

## ***Toxoplasma gondii* Seroprevalence among Pregnant Women in Taiz City, Yemen: Association with Water Source and Feline Exposure**

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## Abstract

Toxoplasmosis poses significant risks to pregnant women and their unborn children. The study aimed at assessing the prevalence and analyzing the risk factors associated with *T. gondii* infection among pregnant women in Taiz City, Yemen. 400 randomly recruited pregnant women were tested for *T. gondii* antibodies (IgM and IgG) by enzyme immunoassay. Bio- and socio-demographic data were gathered using a validated questionnaire, completed during face-to-face interviews. Univariate and multivariate analyses were performed to determine the independent variables associated with *T. gondii* seroprevalence. The study population had an average age of 26 years, with most participants in the second trimester. A significant proportion of participants reported living in rural areas and had exposure to animals, particularly cats. The seroprevalence of *Toxoplasma gondii* infection stands at a notable 36% with 24.5% positive for IgG antibodies only, 1.8% positive for IgM antibodies while, 9.7% positive for IgG and IgM antibodies. The study found a significant association between *T. gondii* seroprevalence and a history of miscarriage, households with unimproved water sources and ownership of cats in the household. Multivariate analysis confirmed that participants who lived in households with unimproved water sources and those who owned cats were significantly more likely to have *T. gondii* seroprevalence. This study highlights a substantial seroprevalence of *T. gondii* infection among pregnant women in Taiz City, Yemen, emphasizing the importance of implementing preventive measures. Promoting access to safe drinking water, advocating for hygiene practices, and raising awareness are essential to mitigate the burden of toxoplasmosis in pregnant women.

**Keywords:** *T. gondii* Infection, Pregnant Women, Seroprevalence, Risk Factors, Toxoplasmosis, Maternal Health, Child Health

## INTRODUCTION

*Toxoplasma gondii* can infect humans and animals including mammals and birds,<sup>1</sup> with cat serving as the definitive host. Human infection occurs through ingestion of food or water contaminated with sporulated oocysts excreted in cat feces, consumption of undercooked meat containing tissue cysts, or, less frequently, via contaminated needles, blood transfusion, organ transplantation, or vertical transmission.<sup>2-4</sup>

The global infection rate of *T. gondii* is over 30%, but varies widely by region, from 10%-90%.<sup>5</sup> For most healthy individuals, this often presents no symptoms but can be severe in immunocompromised persons. The most serious concern is congenital toxoplasmosis resulting from primary infection during pregnancy, which can lead to congenital anomalies, neurological and ocular complications, miscarriage, or stillbirth.<sup>6,7</sup> Higher prevalence is reported among pregnant women in specific regions especially in impoverished countries across Africa and selective regions of the Middle East,<sup>8</sup> influenced by environmental, behavioral, socio-demographic, and obstetric risk factors, including consumption of raw or

undercooked meat, unprotected water sources, direct contact with cats, and history of adverse pregnancy outcomes.<sup>9-13</sup>

Diagnostic methods for *T. gondii* infection include serological assays, molecular techniques, and clinical evaluations. Serological assays detecting IgM and IgG antibodies are widely used for screening and diagnosis.<sup>14</sup> However, interpreting the results is complicated by long term persistence of IgM and lifelong IgG (at low level),<sup>15-17</sup> prompting the introduction of IgG avidity testing to distinguish previous from current infections, and avoid false positive results of IgM testing.<sup>18-20</sup> Despite these limits, IgM/IgG detection remains the most widely used initial screening method,<sup>21,22</sup> with newer assays employing recombinant antigens showing promise for improved accuracy.<sup>23</sup>

Yemen, a country facing numerous healthcare challenges exacerbated by ongoing conflict and limited resources, is particularly vulnerable to infectious diseases<sup>24</sup> such as toxoplasmosis. Previous studies in Yemen reveal varying rates of *T. gondii* infection among pregnant women ranging from 14.4%-64.3% across different regions of the country, but most studies were geographically restricted, based on small samples,

or lacked multivariate analysis to adequately assess risk factors.<sup>12,13,25-29</sup> Given Yemen's diverse geographic conditions and variation in hygiene/cultural practices across regions, it is unclear whether the findings from prior localized research can be generalized nationwide.

