




# *Toxoplasma gondii* Infection in Relation to Pregnancy Characteristics and Bad Obstetric History among Pregnant Women Seeking Healthcare in Sana'a City, Yemen

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## ABSTRACT

**Objective:** To determine the seroprevalence and risk factors associated with *Toxoplasma gondii* among pregnant women seeking healthcare in Sana'a city in relation to pregnancy characteristics and bad obstetric history (BOH).

**Methods:** This hospital-based, cross-sectional study was conducted among 379 pregnant women seeking healthcare in Sana'a city. Data on socioeconomic and pregnancy characteristics, BOH and possible risk factors associated with *T. gondii* infection were collected using a structured, pre-designed questionnaire. Anti-*Toxoplasma* IgG and IgM antibodies were detected using OnSite™ Combo tests. Data were analyzed using the IBM SPSS Statistics using appropriate statistical tests, and a bivariate logistic regression model was used to determine the risk factors possibly associated with *T. gondii* infection among pregnant women. Differences or associations between categorical variables were considered statistically significant at *P* values <0.05.

**Results:** *T. gondii* infection was seroprevalent among 18.7% of pregnant women seeking healthcare in Sana'a. Educational status and household size were the sociodemographic factors significantly associated with anti-*Toxoplasma* IgG seropositivity. Illiterate women were at fourfold higher risk of infection compared to those with higher education (OR = 4.0, 95% CI: 1.69–9.25; *P* = 0.002). Although those with school education were twice more likely to be infected with *T. gondii* compared to those with higher education, the association was on the borderline (OR = 2.0, 95% CI: 0.98–4.06; *P* = 0.055). On the other hand, pregnant women living in households of ≥5 members were 1.7 times more likely to be infected (OR = 1.7, 95% CI: 1.03–2.95; *P* = 0.026). Regarding the BOH among pregnant women, past exposure to infection was significantly associated with premature delivery (*P* = 0.004) and history of fetal deaths (*P* = 0.004). Drinking unboiled water (OR = 2.1, 95% CI = 0.55–8.07; *P* = 0.192) and frequent blood transfusion (OR = 1.8, 95% CI = 0.50–6.1; *P* = 0.288) increased the odds of exposure of pregnant women to infection, but the associations were not statistically significant. On the other hand, other studied factors were not significantly associated with past exposure to infection.

**Conclusions:** The majority (>80.0%) of pregnant women seeking healthcare in Sana'a city are non-immune to primary infection with *T. gondii* during pregnancy as evidenced by their seronegative status, where only 18.7% of them were seropositive for anti-*Toxoplasma* IgG. Higher infection rates were significantly associated with being illiterate and living within households of ≥5 members. With respect to self-reported BOH, *T. gondii* infection was significantly associated with premature delivery and history of fetal deaths. Drinking unboiled water and frequent blood transfusions increased the odds of exposure of pregnant women to infection, though not reaching statistical significance. Further large-scale community-based studies are recommended to determine the risk factors associated with pregnancy characteristics and BOH among pregnant women in the country.

**Keywords:** *Toxoplasma gondii*, Pregnancy, Bad obstetric History, Yemen

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## 1. Introduction

Toxoplasmosis is caused by *Toxoplasma gondii*, a polyxenous tissue cyst-forming coccidium with a heteroxenous life cycle that is able to infect all warm-blooded mammals, including humans.<sup>(1)</sup> It is one of the most prevalent parasitic zoonoses on a global scale, where approximately 30–50% of the world's population has been assumed to have chronic/latent infections.<sup>(2)</sup> Human infection is mainly acquired by ingestion of undercooked or raw meat harboring tissue cysts or oocyst-contaminated food or water.<sup>(3–5)</sup> Transmission may also occur through tachyzoites in transfused blood, tissue transplants or raw milk.<sup>(1)</sup> Congenital transmission occurs predominantly following primary infection of a pregnant woman, but it has also been described from pregnant women infected shortly prior to pregnancy, immunosuppressed women with a reactivated infection during pregnancy or women infected with a different serotype than that previously acquired before pregnancy.<sup>(6)</sup>

Severe complications of toxoplasmosis, such as encephalitis, can occur in immunocompromized patients.<sup>(7)</sup> Moreover, ocular toxoplasmosis, particularly retinochoroiditis, and fatal multivisceral complications associated with atypical parasite genotypes can develop regardless of the immune status of the host.<sup>(8–10)</sup> Congenital toxoplasmosis may lead to spontaneous miscarriage, stillbirth, congenital anomalies in addition to major ocular and neurological consequences.<sup>(11–13)</sup> Although acute infections in immunocompetent pregnant women are usually asymptomatic, 10% of women may experience some symptoms such as lymphadenopathy, flu-like illness, fever, headache, myositis and chorioretinitis.<sup>(14, 15)</sup> The highest incidence rates of congenital toxoplasmosis have been reported from low-income African countries as well as parts of the Middle East.<sup>(16)</sup> Variable *T. gondii* seroprevalence rates among pregnant women or those of childbearing age have been reported from different parts of the world. In a systematic review on the global status of *T. gondii* in-

fection among such women, Pappas et al.<sup>(17)</sup> concluded seroprevalence rates of 6.1–77.5% in the Americas, 8.2–63.2% in Europe, 25.3–75.2% in Africa and 0.8–60% in Asia and Oceania. Globally, the incidence of congenital toxoplasmosis has been estimated to be >190,000 cases (95% confidence interval (CI): 179,300–206,300), with 1.20 million disability-adjusted life years (95% CI: 0.76–1.90).<sup>(16)</sup>

