table 2, the study revealed that 12 (6.0%) specimens tested positive for *T. gondii* IgG antibodies, while the remaining 188 (94.0%) tested negative for both IgG and IgM antibodies.

**Table (2).** Seroprevalence of anti-Toxoplasma gondii IgG and IgM antibodies among the examined women

Antibody types	Examined female students n = 200			
	Positive		Negative	
	No.	%	No.	%
<b>IgG</b>	12	6.0	188	94.0
<b>IgM</b>	00	00	200	100.0

This finding revealed that *T. gondii* infections were significantly higher among the age groups 18–20 and 21–24 years (41.7%), with a non-significant difference ( $P > 0.05$ ). Additionally, a higher rate of *T. gondii* infection was observed among female students living in urban areas (83.3%) and unmarried females (83.8%). Regarding income status, the highest prevalence of *T. gondii* was reported among middle-income participants (66.7%), with no statistically significant differences ( $P = 0.920$ ), as listed in Table 3.

**Table 3.** Prevalence of *Toxoplasma gondii* according to socio-demographic parameters of study subjects

Variables	Categories	<i>Toxoplasma gondii</i> antibodies IgG			P. value*
		Positive	Negative	Total	
		No. (%)	No. (%)	No. (%)	
<b>Age group</b>	20 –18	5.0 (41.7)	64 (34.0)	69 (34.5)	0.877
	24 –21	5.0 (41.7)	106 (56.4)	111 (55.5)	
	28 –25	2.0 (16.6)	18 (9.6)	20 (10.0)	
	<b>Total</b>	12.0 (100)	188 (100)	200 (100)	
<b>Residence</b>	Rural	2.0 (16.7)	15 (8.0)	17 (18.5)	780.2
	Urban	10.0 (83.3)	731 (92.0)	183 (91.5)	
	<b>Total</b>	12.0 (100)	188 (100)	200 (100)	
<b>Marital status</b>	Single	10.0 (83.3)	154 (81.9)	164 (82.0)	0.920
	Married	2.0 (16.7)	34 (18.1)	36 (18.0)	
	<b>Total</b>	12.0 (100)	188 (100)	200 (100)	
<b>Income status</b>	Low	1.0 (8.3)	9.0 (4.8)	10 (5.0)	0.152
	Middle	8.0 (66.7)	167 (88.8)	175 (87.5)	
	High	3.0 (25.0)	12 (6.4)	15 (7.5)	
	<b>Total</b>	12.0 (100)	188 (100)	200 (100)	

P value =  $P < 0.05$ : significant)

The present findings showed that a high rate of *T. gondii* IgG was reported among female students who did not raise cats in their house (66.7%) ( $P = 0.801$ ), did not change cat litter (66.7%) ( $P = 0.990$ ), did not contact cats (75.0%) ( $P = 0.830$ ), did not chew Khat (83.3%) ( $P = 0.566$ ), did not contact soil (75.0%) ( $P = 0.246$ ), and ate food from outside home (75.0%) ( $P = 0.246$ ). There was no statistically significant association between participant behaviors with cats and soil and the prevalence of *T. gondii* infection ( $P > 0.05$ ) (Table 4).

**Table 4.** Prevalence of *T. gondii* infections according to participant behaviors with cats and soil

Variables	Categories	<i>Toxoplasma gondii</i> IgG antibodies			P. value*
		Positive	Negative	Total	
		No. (%)	No. (%)	No. (%)	
Raising cats at home	Yes	4 (33.3)	16 (35.1)	70 (35.0)	0.801
	No	8 (66.7)	122 (64.9)	130 (65.0)	
Changing cat litter	Yes	4 (33.3)	63 (33.5)	67 (33.5)	0.990
	No	8 (66.6)	125 (66.5)	133 (66.5)	
Direct contact with cats	Yes	3 (25.0)	65 (75.0)	68 (34.0)	0.830
	No	9 (75.0)	124 (25.0)	132 (66.0)	
Chewing Khat	Yes	2 (16.7)	45 (23.9)	47 (23.5)	0.566
	No	10 (83.3)	143 (76.1)	153 (76.5)	
Contact with soil	Yes	3 (25.0)	79 (42.0)	82 (41.0)	0.246
	No	9 (75.0)	109 (58.0)	118 (59)	
Eating food from outside the home	Yes	9 (75.0)	162 (86.2)	171 (85.5)	0.288
	No	3 (25.0)	26 (13.8)	29 (14.5)	

