

Brief Original Article

***Toxoplasma gondii* infection among pregnant women in Yemen: Factors associated with high seroprevalence**

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Abstract

Introduction: Although toxoplasmosis is an important public health problem, there is scarcity of data on the disease available from Yemen. A cross-sectional survey was conducted in health facilities to determine seroprevalence of *Toxoplasma gondii* and associated risk factors among pregnant women in Sana'a, the capital city of Yemen.

Methodology: A total of 593 pregnant women were included and examined for anti-*T. gondii* antibodies (Ab) using enzyme-linked immunosorbent assay. Bio and socio-demographic data were collected by pre-tested structured questionnaires through face-to-face interviews. **Results:** The overall seroprevalence of *T. gondii* was 45.4% (95% confidence interval: 41%–49%). The prevalence of anti-*T. gondii* IgG and IgM was 43.7 (95% CI: 40–48%) and 9.1% (95% CI: 7%–12%), respectively. About 7.4 (95% CI: 6%–10%) of pregnant women were seropositive for both IgG and IgM Abs. Multivariate logistic regression analysis identified the following risk factors for toxoplasmosis (IgG and/or IgM): age \geq 25 years (adjusted OR: 2.02, 95% CI: 1.44–2.84, $p < 0.001$), rearing cats in the house (OR: 1.75, 95% CI: 1.20–2.55, $p = 0.004$), and contact with soil (OR: 1.90, 95% CI: 1.32–2.75, $p = 0.001$).

Conclusions: The study reported high seroprevalence among pregnant women in Sana'a, Yemen, with a high proportion of pregnant women having a possibility of acute toxoplasmosis. This highlights the need for including routine screening for *T. gondii* in pregnant women in the country's antenatal clinics. In addition, health education on the mode of transmission of toxoplasmosis should be provided for pregnant women in Yemen.

Key words: toxoplasmosis; seroprevalence; anti-*Toxoplasma gondii* antibodies; pregnant women; risk factors.

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Introduction

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite (phylum: Apicomplexa), which infects almost all warm-blooded animals including humans; up to a third of the world's population is reported to be seropositive [1,2]. The primary host is the domestic cat, and humans are usually infected through ingestion of infective oocysts excreted in cat feces, which could occur through direct contact with cats or through water, food, and soil contaminated with oocysts. Infection can also result from ingestion of tissue cysts when handling or consuming raw or undercooked meat from infected animals [1,3].

Primary infection with *T. gondii* in pregnant women may lead to the development of congenital toxoplasmosis [4-6]. 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 [6]. The risk for toxoplasmosis increases with age, lower socio-economic status, low education level, contact with cats, and poor hygiene practices [6-8]. Diagnosis of toxoplasmosis is usually based on the levels of immunoglobulin G (IgG) Ab for previous infection and immunoglobulin M (IgM) Ab for active infection [8,9].

In Yemen, which has been ranked as one of the least-developed countries, high seroprevalence of toxoplasmosis among pregnant women has been reported previously [10-12]. However, toxoplasmosis is not included in the routine investigations of pregnant women in Yemen. Furthermore, the underlying predictors of toxoplasmosis in the country are still not well identified. The present study aimed to determine the prevalence of toxoplasmosis in pregnant women and to identify risk factors associated with seroprevalence in Sana'a, Yemen.

Methodology

Samples and serological testing

Blood samples were collected from pregnant women seeking health care in public and private health facilities in Sana'a city, the capital of the Republic of Yemen, from December 2010 to April 2011. Anti-*T. gondii* IgG and IgM Abs were detected using an enzyme-linked immunosorbent assay (ELISA) kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's protocol. The sera were considered positive if IgG and/or IgM Ab were detected.

Data collection

Data were collected using pre-tested structured questionnaires through face-to-face interviews with the study participants. The questionnaire included socio-demographic data such as age, education level, and the number of children for each participant. Questions on knowledge and practices toward toxoplasmosis were also included. The knowledge of the study participants was assessed through questions regarding meanings of the term *toxoplasmosis* (in Arabic) or the common local term used (Al-gerthoumah or *the germ*).

The interviewers were trained simultaneously and discussed the questionnaire among themselves to decrease inter-observer variation. Participation was voluntary and all participants signed informed consent after being given a clear explanation of the objectives of the study. The ethical committee of Sana'a University, Republic of Yemen, approved the study protocol.

Data analysis

Data were analyzed using Statistical Package for Social Sciences for Windows (SPSS) version 21 (SPSS Inc., Chicago, USA). Variables were presented as proportions and patients seropositive for anti-*T. gondii*. IgG and/or IgM Ab were considered as the outcomes. The association between explanatory variables and outcome were tested using the Pearson Chi-square test.