In Sana'a city, the capital of Yemen, anti-*Toxoplasma* IgG and IgM antibodies were reported among 43.7% and 9.1% of pregnant women, respectively.<sup>(18)</sup> Comparable rates of 44.0–46.2% and 3.3% were also reported for anti-*Toxoplasma* IgG and IgM antibodies, respectively, among pregnant women in Taiz governorate.<sup>(19, 20)</sup> On the other hand, seroprevalence rates of 31.0% and 14.0% were found among women attending private clinics and hospitals in Aden city for anti-*Toxoplasma* IgG and IgM antibodies, respectively.<sup>(21)</sup>

Given that the most recently published study on *T. gondii* infection among pregnant women in Sana'a city was conducted in 2010–2011,<sup>(18)</sup> there is a need for a relook at the epidemiologic situation of the infection. Moreover, studies on *T. gondii* among pregnant women with a focus on its association with bad obstetric history (BOH) are few in the country. Therefore, the present study aimed to determine the seroprevalence and risk factors associated with *T. gondii* among pregnant women seeking healthcare in Sana'a city in relation to pregnancy characteristics and BOH.

## 2. Methods

### 2.1. Study design, setting and population

This was a hospital-based, cross-sectional study conducted among randomly selected pregnant women seeking healthcare care in five public and private hospitals and health centers in Sana'a city. Inclusion criteria were pregnant women of any age seeking healthcare in outpatient clinics, having no pregnancy complications and giving in-



formed consent to participate in the study on a voluntary basis.

## 2.2. Sample size calculation

Based on a seroprevalence rate of 43.7% for anti-*Toxoplasma* IgG antibody among pregnant women in Sana'a,<sup>(18)</sup> a minimum sample size of 379 was calculated at a confidence level of 95% and an accepted marginal error of 5%.

## 2.3. Data and blood sample collection

Data on sociodemographic and pregnancy characteristics, self-reported BOH and possible risk factors associated with *T. gondii* infection were collected using a structured, pre-designed questionnaire. Blood samples of 3–5 ml were collected into pre-labeled plain test tubes and left to clot, and sera were separated by centrifugation at 3000 rounds per minute for 5 min. Sera were then either tested immediately or transferred into Eppendorf tubes and stored at 8°C until the detection of anti-*Toxoplasma* IgG and IgM antibodies.

## 2.4. Serological investigations

Sera were tested for anti-*Toxoplasma* IgG and IgM antibodies using OnSite™ Toxo IgG/IgM Combo tests (CTK Biotech, Inc., San Diego, CA, USA) according to the instructions of the manufacturer. As per the manufacturer, the OnSite™ detects and differentiates *Toxoplasma* IgM and IgG with an overall diagnostic accuracy of 97.8% and 94.9%, respectively, compared to enzyme-linked immunosorbent assay (ELISA) and no false-positive cross-reactivity with other infectious or autoimmune conditions.

In a blinded evaluation of diagnostic tests for the detection of anti-*Toxoplasma* IgG and IgM against gold-standard testing performed at the *Toxoplasma* Serology Laboratory of Palo Alto Medical Foundation in the United States, OnSite™ Toxo IgG/IgM Combo test showed sensitivity of 100% (95% CI: 97.8–100) and specificity 98.8% (95% CI: 93.2–99.9) for the detection of *Toxoplasma*

IgG.<sup>(22)</sup> Despite its low sensitivity for the detection of anti-*Toxoplasma* IgM,<sup>(22)</sup> the main goal of the present study was not to detect recent infection (IgM seropositivity) but to detect past exposure to the parasite (IgG seropositivity).

## 2.5. Statistical analysis

Data were entered and verified using Excel spreadsheets. Coded data were then exported and analyzed using the IBM SPSS Statistics for Windows®, version 21.0 (IBM Corp., Armonk, NY, USA). Chi-square and Fischer's exact tests, whichever suitable, were used to test the differences or associations between categorical variables. A bivariate logistic regression model was used to determine the risk factors possibly associated with *T. gondii* infection among pregnant women, where the results were presented as odds ratios (ORs) and their corresponding 95% CIs. Differences and associations were considered statistically significant at *P* values <0.05.

## 3. Results

### 3.1. Sociodemographic characteristics of the study population

Table (1) shows that more than half of pregnant women seeking healthcare in hospitals of Sana'a city were aged >25 years (53.3%; 202/379), with a mean age of 26.89 ± 5.47 years (range: 17–43). In addition, the majority of women were urban residents (82.8%; 314/379), unemployed (89.7%; 340/379), while about a half of them were living within households of ≥5 members (50.9%; 193/379). On the other hand, 14.0% (53/379) of pregnant women seeking healthcare in the hospitals of Sana'a city were illiterate, whereas comparable proportions of the educated women had primary, secondary or higher educational levels.