95% CI = Confidence interval; P value =  $P < 0.05$ : significant

The current findings indicate a significant increase in *T. gondii* infections among female students who regularly wash their kitchen knives ( $P = 0.132$ ), refrain from consuming raw or undercooked meat ( $P = 0.566$ ), avoid untreated water ( $P = 0.316$ ), wash vegetables before eating ( $P = 0.134$ ), avoid drinking unpasteurized milk ( $P = 0.216$ ), and do not receive blood transfusions ( $P = 0.658$ ). However, there was no statistically significant association between these behaviors and the presence of *T. gondii* infection ( $P > 0.05$ ), as presented in Table 5.

**Table 5.** The prevalence of *Toxoplasma gondii* infections according to risk factors.

Variables	Categories	<i>Toxoplasma gondii</i> IgG antibodies			P. value*
		Positive	Negative	Total	
		No. (%)	No. (%)	No. (%)	
Regularly washing kitchen knives.	Yes	9 (75.0)	168 (89.4)	177 (88.5)	0.132
	No	3 (25.0)	20 (10.6)	23 (11.5)	
Consumed raw or undercooked meat	Yes	2 (16.7)	45 (23.9)	47(23.6)	0.556
	No	10 (83.3)	143(76.1)	153(79.5)	
Consumed untreated water	Yes	2 (16.7)	57 (30.3)	59(29.5)	0.316
	No	10 (83.3)	131 (69.7)	141(70.5)	
Washing vegetables before eating	Yes	12 (100)	158 (84.0)	170(85.0)	0.134
	No	00 (0.0)	30 (16.0)	30(15.0)	
Drinking unpasteurized milk	Yes	2 (16.7)	64 (34.0)	66(33.0)	0.216
	No	10 (83.3)	124 (66.0)	134(67.0)	
History of blood transfusion	Yes	1 (8.3)	10 (5.3)	11 (5.5)	0.658

Variables	Categories	<i>Toxoplasma gondii</i> IgG antibodies			P. value*
		Positive	Negative	Total	
		No. (%)	No. (%)	No. (%)	
	No	11 (91.7)	178 (49.7)	189(94.5)	

P value = P < 0.05: significant)

### Risk factors associated with *Toxoplasma gondii* infections

A high risk of *Toxoplasma gondii* infection was observed in female students who washed vegetables before eating (OR = 4.810; 95% CI = 83.432-0.277), followed by raising cats in the house (OR=3.812; CI=1.030-14.108), and a history of blood transfusion (OR=1.618; CI=0.189-13.808), as listed in Table 6.

Table 6. The major of *Toxoplasma gondii* infections among female students.

Variables	OR	95% CI	P-value
Raising cats in the house	3.812	1.030-14.108	0.045
Changing cat litter	0.992	0.2877-3.420	0.989
Direct contact with the cat	0.635	0.166-2.430	0.508
Chewing khat	0.635	0.134-3.008	0.567
Contact with soil	0.459	0.120-1.753	0.255
Eating food from outside the home	0.481	0.122-1.896	0.296
Regulatory washing kitchen knife	0.357	0.089-1.428	0.145
Consumed raw or undercooked meat	0.635	0.134-3.008	0.567
Consumed untreated water	0.459	0.097-2.165	0.325
Washing vegetables before eating	4.810	0.2774_ 83.432	0.280
Drinking unpasteurized milk	0.387	0.082-1.821	0.230
History of blood transfusion	1.618	0.189-13.808	0.659

95% CI = Confidence interval; P value = P < 0.05: significant)

### Discussion

*Toxoplasma gondii* is an opportunistic intracellular parasitic protozoan that can infect intermediate hosts (including humans, ruminants, rodents, and birds) [25–27]. There are various explanations behind Yemen's high prevalence of infections, particularly. These challenges encompass living situations, economic and environmental factors, a lack of public health awareness, a lack of sanitary facilities and infrastructure, and a lack of access to safe drinking water [28–29].