Odds ratios (OR) and their 95% confidence intervals (CI) were reported in the bivariate analysis. Multivariate analysis using stepwise forward logistic regression model was applied on variables with $p < 0.05$ in bivariate analysis.

Results

A total of 593 pregnant women were included. Their ages ranged from 15 to 48 years, with a median age of 25 years (interquartile range: 21–32 years). The overall prevalence of anti-*T. gondii* Abs (IgG and/or IgM) was 45.4% (269/593) (95% confidence interval: 41%–49%). The prevalence of IgG and IgM was 43.7% (259/593) (95% CI: 40%–48%) and 9.1% (54/593) (95% CI: 7%–12%), respectively. A total of 44 (7.4%) pregnant women had positive sera for both anti-*T. gondii* IgG and IgM Abs. The study results showed an increasing prevalence of previous infection by age (Table 1).

Bivariate analysis identified the following factors to be significantly associated with toxoplasmosis (IgG and/or IgM): age ≥ 25 years, education level, number of children, cat rearing, contact with soil, use of unwashed kitchen knife, and eating unwashed vegetables (Table 2). Independent factors in the multivariate logistic regression analysis included age ≥ 25 years, cat rearing, and contact with soil (Table 3).

Discussion

Our data showed a high prevalence of both previous and a possibility of active toxoplasmosis in the study population. About 9% of pregnant women attending antenatal clinics had IgM, which indicates the possibility of early or acute infection of *T. gondii*, highlighting the high risk of placental transmission to the fetus and, consequently, congenital toxoplasmosis. The presence of IgM without IgG Ab may indicate an early infection, while the presence of both IgG and IgM Abs suggests acute infection [13]. However, it has been reported that IgM may remain positive for up to 18 months after infection [14].

Table 1. Prevalence of anti-*Toxoplasma gondii* Ab stratified by age group in Sana'a, Yemen.

Age (years)	n	IgG	95% CI	IgM	95% CI
15–20	127	41 (32.3)	(25–41)	12 (9.4)	(5–16)
21–25	205	77 (37.6)	(31–44)	18 (8.8)	(6–13)
26–30	156	76 (48.7)	(41–56)	14 (9.0)	(5–14)
31–35	66	38 (57.6)	(46–69)	5 (7.6)	(3–17)
36–40	30	20 (66.7)	(49–81)	3 (10)	(3–26)
> 40	9	7 (77.8)	(45–94)	2 (22)	(6–55)
Total	593	259 (43.7)	(40–48)	54 (9.1)	(7–12)

Table 2: Bivariate analysis of factors associated with seroprevalence of toxoplasmosis (n = 593).

Exposure variable	n	n positive	OR (95% CI)	P value
<u>Socio-demographic factors</u>				
Age (years)				
≥ 25	261	144 (55.2)	2.06 (1.48–2.87)	< 0.001
< 25	332	124 (37.4)	1	
Education				
Uneducated	181	102 (56.4)	1.91 (1.34–2.72)	< 0.001
Educated	412	166 (40.3)	1	
No. of children				
> 2	182	98 (53.9)	1.66 (1.17–2.36)	0.003
≤ 2	410	169 (41.2)	1	
<u>Knowledge</u>				
Knowing toxoplasmosis				
No	222	94 (42.3)	0.83 (0.59–1.16)	0.160
Yes	371	174 (46.9)	1	
Knowing the mode of transmission				
No	396	180 (45.5)	1.03 (0.73–1.45)	0.463
Yes	197	88 (44.7)	1	
<u>Practices</u>				
Rearing cats in house				
Yes	189	110 (58.2)	2.16 (1.52–3.08)	< 0.001
No	404	158 (39.1)	1	
Contact with soil				
Yes	218	126 (57.8)	2.23 (1.59–3.14)	< 0.001
No	374	142 (38.0)	1	
Eating food from outside home				
Yes	423	200 (47.3)	1.34 (0.93–1.93)	0.064
No	170	68 (40.0)	1	
Regularly washing kitchen knife				
No	103	57 (55.3)	1.63 (1.06–2.51)	0.015
Yes	490	211 (43.1)	1	
Cooking meat well				
No	24	15 (62.5)	2.08 (0.89–4.83)	0.063
Yes	569	253 (44.5)	1	
Washing vegetables before eating				
No	22	15 (68.2)	2.69 (1.08–6.70)	0.023
Yes	571	253 (44.3)	1	

Table 3. Multivariable analysis of factors associated with seroprevalence of toxoplasmosis in pregnant women.