**Table 1.** Characteristics of pregnant women included in the study\*

Characteristic	Frequency (%)
<b>Age (years)</b>	
Mean ± SD	26.89 ± 5.47 years
Range	17–43
≤25	177 (46.7)
>25	202 (53.3)
<b>Residence</b>	
Rural	65 (17.2)
Urban	314 (82.8)
<b>Household size (members)</b>	
<5	186 (49.1)
≥5	193 (50.9)
<b>Educational status</b>	
Illiterate	53 (14.0)
Primary education	109 (28.8)
Secondary education	114 (30.0)
Higher education	103 (27.2)
<b>Employment status</b>	
Employed	39 (10.3)
Unemployed	340 (89.7)

\* Total number of women in the study was 379.

### 3.2. Distribution of pregnant women in relation to pregnancy characteristics and BOH

Table (2) shows that the mean gestational age of pregnant women seeking healthcare in Sana'a city was 21.4±10.19 weeks. The majority of pregnant women seeking healthcare in Sana'a city were in the third trimester of pregnancy (37.2%; 141/379) followed by those in the second and first trimesters, being 35.6% and 27.2%, respectively. In addition, the majority of pregnant women were multigravid (73.6%; 279/379), and less than half of multigravid women (46.6%; 130/279) reported a history of miscarriage. Of whom, 80.8% reported <3 miscarriages.

The majority of women delivering live births (87.0%; 220/253) reported the delivery of <5 births, while only 7.5% (19/253) of them experienced premature births. Of whom, 26.3% (5/19) experienced it more than once. In addition, a small proportion (7.5%; 21/279) of multigravid women reported history of fetal deaths (Table 2).

### 3.3. Seroprevalence of *T. gondii* infection among pregnant women

Table (3) shows that the majority of pregnant women had not been exposed to infection with *T. gondii*, where anti-*Toxoplasma* IgG antibodies were detected among 18.7% (71/379) of women. However, only one woman was found to be seropositive for anti-*Toxoplasma* IgM.

**Table 2.** Distribution of pregnant women seeking healthcare in Sana'a city according to pregnancy characteristics and BOH (2018-2019)\*

Characteristic	Frequency (%)
<b>Gestational age</b>	
Mean ± SD (weeks)	21.4±10.91
Range	1–40
First trimester	103 (27.2)
Second trimester	135 (35.6)
Third trimester	141 (37.2)
<b>Gravidity</b>	
Primigravid	100 (26.4)
Multigravid	279 (73.6)
<b>Parity <sup>a</sup></b>	
<5	220 (87.0)
≥5	33 (13.0)
<b>History of miscarriage <sup>b</sup></b>	
Yes	130 (46.6)
No	149 (53.4)
<b>Frequency of miscarriages <sup>c</sup></b>	
<3	105 (80.8)
≥3	25 (19.2)
<b>History of premature delivery <sup>a</sup></b>	
Yes	19 (7.5)
No	234 (92.5)
<b>Frequency of premature delivery <sup>d</sup></b>	
Once	14 (73.7)
More than once	5 (26.3)
<b>History of having children with eye problems <sup>b</sup></b>	
Yes	3 (1.1)
No	276 (98.9)
<b>History of having children with mental retardation <sup>b</sup></b>	
Yes	4 (1.4)
No	275 (98.6)
<b>History of fetal death <sup>b</sup></b>	
Yes	21 (7.5)
No	258 (92.5)

\* The total number of women participating in the study was 379; BOH, bad obstetric history; <sup>a</sup>, calculated for 253 women reporting the delivery of live births; <sup>b</sup>, calculated for multigravid women; <sup>c</sup>, calculated for women reporting miscarriage; <sup>d</sup>, calculated for women reporting premature births.

**Table 3.** Seroprevalence of *T. gondii* infection among pregnant women seeking healthcare in Sana'a city (2018–2019)\*

Serostatus	n	(%)
Seronegative	307	(81.0)
IgG-seropositive	71	(18.7)
IgM-seropositive	1	(0.3)

\* Total number of pregnant women examined for *T. gondii* was 379; n, number positive for *T. gondii*.

### 3.4. Association of sociodemographic characteristics of pregnant women with anti-*Toxoplasma* IgG seropositivity

Table (4) shows statistically significant associations between the educational status ( $P < 0.05$ ) and household size ( $P = 0.026$ ) of pregnant women seeking healthcare in Sana'a city and anti-*Toxoplasma* IgG seropositivity. Illiterate women and those with school education were four times more likely to be infected with *T. gondii* compared to those with higher education (OR = 4.0, 95% CI:





1.69–9.25;  $P = 0.002$ ). Although pregnant women with school education were twice more likely to be infected with *T. gondii* infection than those with higher education, the association was on the borderline ( $P = 0.055$ ). On the other hand, women living in households of  $\geq 5$  members were 1.7 times more likely to be infected compared to those living within households of  $< 5$  members (OR = 1.7, 95% CI: 1.03–2.95;  $P = 0.026$ ). However, age ( $P = 0.463$ ) and residence ( $P = 0.206$ ) were not associated with anti-*Toxoplasma* seropositivity. Although unemployed women were 2.2 times more likely to be infected with *T. gondii* compared to employed women (OR = 2.2, 95% CI: 0.74–6.25), the difference did not reach the level of statistical significance ( $P = 0.108$ ).