Toxoplasmosis remains a neglected infection in Yemen, as it is not part of the regular screening tests for pregnant women. This study aimed to investigate the seroprevalence of *T. gondii* infection among pregnant women in Taiz city, Yemen, and explore the associated risk factors. By examining environmental exposures, demographic characteristics, and obstetric histories, we seek to identify determinants of *T. gondii* infection in this population. Through this research, we endeavor to contribute to the evidence base for informed decision-making and the development of targeted interventions to prevent and control *T. gondii* infection in pregnant women, ultimately improving maternal and child health outcomes in Yemen and beyond.

## MATERIALS AND METHODS

### Study design and setting

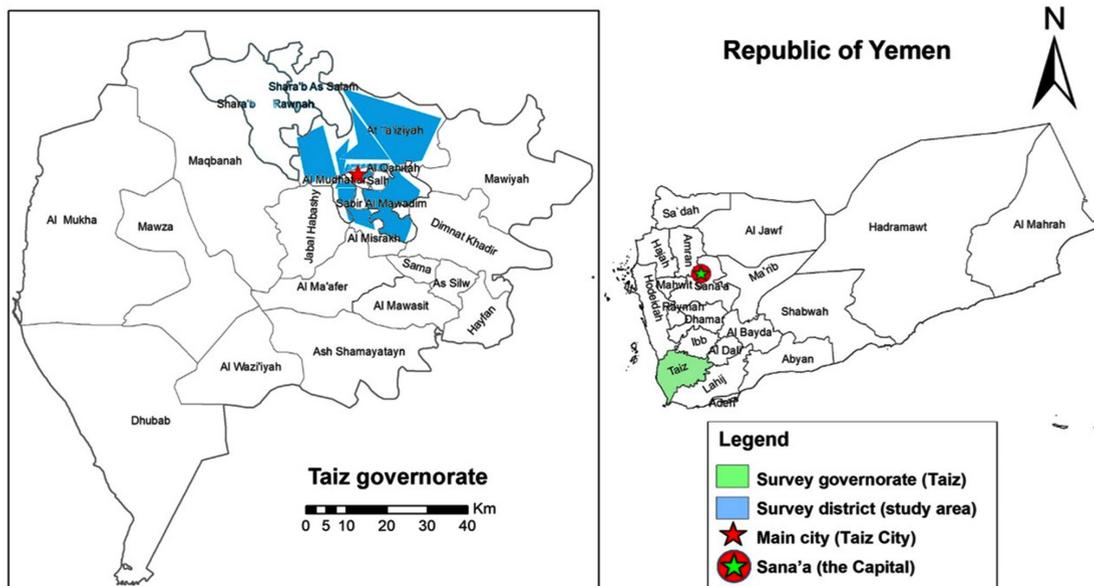
A cross-sectional study was carried out in Taiz, Yemen, from October 2023 to

February 2024. Data were collected using a pre-tested questionnaire first developed in English and translated into Arabic. Pregnant women attending routine antenatal visits underwent standard clinical assessments, including physical examination, history-taking, laboratory testing, and ultrasound.

Taiz is a densely inhabited city in Yemen and rises at about 1,400 meters above the Red Sea (Figure). It has minimal seasonal temperature variation (20-30 °C) and moderate humidity (40%-60%).<sup>30</sup> This study was conducted in a maternal and child health facility serving around 10,000 patients annually, selected for its accessibility and comprehensive antenatal services.

### Study population and sampling

Participants were pregnant women seeking antenatal care. The sample size (n = 400) was determined using the WHO formula for health studies,<sup>31</sup> based on a 46.2% previously reported toxoplasmosis prevalence.<sup>26</sup> Every pregnant woman present on the day of recruitment was eligible for inclusion, ensuring a representative sample from the target population, and random selection was utilized to minimize selection bias and enhance the generalizability of the study findings.



**Figure.** A geographical map showing the study area (Taiz City) in Taiz governorate

### Data collection procedures

Trained healthcare professionals conducted interviews and gathered pertinent data. Training included standardized instruction on questionnaire administration, specimen collection, and confidentiality procedures. A standardized questionnaire was utilized to gather data on the participants' age, gestational age, educational level, employment status, household water sources, animal husbandry, and khat chewing habits, i.e., chewing the green leaves of the *Catha edulis* shrub for social and psychological purposes, a common practice in Yemen and East Africa. Water sources were classified as improved or unimproved following UNICEF/WHO Joint Monitoring Program for Water Supply and Sanitation.<sup>32</sup>