Variables	Adjusted OR	95% CI	P value
Age ≥ 25 years	2.02	(1.44–2.84)	< 0.001
Rearing cats in house	1.75	(1.20–2.55)	0.004
Contact with soil	1.90	(1.32–2.75)	0.001

Furthermore, false positives for IgM are expected, which necessitates repeating the test after two weeks before considering the patient infected [15]. The presence of IgG Ab, in our study about 44%, may indicate that infection with toxoplasmosis among these pregnant women had occurred in the past very commonly (chronic infection). A previous study conducted in the south of Yemen reported higher overall seroprevalence of *T. gondii* (64.3%), with 14% of pregnant women seropositive for IgM Abs [12]. Compared to other studies conducted in the region, a study conducted in Sudan showed a seroprevalence of IgG Ab of 34.1% and a higher prevalence of IgM Ab (14.3%) [16]. High seroprevalence of IgG (46.1%) and IgM (18.4%) was also reported from Egypt [17]. In Saudi Arabia, a neighboring country of Yemen, the prevalence of IgG and IgM Abs was 29.4% and 5.6%, respectively [18]. In Ethiopia, the overall seroprevalence of *T. gondii* was 81.4%, with 3.1% and 4% of pregnant women seropositive for both IgG and IgM, and IgM alone, respectively [19]. However, it should be noted that comparing the seroprevalence of *T. gondii* between the different studies is problematic due to the different populations tested and the different serological tests and cut-offs used.

Bivariate analysis showed significant association between illiteracy and seroprevalence of *T. gondii* infection, which is in agreement with previous studies [12,20,21]. It is expected that pregnant women who are illiterate may not be aware of the prevention and mode of transmission of toxoplasmosis. Pregnant women who had more than two children were more exposed to toxoplasmosis in this study. Similar findings have been reported in several studies [5,22,23]. However, multiparity, which increases the prevalence of toxoplasmosis, is still unknown [5]. Not washing kitchen knives after cutting raw meat and not washing vegetables before eating were significantly associated with the seroprevalence of *T. gondii* in the bivariate analysis. These findings are in agreement with recent studies [19,21,24,25]. The use of unwashed kitchen knives and eating unwashed vegetables may play a significant role in disease transmission [25,26]. However, these factors were not identified as independent risk factors of *T. gondii* infection in multivariate analysis in this study.

Multivariate analysis identified that pregnant woman ≥ 25 years of age are at high risk of having anti-*T. gondii* Abs compared to younger (15–24 year-old) women. This finding is consistent with several previous reports [5,10,12,27]. In Yemen, women ≥ 25 years of age are more likely to be involved in agricultural

activities, rearing of animals, and taking care of children, and therefore have a higher chance of being exposed to *T. gondii*, which may explain the higher seroprevalence within this age group in our study.

The present study also indicated that rearing cats in the home is a significant risk factor for seropositivity. Previous studies conducted in Aden, Yemen [12], Ethiopia [4], Tunis [24], Brazil [27] and East Asia [21] have reported a significant association between contact with cats and toxoplasmosis. The cat is the definitive host of *T. gondii* [27] and avoidance of contact with cats is considered to be the key for control of toxoplasmosis [28].

In this study, contact with soil was significantly associated with anti-*T. gondii* Abs, highlighting the importance of this route of transmission, as reported in previous studies [29-31]. This significant risk factor could be explained by the habit of keeping cats in the Yemeni community, which acts as a potential source for contamination of the soil, and the Yemeni climate, which sustains the infectivity of oocysts. The oocysts of *T. gondii* can remain infective for many months in suitable environment conditions such as warm temperature, moisture, and shade [32,33], which is the typical and relatively stable environment in Yemen. Studies in areas of colder or unfavorable climate for oocyst survival showed no risk for contact with soil [34,35]. This study is limited by the fact that it is clinic based; however, it should be noted that these clinics are public and are where most pregnant women attend seeking health care. Furthermore, the diagnosis of acute infection is limited by the fact that repeating the serological test for samples positive for IgM after two weeks of the first test was not conducted due to difficulties in following the patients and the cost of the test. Thus, false positives for IgM are possible.

Conclusions

A high seroprevalence of both IgM and IgG in pregnant women attending antenatal clinics in Sana'a city, Yemen, was found, indicating a high risk for the offspring. The identified risk factors should be taken into consideration in the control of toxoplasmosis in Yemen. Community health campaigns should be formed to educate the women on the risk factors and modes of prevention. The study's findings also emphasize the importance of including testing for toxoplasmosis as routine screening in all antenatal clinics.

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Authors' contributions

All authors contributed substantially to the conception and design of the study, and/or acquisition of data, and/or analysis of the data, interpretation of data, drafting the article and/or revising it critically.

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