### 3.5. *T. gondii* infection among pregnant women in relation to pregnancy characteristics and BOH

Table (5) shows that anti-*Toxoplasma* IgG seropositivity was significantly associated with history of premature delivery and history of fetal death among pregnant women. Women with premature deliveries were 4.3 times more likely to be infected with *T. gondii* (OR = 4.3; 95% CI = 1.67–11.43;  $P = 0.004$ ), while women with history of fetal deaths were 3.6 times more likely to be infected (OR = 3.6; 95% CI = 1.41–8.93;  $P = 0.009$ ). In contrast, gestational age ( $P > 0.05$ ), gravidity ( $P = 0.361$ ), parity ( $P = 0.465$ ), history of miscarriage ( $P = 0.542$ ), frequency of miscarriage ( $P = 0.069$ ), frequency of premature births ( $P = 0.444$ ), history of delivery of babies with eye problems ( $P = 0.097$ ) and frequency of fetal deaths ( $P = 0.586$ ) were not significantly associated with *T. gondii* infection among pregnant women. It is noteworthy that four women (three IgG-negative and one IgG-positive) reported having children with mental disabilities, while one IgG-negative woman reported having a child with hearing loss (data not shown).

**Table 4.** Association of certain sociodemographic characteristics with anti-*Toxoplasma* IgG seropositivity among pregnant women seeking healthcare in Sana'a city (2018–2019)

Characteristic	N	n (%)	OR (95% CI)	P value
<b>Age (years)</b>				
≤25	177	34 (19.2)	Ref.	
>25	202	37 (18.3)	0.9 (0.56–1.58)	0.463
<b>Residence</b>				
Rural	65	15 (23.1)	1.1 (0.93–1.21)	
Urban	314	56 (17.8)	Ref.	0.206
<b>Educational status</b>				
Illiterate	53	17 (32.1)	4.0 (1.69–9.25)	0.002
School education*	223	43 (19.3)	2.0 (0.98–4.06)	0.055
Higher education	103	11 (10.7)	Ref.	
<b>Employment status</b>				
Employed	39	4 (10.3)	Ref.	
Unemployed	340	67 (19.7)	2.2 (0.74–6.25)	0.108
<b>Household size (members)</b>				
<5	186	27 (14.5)	Ref.	
≥5	193	44 (22.8)	1.7 (1.03–2.95)	0.026

N, number examined; n, number positive for IgG; OR, odds ratio; CI, confidence interval; \* This includes primary and secondary education.

**Table 5.** Association of *T. gondii* infection with certain pregnancy characteristics and BOH among pregnant women seeking healthcare in Sana'a city (2018–2019)\*

Characteristic	N	n (%)	OR (95% CI)	P value
<b>Gestational age</b>				
First trimester	103	22 (21.4)	1.4 (0.72–2.69)	0.320
Second trimester	135	22 (16.3)	Ref.	
Third trimester	141	27 (19.1)	1.2 (0.65–2.26)	0.536
<b>Gravidity</b>				
Primigravid	100	17 (17.0)	Ref.	
Multigravid	54	279 (19.4)	1.2 (0.94–2.14)	0.361
<b>Parity</b>				
<5	220	42 (19.1)	0.9 (0.44–1.83)	
≥5	33	7 (21.2)	Ref.	0.465
<b>History of miscarriage</b>				
Yes	130	25 (19.2)	1.0 (0.54–1.79)	
No	149	29 (19.5)	Ref.	0.542
<b>Frequency of miscarriages</b>				
<3	105	17 (16.2)	Ref.	
≥3	25	8 (32.0)	2.4 (0.91–6.54)	0.069
<b>Premature delivery</b>				
Yes	19	9 (47.4)	4.3 (1.67–11.43)	
No	234	40 (17.1)	Ref.	0.004
<b>Frequency of premature births</b>				
Once	14	6 (42.9)	Ref.	
More than once	5	3 (60.0)	2.0 (0.25–16.00)	0.444
<b>History of delivery of babies with eye problems</b>				
Yes	3	2 (66.7)	8.6 (0.77–96.82)	
No	276	52 (18.8)	Ref.	0.097
<b>History of fetal death</b>				
Yes	21	9 (42.9)	3.6 (1.41–8.93)	
No	258	45 (17.4)	Ref.	0.009
<b>Frequency of fetal deaths</b>				
Once	17	7 (41.2)	Ref.	
More than once	4	2 (50.0)	1.4 (0.16–12.70)	0.586

BOH, bad obstetric history; N, number examined; n, number positive for IgG; OR, odds ratio; CI, confidence interval.

### 3.6. Risk factors associated with *T. gondii* infection

Table (6) shows that pregnant women who did not boil unbottled drinking water were approximately twice more likely to be seropositive for an-



ti-*Toxoplasma* IgG than those who boiled drinking water (OR = 2.1, 95% CI = 0.55–8.07), but the difference was not statistically significant ( $P = 0.192$ ). However, neither the presence of cat(s) inside the house (OR = 0.7, 95% CI = 0.36–1.39;  $P = 0.197$ ) nor cleaning the cat litter (OR = 0.5, 95% CI = 0.06–4.62;  $P = 0.487$ ) was significantly associated with anti-*Toxoplasma* seropositivity.