### Serological analysis

Antibodies against *T. gondii* were analyzed using an electrochemiluminescence immunoassay (ECLIA) on the fully automated Elecsys immunoassay analyzer (Roche Diagnostics, Mannheim, Germany), employing recombinant antigens (SAG1, GRA1, GRA7). IgG and IgM positivity was defined as a signal/cutoff of  $\geq 1.0$ . Reported sensitivities and specificities were 100%/99.8% for IgG and 96.7%/99.8% for IgM.<sup>33,34</sup> Quality control included manufacturer-provided controls and retesting of samples suspected of prozone effect. This ECLIA method has been well validated for toxoplasmosis screening and showed excellent agreement with reference immunofluorescence and ELISA methods.<sup>35</sup>

### Ethical considerations

Ethical approval was obtained from the Faculty of Medicine and Health Sciences, Taiz University (IRB-2023-05-12). Written informed consent was secured from all participants, and confidentiality was maintained throughout the study.

## RESULTS

### Characteristics of the study population

This study involved 400 pregnant women aged 15-46 years, with an average age of 26 years (interquartile range: 21-31 years). Gestational age was distributed as follows: 26.5% in the first

trimester, 38.3% in the second trimester and 35.3% in the third trimester. In addition, about 26.2% of the participants had no formal education. In addition, 66% and 48.5% of the women stated that they kept animals and had cats in the household, respectively. 37.3% (149/400) of the women reported a spontaneous abortion in the past. Furthermore, the majority of pregnant women had access to unimproved water sources (61.8%, 247/400), as shown in Table 1.

### Associated risk factors and seropositivity of *T. gondii*

In the study, a general seroprevalence of anti-*T. gondii* antibodies (IgG and/or IgM) was

**Table 1.** Characteristics of study participants in Taiz, Yemen (N = 400)

Characteristics	N (%)
<b>Age groups (years)</b>	
15-29	180 (45)
$\geq 30$	220 (55)
<b>Number of children</b>	
<3	146 (36.5)
$\geq 3$	254 (63.5)
<b>Gestational age</b>	
First trimester	106 (26.5)
Second trimester	153 (38.3)
Third semester	141 (35.3)
<b>Residence</b>	
Urban	152 (38)
Rural	248 (62)
<b>Education</b>	
Primary school or above	295 (73.8)
No formal education	105 (26.2)
<b>History of spontaneous abortion</b>	
No	251 (62.75)
Yes	149 (37.3)
<b>Employment</b>	
Employed	217 (54.2)
Unemployed	183 (45.8)
<b>Type of household water source</b>	
Improved	153 (38.2)
Unimproved	247 (61.8)
<b>Rearing animals</b>	
No	136 (34)
Yes	264 (66)
<b>Having cats at home</b>	
No	206 (51.5)
Yes	194 (48.5)

found in 36% (144/400) of pregnant women. Specifically, 24.5% (98/400) showed reactivity to IgG antibodies only, 9.7% (39/400) showed reactivity to IgG and IgM antibodies, and 1.8% (7/400) showed reactivity to IgM antibodies only. Chi-square test was employed in identifying the factors associated with *T. gondii* seropositivity.

As shown in the Chi-square analysis (Table 2), a significant association was found between *T. gondii* infection and a history of spontaneous abortion in pregnant women (OR = 1.61, 95% CI = 1.06-2.45, P = 0.026). Similarly, pregnant women living in households without access to improved water sources had more than double the risk of

**Table 2.** Bivariate analysis of factors associated with seroprevalence of toxoplasmosis (N = 400)

Variables	<i>Toxoplasma</i> seroprevalence			
	N participants	N (%)	COR (95% CI)	P-value
<b>Age groups (years)</b>				
15-29	180	56 (31.1)	1	
≥30	220	88 (40.0)	0.68 (0.45-1.03)	0.065
<b>Number of children</b>				
<3	146	61 (41.8)	1	
≥3	254	83 (32.7)	0.68 (0.44-1.03)	0.068
<b>Education</b>				
Primary school or above	295	111 (37.6)	1	
No formal education	105	33 (31.4)	0.76 (0.47-1.22)	0.256
<b>Employment</b>				
Employed	183	58 (31.7)	1	
Unemployed	217	86 (39.6)	0.71 (0.47-1.06)	0.099
<b>Gestational age</b>				
First trimester	106	46 (43.4)	1	
Second trimester	153	50 (32.7)	0.63 (0.38-1.05)	0.080
Third trimester	141	48 (34.0)	0.67 (0.40-1.13)	0.135
<b>History of spontaneous abortion</b>				
No	251	80 (31.9)	1	
Yes	149	64 (43.3)	1.61 (1.06-2.45)	<b>0.026*</b>
<b>Residence</b>				
Urban	152	61 (40.1)	1	
Rural	248	83 (33.5)	0.75 (0.49-1.14)	0.178
<b>History of blood transfusion</b>				
No	223	83 (37.2)	1	
Yes	177	61 (34.5)	0.89 (0.59-1.34)	0.568
<b>Type of household water source</b>				
Improved	153	36 (23.5)	1	
Unimproved	247	108 (43.7)	2.53 (1.61-3.96)	<b>0.001*</b>
<b>Handling fresh meat</b>				
No	131	41 (31.3)	1	
Yes	269	103 (38.3)	0.73 (0.47-1.14)	0.172
<b>Rearing animals</b>				
No	136	56 (41.2)	1	
Yes	264	88 (33.3)	0.714 (0.47-1.09)	0.122
<b>Having cats at home</b>				
No	206	62 (30.1)	1	
Yes	194	82 (42.3)	1.70 (1.13-2.57)	<b>0.011*</b>