The overall seroprevalence of *T. gondii* infection reported by this study was (6.0%), which is lower than the study carried out in women of childbearing age in the faculty of medicine and health sciences in Hodeida City, Yemen, who were seropositive for *T. gondii* IgG (14.5%) [30], and this rate is lower than that reported in other countries such as Brazil (71%), Lebanon (82.6%), Ethiopia (85.3%), Ghana (92.5%), Libya (32.7%) [31–35]. The variations in toxoplasmosis seroprevalence may be due to differences in geographical location and characteristics of study subjects, such as age, educational level, cat handling, hygiene, and feeding habits. The use of different serological methods may also be responsible for discrepancies.

Also, all the positive cases concerning *T. gondii* have IgG antibodies, but IgM antibodies were not found among the positive cases, which is the same as the study carried out among women in Aden city [16]. The study showed that there was no significant association between positive *T. gondii* cases and the increasing age since old female students showed a lower ratio of *T. gondii* than young ones, which was inconsistent with several previous studies that reported a significant increase in the level of toxoplasmosis infection with increasing age [36–38]. Al-Eryani et al. [15] state that, in Yemen, old women have a higher chance of being exposed to *T. gondii* since they are responsible for agricultural activities and the raising of animals [17]. The present study showed that there was a high prevalence of *T. gondii* among female students who reside in urban areas, and this is consistent with other studies [19, 23], and at the same time, it is inconsistent with other studies [23, 39].

The current study showed the high prevalence of *T. gondii* among female students who don't have contact with cats and don't change cat litter. This finding was in disagreement with several previous studies that pointed to the existence of a significant association between *T. gondii* and contact with cats and raising them at home [40-41]. This could be explained by the fact that the presence of a cat at home can cause zoonosis facilitated by the handling of cat litter.

There was a high prevalence of *T. gondii* among the female students who eat food from outside the home, who don't consume untreated water, and who don't chew Khat. This is in contrast with previous studies, which state that there was no significant association of *T. gondii* with these factors [19, 42]. The findings showed that all prevalence cases of *T. gondii* are among female students who regularly wash kitchen knives and who don't wash vegetables before eating. And that is by previous studies [43-44].

In the present study, the findings showed that there was a high prevalence of *T. gondii* among female students who don't consume raw or undercooked meat. This was in contrast with previous studies, which refer to the association of *T. gondii* with consuming raw or undercooked meat [45-46]. The high prevalence of *Toxoplasma gondii* among female students who don't drink unpasteurized milk and the high prevalence of *T. gondii* among single female students, who are from the middle class, and who don't have blood transfusion history. These findings are inconsistent with another study [23]. This result corroborates that reported by Hamaichat in Morocco, who found a positive correlation between the consumption of undercooked or uncooked meat and the seroprevalence of toxoplasmosis [47]. This is probably because undercooked or uncooked meat contains tissue cysts of the *T. gondii* parasite. This difference could be explained by the different dietary habits of the participants in each study. It could also be explained by the analytical methods used in these studies.

The high risk of *T. gondii* infection was in the female students who were washing vegetables before eating (OR = 4.810; 95% CI = 83.432-0.277), followed by raising cats in the house (OR = 3.812; CI = 1.030-14.108), and had a had a history of blood transfusion (OR = 1.618; CI = 0.189-13.808). These findings are inconsistent with another study [48, 23].

#### **Limitations of the study**

The limitations of this study include a small sample size, serologic tests performed using rapid assays, and a lack of more advanced diagnostic techniques such as enzyme-linked immunosorbent assay (ELISA), which is highly accurate and reliable due to limited resources.

#### **Conflict of Interest**

The authors have no conflict of interest.

#### **Acknowledgments**

The authors would like to thank the team investigators, including Eman Sailan, Hanan Al-Taheri, Taghreed Rabe'a, Maha Al-Kamim, Nosiba Althujiry, and Noora Hadi, for their generous help in collecting data and specimens. All authors would like to express their gratitude to the female students of Al-Yemenia University, teachers, doctors, and Al-Yemenia University chairman for their contributions and support during the various aspects of the study.

#### **Author Contributions**

Al-Wajeeh and Al-Hadheq conceived and designed the experiments; Al-Wajeeh performed the experiments; Al-Wajeeh and Nomaan analyzed the data and wrote the first draft of the manuscript. Edress contributed to writing the manuscript. Al-Wajeeh and Al-Shehari agreed with the results and conclusions of the manuscript. All authors read, revised, and approved the final manuscript.

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