Because none of the pregnant women in the present study reported eating raw or semi-cooked meat/poultry, its association with anti-*Toxoplasma* IgG seropositivity could not be tested. In addition, there was no statistically significant association between the type meat most frequently consumed and anti-*Toxoplasma* IgG seropositivity (OR = 1.4, 95% CI = 0.78–2.50;  $P = 0.189$ ).

Similarly, other factors related to food hygiene; namely, not regularly washing vegetables and fruits before eating ( $P = 0.536$ ), not wearing hand gloves when handling meat or poultry (OR = 0.6, 95% CI = 0.34–1.11;  $P = 0.101$ ), using the same knife or cutting board used for meat or poultry for chopping vegetables (OR = 1.4, 95% CI = 0.80–2.30;  $P = 0.161$ ), not boiling raw milk before drinking (OR = 1.0, 95% CI = 0.66–1.65;  $P = 0.485$ ) and not washing hands regularly before eating (OR = 0.9, 95% CI = 0.10–8.37;  $P = 0.648$ ) were not significantly associated with anti-*Toxoplasma* IgG seropositivity among pregnant women. Contact with soil (OR = 0.9, 95% CI = 0.47–1.85;  $P = 0.373$ ), not wearing hand gloves before contact with soil (OR = 0.4, 95% CI = 0.18–1.12;  $P = 0.068$ ) and not washing hands regularly after contact with soil (OR = 0.8, 95% CI = 0.09–7.77;  $P = 0.615$ ) were not significantly associated with anti-*Toxoplasma* IgG seropositivity among pregnant women (Table 6).

The odds of anti-*Toxoplasma* IgG seropositivity increased among pregnant women with history of blood transfusion (OR = 1.6, 95% CI = 0.84–3.22;  $P = 0.104$ ) and its frequency (OR = 1.8, 95% CI = 0.50–6.15;  $P = 0.288$ ), but the associations were not statistically significant (Table 6).

**Table 6.** Risk factors associated with *T. gondii* IgG seropositivity among pregnant women seeking health care in Sana'a city (2018–2019)

Variable	N	n (%)	OR (95% CI)	P value
<b>Presence of cat(s) inside the house</b>				
Yes	81	12 (14.8)	0.7 (0.36–1.39)	0.197
No	298	59 (19.8)	Ref.	
<b>Cleaning the cat litter</b>				
Yes	11	1 (9.1)	0.5 (0.06–4.62)	0.487
No	70	11 (15.7)	Ref.	
<b>Regular washing of vegetables and fruits before eating</b>				
Yes	376	71 (18.7)	NA	0.536
No	3	0 (00.0)		
<b>Eating raw or semi-cooked meat/chicken</b>				
Yes	0	00 (00.0)	NA	1.000
No	379	71 (18.7)		
<b>Type of most frequently consumed meat*</b>				
Animal meat	39	10 (25.6)	1.4 (0.78–2.50)	0.189
Poultry meat	321	59 (18.4)	Ref.	
<b>Wearing gloves when handling meat or poultry</b>				
Yes	31	9 (29.0)	Ref.	0.101
No	348	62 (17.8)	0.6 (0.34–1.11)	
<b>Using the same knife/cutting board used for meat/poultry for chopping vegetables</b>				
Yes	133	29 (21.8)	1.4 (0.80–2.30)	0.161
No	246	42 (17.1)	Ref.	
<b>Boiling unbottled drinking water**</b>				
Yes	20	2 (10.0)	Ref.	0.192
No	190	40 (21.1)	2.1 (0.55–8.07)	
<b>Boiling raw milk before drinking</b>				
Yes	121	22 (18.2)	Ref.	0.485
No	258	49 (19.0)	1.0 (0.66–1.65)	
<b>Contact with soil (agriculture, gardening, etc.)</b>				
Yes	94	16 (17.0)	0.9 (0.47–1.85)	0.373
No	285	55 (19.3)	Ref.	
<b>Wearing hand gloves before contact with soil</b>				
Yes	40	10 (25.0)	Ref.	0.068
No	54	11 (11.1)	0.4 (0.18–1.12)	
<b>Washing hands regularly after contact with soil</b>				
Yes	89	15 (16.9)	Ref.	0.615
No	5	1 (20.0)	0.8 (0.09–7.77)	
<b>Washing hands regularly before eating</b>				
Yes	374	70 (18.7)	0.9 (0.10–8.37)	0.648
No	5	1 (20.0)	Ref.	
<b>History of blood transfusion</b>				
Yes	54	14 (25.9)	1.6 (0.84–3.22)	0.104
No	325	57 (17.5)	Ref.	
<b>Frequency of blood transfusions</b>				
Once	36	8 (22.2)	Ref.	0.288
More than once	18	6 (33.3)	1.8 (0.50–6.15)	

N, Number examined; n, number positive for IgG; OR, odds ratio; CI, confidence interval; \* 19 cases were missing; \*\* Calculated for 210 women reporting the drinking of unbottled water.