COR: Crude Odds ratio; CI: Confidence interval; \*: Significant association (P < 0.05)

**Table 3.** Multivariable analysis of factors associated with seroprevalence of toxoplasmosis in pregnant women (N = 400)

Variables	AOR	95% CI	P-value
<b>History of spontaneous abortion</b>			
No	1		
Yes	0.742	0.26-0.65	0.184
<b>Type of household water source</b>			
Improved	1		
Unimproved	2.65	1.68-4.17	<b>0.001*</b>
<b>Having cats at home</b>			
No	1		
Yes	1.65	1.08-2.51	<b>0.021*</b>

*T. gondii* infection than those who had access to improved water sources (OR = 2.53; 95% CI = 1.61-3.96, P = 0.001). In addition, the risk of *T. gondii* infection was more than 1.7 times higher in pregnant women who keep cats at home than in women who do not have cats at home (OR = 1.70; 95% CI = 1.13-2.57, P = 0.011). However, the study found no statistically significant association between *T. gondii* infection and the participants' gestational age (P = 0.135), age group, employment and education status, number of children, history of blood transfusions, handling of fresh meat, and pet ownership.

A multivariate logistic regression analysis was performed to investigate the association between the seroprevalence of *T. gondii* infection and possible risk factors. Variables entered into the multiple logistic regression were history of spontaneous abortion, lack of access to improved water sources, and having cats at home. The results showed that lack of access to improved water sources (AOR = 2.65; 95% CI = 1.68-4.17, P = 0.001), and the presence of cats in the house (AOR = 1.65; 95% CI = 1.08-2.51, P = 0.021) were significant independent risk factors for *T. gondii* infection (Table 3).

## DISCUSSION

Toxoplasmosis can be fatal and lead to long-term complications, especially if it is congenital or occurs in immunocompromised individuals.<sup>36</sup> The findings underscore the

significance of understanding the epidemiology of *T. gondii* infection, given its potential for severe consequences, especially in congenital cases or among immunocompromised individuals.

Overall, the prevalence of *T. gondii* infection in this study was 36%, which is lower compared to previous studies conducted in different parts of Yemen.<sup>13,25-27</sup> For example, in the rural community of Taiz governorate, 46.2% (166/359) of pregnant women were seropositive for *T. gondii* infection.<sup>26</sup> In Aden governorate in southern Yemen, 64.3% (431/670) of those who were pregnant and sought care at private clinics and hospitals were also seropositive.<sup>29</sup> However, a recent study of 90 pregnant women in the Hodeida governorate reported a lesser prevalence (14.4%) of *T. gondii* infection.

The rate of seropositivity observed in this study aligns with previous findings conducted in Sudan (34.1%) and Tobago (39.3%).<sup>37,38</sup> In contrast, Lebanon (82.6%) and Ethiopia (85.3%) reported a higher seroprevalence rate.<sup>39,40</sup> In addition, Saudi Arabia (8.3%), Zambia (5.9%) and Sri Lanka (12.3%) exhibited a lower rate of seropositivity for the infection than the rates revealed in the current study.<sup>41-43</sup> Several studies indicate that the seroprevalence of *T. gondii* infection varies significantly by country, region and community and may be influenced by factors such as diet, socioeconomic status, culture, water quality, and sanitation.<sup>10,44</sup>