## 4. Discussion

The present study revealed that the seroprevalence rate of anti-*Toxoplasma* IgG among pregnant women seeking healthcare in Sana'a city was 18.7%. This rate is lower than those reported among pregnant women seeking healthcare in Sana'a (41.9–43.7%) in studies conducted between the years 2007 and 2011.<sup>(18, 23)</sup> In addition, it is lower than the rates reported among pregnant



women residing in rural areas (46.2%) and those seeking antenatal care (44.0%) in Taiz.<sup>(19, 20)</sup> On the other hand, it is comparable to that reported among pregnant women from the southern region of Saudi Arabia (20.0%),<sup>(24)</sup> but it is lower than the rates reported among pregnant women from Palestine (27.9%),<sup>(25)</sup> Egypt (30.2–67.5%),<sup>(26–28)</sup> Iraq (31.5%),<sup>(29)</sup> Sudan (34.1%),<sup>(30)</sup> Jordan (47.1%),<sup>(31)</sup> Tunisia (47.7–58.4%),<sup>(32, 33)</sup> Algeria (47.8%),<sup>(34)</sup> Morocco (50.6%),<sup>(35)</sup> different regions of Saudi Arabia (29.4–61.4%),<sup>(36–40)</sup> Libya (44.8%),<sup>(41)</sup> and Kuwait (53.1%).<sup>(42)</sup>

Variations in *T. gondii* seroprevalence could be attributed to climate conditions, differences in cultural and socioeconomic factors as well as hygienic and feeding habits. The role of adopting different methods of detection in the variations in the seroprevalence rates reported from different countries or regions within the same country could not also be ruled out. The use of the test kits in the present study is justified by their absolute sensitivity (100%) and very high specificity (98.8%) for the detection of anti-*Toxoplasma* IgG antibodies as established by the *Toxoplasma* Serology Laboratory.<sup>(22)</sup> Moreover, the main goal of the study did not target the detection of anti-*Toxoplasma* IgM as a marker of probable recent infections but to investigate the prevalence of anti-*Toxoplasma* IgG as a marker of past exposure to infection.

In fact, the large proportion of *Toxoplasma*-seronegative, non-immune pregnant women in the present study is quite alarming because of the potential risk of primary infection with *T. gondii* during pregnancy, which can lead to congenital toxoplasmosis if not promptly diagnosed and treated.<sup>(11–13)</sup> In IgG-seropositive women, however, preconception infection with *T. gondii* confers little or no risk to the fetus.<sup>(43)</sup> The low IgM seropositivity rate (0.3%) in the present study is consistent with the low rate of 3.3% among pregnant women in rural areas of Taiz.<sup>(19)</sup> However, the low sensitivity of the tests used in the present study in

the detection of anti-*Toxoplasma* IgM could not rule out the presence of false negativity. Therefore, it is difficult to compare the rate of anti-*Toxoplasma* IgM in the present study with studies adopting ELISA or other serological methods. On the other hand, it remains uncertain whether the single IgM-seropositive woman in the present study had an acute infection because neither seroconversion nor IgG avidity testing was performed on the serum.

Of the studied sociodemographic factors, the educational status and household size were significantly associated with past exposure to *T. gondii* infection among pregnant women. Illiterate women and those living in households of  $\geq 5$  members had significantly higher rates of infection with *T. gondii* than their counterparts. In line with this finding, being uneducated or illiterate was a significant risk factor for infection with *T. gondii* among pregnant women from Sana'a and Aden.<sup>(18, 21)</sup> In contrast, education of pregnant women was not found to be significantly associated with past exposure to *T. gondii* infection among rural pregnant women from Taiz.<sup>(19)</sup>

In the present study, however, no significant association was found between past exposure to *T. gondii* infection among pregnant women and age, residence, or employment status. In contrast, age of  $\geq 25$  years was an independent significant predictor associated with *T. gondii* infection among pregnant women seeking healthcare in Sana'a city (2010–2011) and rural pregnant women from Taiz.<sup>(18, 19)</sup> Increasing age was also found to be significantly associated with past exposure to *T. gondii* among pregnant women from Jordan and Saudi Arabia.<sup>(24, 31, 37, 39, 40)</sup> However, it was not found to be a significant predictor of *T. gondii* infection among Sudanese pregnant women.<sup>(30)</sup> The association between educational status with past exposure to *T. gondii* is inconsistent with the finding recently reported among rural pregnant women from Taiz.<sup>(19)</sup> In contrast to the finding of the present study, no statistically significant differ-



ence in anti-*Toxoplasma* IgG seroprevalence was found between educated and non-educated pregnant women from Saudi Arabia.<sup>(37)</sup>

Although *T. gondii* infection was not significantly associated with the gestational age in the present study, a large proportion of seronegative, or non-immune, women are still at high risk of primary infection during their first trimester. It is noteworthy that the placenta acts as an efficient barrier to parasite transmission in the first trimester, but its permeability increases at the late stages of pregnancy.<sup>(44)</sup> The majority of primary maternal *T. gondii* infections can be asymptomatic and approximately half of infected pregnant women giving birth to congenitally infected infants may not exhibit any symptoms or report risk factors suggestive of infection.<sup>(45, 46)</sup> Congenital *T. gondii* infection can lead to more severe clinical outcomes if primary maternal infection occurs during the first trimester than in the third trimester.<sup>(47–49)</sup> The lack of association between *T. gondii* and gestational age is consistent with that recently reported among pregnant women residing in rural areas and those seeking antenatal care in Taiz.<sup>(19, 20)</sup> In addition, the finding of the present study is in agreement with the findings reported among pregnant women from southwestern and Makkah in Saudi Arabia.<sup>(37, 40)</sup>

The present study did not reveal any statistically significant difference in the past exposure to *T. gondii* infection with respect to the pregnancy characteristics of gravidity and parity. Similarly, parity was not found to be a significant predictor of exposure to *T. gondii* among rural pregnant women and those seeking antenatal care in Taiz.<sup>(19, 20)</sup> In contrast, having >2 children was significantly associated with past exposure to *T. gondii* infection among pregnant women seeking healthcare in the hospitals of Sana'a in 2010–2011.<sup>(18)</sup> In contrast to the finding of the present study, a statistically significant association between anti-*Toxoplasma* IgG seroprevalence and gravidity was reported from Saudi Arabia and

Egypt.<sup>(39, 40, 50)</sup> Parity of >2 births was found to be significantly associated with *T. gondii* infection among pregnant women from southwestern Saudi Arabia,<sup>(40)</sup> but not among Sudanese pregnant women.<sup>(30)</sup>

With regard to BOH, past exposure to *T. gondii* was found to be significantly associated with premature delivery and history of fetal death. Up to the best of our knowledge, this is the first study to report the association of *T. gondii* infection with the delivery of preterm births in Yemen. In another context, a multicenter prospective cohort study on the association of congenital toxoplasmosis with preterm births in ten European countries concluded that infected babies were born or delivered earlier than uninfected ones,<sup>(51)</sup> though the exact mechanism shortening the length of gestation remains unknown. However, no statistically significant association was found between past exposure to *T. gondii* infection and self-reported miscarriages or fetal deaths in the present study. This, in turn, highlights the importance of the study of the TORCH (*T. gondii*, rubella virus, cytomegalovirus, and herpes simplex virus) panel among women with BOH to determine the relative contribution of these pathogens to BOH among Yemeni women.

The lack of significant association of anti-*Toxoplasma* IgG seroprevalence with history of miscarriage in the present study is consistent with that reported by Mahdy et al.,<sup>(19)</sup> where no statistically significant association between the past exposure to *T. gondii* and history of miscarriage among pregnant women at the rural community level of Taiz. In contrast, a significant association was reported between past exposure to *T. gondii* and abortion among pregnant women seeking antenatal care in Taiz and Aden.<sup>(20, 21)</sup> In agreement with the present study, past exposure to *T. gondii* was not found to be significantly associated with abortion among pregnant women from Palestine and southwestern region and Makkah in Saudi Arabia.<sup>(25, 37, 40)</sup> It is noteworthy that a number of





studies reported statistically insignificant differences in the seroprevalence of *T. gondii* between pregnant women with and without BOH from Egypt and India.<sup>(52-54)</sup> In contrast to the present study, an association between *T. gondii* infection and history of abortion was found among pregnant women from Egypt, Saudi Arabia, Sudan and Iraq.<sup>(28-30, 39, 50, 55)</sup>

Although cats, only known definitive hosts of *T. gondii*, play a critical role in disease epidemiology via environmental contamination with oocysts,<sup>(56-58)</sup> presence of cats in houses was not significantly associated with the *T. gondii* infection in the present study. This finding is in agreement with that reported among pregnant women from Taiz,<sup>(20)</sup> but it disagrees with those reported among pregnant women seeking healthcare in Sana'a and Aden.<sup>(18, 21)</sup> The lack of association in the present study could be attributed to the fact that only a small proportion of the population of Sana'a rear cats inside houses. Moreover, not only domestic cats are present in urban areas of the country, but also stray cats are largely distributed in the city. Therefore, unowned and stray cats contribute to the environmental contamination and obscure the individual role played by domestic ones in the dissemination of *T. gondii* infection. In parallel with the findings of the present study, ownership or contact with cats was not found to be a significant risk factor for infection with *T. gondii* among pregnant women in studies from Egypt, Saudi Arabia, Palestine and Sudan.<sup>(24, 25, 28, 30, 37)</sup> In contrast, contact with cats was found to be significantly associated with past exposure to *T. gondii* infection among pregnant women from Algeria.<sup>(34)</sup>

Contact with soil and its associated poor practices of not wearing gloves before or washing hands after dealing with it were not significantly associated with past exposure to *T. gondii* infection among pregnant women in the present study. This is in contrast to a previous finding among pregnant women seeking healthcare in Sana'a,

where contact with soil was found to be an independent risk factor for infection with the parasite.<sup>(18)</sup> It is also in contrast to the existence of an association reported from Menoufia - Egypt.<sup>(27)</sup> In line with the finding of the present study, a statistically significant association was found between contact with soil and past exposure to *T. gondii* infection among pregnant women from six Egyptian governorates and Jordan.<sup>(28, 31)</sup>

The local preferences of the majority of Yemen's population in eating meat/chicken cooked well under high pressure made it difficult to test the association of eating raw or semi-cooked meat/poultry with *T. gondii* infection in the present study. In line with this fact, all women in the present study reported eating well-cooked meat/poultry. It is worth mentioning that the lack of significant association between eating raw/undercooked meat and past exposure to infection with *T. gondii* was reported among pregnant women from Makkah, Saudi Arabia.<sup>(37)</sup> On the contrary, a significant association was confirmed between the consumption of undercooked or poorly cooked meat and *T. gondii* infection among pregnant women in other studies from the Arab region, including Egypt,<sup>(28, 50, 54)</sup> Jordan,<sup>(31)</sup> Tunisia,<sup>(33)</sup> Algeria<sup>(34)</sup> and eastern region of Saudi Arabia.<sup>(39)</sup> In the southern region of Saudi Arabia, eating raw or undercooked minced meat was found to increase the odds of *T. gondii* infection among pregnant women by approximately two-fold, but without statistical association.<sup>(24)</sup> In Sudan, however, eating raw meat such as liver but not undercooked meat was found to be significantly associated with *T. gondii* infection among pregnant women.<sup>(30)</sup>