The study's result revealed 11.5% of the pregnant women tested had IgM antibodies. Of these, 9.7% detected positive for both anti-*T. gondii* IgM and IgG antibodies, while 1.8% tested positive for IgM antibodies only. These results align with the outcomes of earlier studies on pregnant women in Dhamar governorate.<sup>12</sup> However, it was reported that the seroprevalence of anti-*T. gondii* IgM antibodies was lower in pregnant women living in a rural community in Taiz, southern Yemen.<sup>26</sup> When the Toxoplasma IgM antibodies are present with negative IgG antibodies, this may suggest early infection, but the concurrent detection of IgM and IgG antibodies indicates acute infection. In the present study, however, these results were not confirmed by seroconversion tests and/or IgG avidity tests. It is important to note that the detection of IgM antibodies cannot definitely

confirm an acute *Toxoplasma* infection, as IgM antibodies can remain positive for up to two years in chronic infections.<sup>14</sup>

The results of the study suggest that, in accordance with previous global studies, history of miscarriage and feline exposure were significant independent predictors of *T. gondii* infection.<sup>45-47</sup> Consistent with prior global research, the study results indicate that a history of miscarriage and exposure to felines were the key factors linked with infection by *T. gondii*,<sup>45-47</sup> which has a causal link to miscarriages in the first trimester and IgM seropositivity.<sup>48</sup> In addition, domestic cats are known to be a source of contamination with *T. gondii* sporocyst.<sup>49</sup> Furthermore, based on the univariate analysis, a statistically significant association between toxoplasmosis and the use of unimproved drinking water sources among participants in Taiz. Pregnant women who used unimproved drinking water sources were about twice as likely to be infected as those who used improved sources. This observation aligns with the report from rural communities in Taiz, which demonstrated a remarkable association between the high prevalence of *T. gondii* infection and the consumption of unimproved drinking water.<sup>26</sup>

The age of the participants, their level of education, place of residence, employment status, number of children, handling of fresh meat, history of blood transfusion and presence of animals (except cats) in the household did not significantly affect the seroprevalence of known *T. gondii* infection in the present study. These findings are consistent with those from other researches.<sup>50,51</sup> In contrast, consumption of untreated water is generally not considered a significant risk factor for *T. gondii* in pregnant women in Dhamar, Yemen.<sup>12</sup> The differences in the results are possibly due to the differing climatic conditions in the Dhamar district and Taiz governorate in Yemen. Dhamar, which is situated in the mountains, has a colder and drier climate, while Taiz has a warmer and more humid climate. The warm and humid environment in Taiz could provide a more favorable atmosphere for the oocysts of *T. gondii* to remain infectious longer in the water, which could explain the higher incidence of infection compared to Dhamar.<sup>9</sup>

This study has some limitations that should be acknowledged. As a hospital-based

cross-sectional analysis, we could not determine causality or the time of infection. This may limit the depth of understanding regarding the factors influencing *T. gondii* infection, hence cautious consideration is needed when interpreting the results and generalizing findings. To mitigate these limitations, future research endeavors should prioritize longitudinal cohort and community-based designs, incorporate avidity testing and direct detection methods like PCR, and broaden recruitment strategies to encompass a more diverse participant pool. These approaches would enhance the validity and generalizability of findings, thus providing a more comprehensive understanding of *T. gondii* infection dynamics among pregnant women in Taiz city, Yemen, and facilitating the development of targeted interventions to mitigate its impact on maternal and child.

## CONCLUSION

The study suggests that 36% of pregnant women interviewed in Taiz city had evidence of previous exposure to *T. gondii* infection indicating a substantial number of women were exposed to the parasite. Further analysis revealed two key risk factors associated with infection: lack of access to improved water sources and presence of cats in households. Identifying these risk factors may help in the development of public health strategies to avert and control *T. gondii* infection in pregnant women. Promoting safe drinking water sources, encouraging good hygiene practices, and providing education about the risks linked to handling cats and contact with their feces are recommended measures to decreased the risk of *T. gondii* infection in pregnant women.

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None.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

TA, JCA, JD, RH, ARA, FDH, IA and CPC conceptualized the study. TA and IA performed data collection. TA, JCA, JD, RH, ARA, FDH, IA and

CPC applied methodology. TA, JCA, JD, RH, ARA and FDH performed formal analysis. TA, JCA and IA performed visualization. TA, JCA, JD, RH, ARA, FDH, IA and CPC performed validation. TA and JCA performed project administration. TA, JCA, JD, RH, IA and CPC wrote the manuscript. All authors read and approved the final manuscript for publication.

## FUNDING

None.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Faculty of Medicine and Health Sciences, Taiz University, Taiz, Yemen (IRB-2023-05-12).

## INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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