In the present study, pregnant women who did not boil drinking water were approximately twice more likely to be exposed to infection with *T. gondii*, but without statistically significant association. This is in contrast to the finding reported among pregnant women from rural Taiz and Aden city,<sup>(19, 21)</sup> where a significant association was



found between unimproved water sources and the seroprevalence of *T. gondii* infection. In line with the finding of the present study, however, the type of water supply was not found to be significantly associated with *T. gondii* infection among pregnant women from Makkah- Saudi Arabia.<sup>(37)</sup> On the other hand, the lack of significant association of the consumption of unboiled raw milk with *T. gondii* infection among pregnant women in the present study is in disagreement with that reported among pregnant women from Aden.<sup>(21)</sup> In another context, it is consistent with that reported among pregnant women from Sudan,<sup>(30)</sup> but inconsistent with that reported among pregnant women from Menoufia - Egypt.<sup>(27)</sup>

The insignificant association of eating unwashed vegetables or fruits with past exposure to infection with *T. gondii* in the present study could be attributed to the very low proportion (<1%) of pregnant women who reported irregular washing of vegetables/fruits before eating. This finding is in contrast to that reported among pregnant women from Sana'a,<sup>(18)</sup> where eating unwashed vegetables was significantly associated with *T. gondii* infection. However, it was not found to be an independent predictor of infection on multivariable analysis.<sup>(18)</sup> It is also in contrast to the association between eating unwashed raw vegetables/fruits and *T. gondii* infection among pregnant women from Aden.<sup>(21)</sup> Additionally, it disagrees with those reported among pregnant women elsewhere in the region.<sup>(27, 33, 50)</sup> In another context, chewing Kath was not found to be a significant predictor of *T. gondii* infection among pregnant women from rural Taiz.<sup>(19)</sup>

Up to the best of our knowledge, no published studies assessed the association between blood transfusion and the risk of exposure to *T. gondii* infection among pregnant women in Yemen. In the present study, the odds of past exposure to infection increased, though statistically insignificant, by >1.5 times among pregnant women with history frequent transfusion of blood. This finding

highlights the importance of designing large-scale studies to assess such an association. It is well documented that blood transfusion could be a potential risk factor for *T. gondii* infection.<sup>(59)</sup> Meanwhile, blood can be transfused frequently to pregnant women, posing those seronegative to the risk of contracting primary infections.

This study is limited by the fact that it was a cross-sectional study based on cassette testing of the sera of pregnant women. However, the sensitivity and specificity of the kits used were high for the detection of anti-*Toxoplasma* IgG antibodies, enabling the study of the possible associations of past infection with BOH as well as the identification of risk factors associated with infection. Although the findings of this hospital-based study may not be generalizable at the community level, it highlights the existence of a large proportion of pregnant women not immune to primary infection during pregnancy, which raises concerns about the importance of including prenatal *T. gondii* serological screening and monitoring as part of the routine antenatal care.

## 5. Conclusions

Only 18.7% of pregnant women seeking healthcare in Sana'a city are seropositive for anti-*Toxoplasma* IgG. This indicates that the majority (>80.0%) of pregnant women are non-immune to primary infection during pregnancy as evidenced by their seronegative status. Higher infection rates are significantly associated with being illiterate and living within households of  $\geq 5$  members. With respect to self-reported BOH, *T. gondii* infection was significantly associated with premature delivery and history of fetal deaths. On the other hand, drinking unboiled water and frequent blood transfusion increased the odds of exposure to *T. gondii* infection among pregnant women, though not reaching statistical significance.

Further large-scale, community-based studies are recommended to determine the seropreva-



lence and risk factors associated with pregnancy characteristics and BOH among Yemeni women, preferably as part of the TORCH panel. The high seronegativity necessitates the implementation of health education programs and prenatal screening as preventive strategies against primary infection during pregnancy, which may pose a potential risk for the development of congenital toxoplasmosis. Longitudinal studies on the relationship between *T. gondii* infection and pregnancy outcomes are recommended to determine the contribution of congenital toxoplasmosis to such adverse outcomes among pregnant women in the country.

### Ethical considerations

The ethical clearance of this study was obtained from the Ethics Committee of the University of Science and Technology, Sana'a. Informed consent was obtained from pregnant women, who were asked to participate voluntarily after explaining to them the objectives of the study. Confidentiality of the study participants' data was assured.

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### Authors' contributions

RA designed the study, analyzed the data, supervised the whole work and wrote the manuscript. AA-N, AY, AA-H, AA, EMJ, HB, NA-D and NS collected the data and implemented the laboratory investigations. All authors read, revised and approved the final draft of the manuscript.

### Competing interests

The authors declare that they have no competing interests associated with this article.

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