Trans R Soc Trop Med Hyg 2023; **0**: 1–8
https://doi.org/10.1093/trstmh/trad053 Advance Access publication 0 2023

ORIGINAL ARTICLE

Seroprevalence and risk factors for *Toxoplasma gondii* infection among pregnant women at Debre Markos Referral Hospital, northwest Ethiopia

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Received 11 April 2023; revised 5 July 2023; editorial decision 23 July 2023; accepted 1 August 2023

Background: To assess the seroprevalence and risk factors of *Toxoplasma gondii* infection in pregnant women at the Debre Markos Referral Hospital, northwest Ethiopia.

Methods: A facility-based cross-sectional study was undertaken among pregnant women from March 2020 to May 2021. Sociodemographic and clinical data were collected from randomly selected participants. Five millilitres of blood was collected and an enzyme-linked immunosorbent assay kit was used to test for *T. gondii* immunoglobulin G (IgG) antibodies. A logistic regression model was computed to identify the risk factors. The adjusted odds ratio (aOR) was estimated along with the 95% confidence interval (CI). A statistically significant association was defined as $p<0.05$.

Results: *T. gondii* IgG antibody positivity was found in 38.8% (n=132) of 340 pregnant women. Contact with cats (AOR 2.5 [95% CI 1.5 to 4.2]), eating raw/undercooked meat (AOR 5.7 [95% CI 3.2 to 10.3]), consuming unwashed vegetables (AOR 4.1 [95% CI 2.1 to 8.0]), a history of abortion (AOR 1.9 [95% CI 1.1 to 3.3]) and drinking water sources (AOR 2.5 [95% CI 1.2 to 5.2]) demonstrated a statistically significant association with *T. gondii* infection.

Conclusions: Toxoplasmosis was found to be fairly common in pregnant mothers. Proper cat excreta disposal, not eating raw/undercooked meat, maintaining hand cleanliness and following environmental sanitation protocols could be important to decrease *T. gondii* infection.

Keywords: ELISA, pregnancy, seropositivity, *Toxoplasma gondii*, toxoplasmosis

Introduction

Up to one-third of people on the planet are infected with *Toxoplasma gondii*, the protozoan parasite that causes toxoplasmosis. Primary infection is asymptomatic, but eye disease or cervical lymphadenopathy may occur.[1] Typically, *T. gondii* infection is asymptomatic and dormant. The protozoa *Ctenodactylus gundi* was recognized by Nicolle and Manceaux in 1908; it was subsequently termed *T. gondii* in 1909.[2] When a person has a compromised immune system or is extremely young, toxoplasmosis can be fatal. Toxoplasmosis is the major cause of death for many human immunodeficiency virus (HIV)-positive people.[3] *T. gondii* is linked to congenital infection and abortion in a few of its hosts. *T. gondii* can also cause encephalitis or systemic infections in people with impaired immune systems, particularly those with HIV/acquired immunodeficiency syndrome.[4] One of the more widespread parasitic zoonoses globally is toxoplasmosis. *T. gondii* is a polyxenous, facultatively heteroxenous protozoon that has developed a number of potential transmission pathways both in and between various host species.[5]

Toxoplasmosis is a severe disease affecting veterinary and human medicine, causing reproductive reductions and public health issues due to contaminated food consumption.[6] *T. gondii* has three infectious phases: bradyzoites, tachyzoites and sporozoites in oocysts. Transitioning from acute primary infection to chronic toxoplasmosis requires developmental differences in tachyzoites and dormant bradyzoites.[7] *T. gondii* infection is most commonly acquired by eating raw or undercooked meat. It can also be contracted by consuming unwashed vegetables and fruits, as well as drinking water contaminated with oocysts.[8] *T. gondii* infections during pregnancy can result in abortion or congenital defects[9] that primarily affect the central nervous system.[10]

The importance of this parasite is mainly in pregnancy, as it can cross the placental barrier to infect foetal tissues, leading to spontaneous miscarriage, death of the foetus in utero or severe congenital defects such as hydrocephaly, mental retardation or chorioretinitis.[11,12]

This study aimed to determine the prevalence of *T. gondii* infection among pregnant women at the Debre Markos Referral Hospital (DMRH) in northwest Ethiopia, addressing data gaps.

Methods

Study setting

The study was conducted among pregnant mothers at the DMRH, located 295 km north of Ethiopia's capital, Addis Ababa, and 265 km south of Bahirdar, the capital of the Amhara National Regional State. The town has one governmental referral hospital and four health centres. The DMRH serves >5 million people in the East Gojjam Zone and four districts of the West Gojjam Zone.

Study design and period

A facility-based cross-sectional study was employed from March 2020 to May 2021.

Enrolment procedure

All non-follow-up, consecutive, first-visit antenatal care (ANC) pregnant mothers were included in the study with the exception of non-volunteer pregnant mothers, who were excluded.

Sample size estimation and sampling procedure

The sample size was estimated using the single population proportion formula, taking into account the estimated seroprevalence of *T. gondii* among pregnant women (71.2% from a previous study conducted in Adwa, Ethiopia[13]), the 95% CI (0.29), the value of Z (the selected critical value; 1.96) and d (5%). As a result of these assumptions (n=316), the ultimate sample size was 347, with a 10% contingency (non-response rate).

Information was gathered from participants in order of registration from any newly pregnant woman who came to the ANC ward.

Study variables

The presence of *T. gondii* antibody was the dependent variable. Age, occupation, residence, education level, history of abortion, history of blood transfusion, history of needle stick injury, presence of a domestic cat, raw meat consumption, raw milk consumption, raw vegetable consumption, hand washing after contact with garden soil or domestic animals, source of drinking water, history of contact with sheep/goats and history of contact with dogs were the independent variables.

Data collection

Both questionnaire-based and laboratory-based data were collected from each study participant. To collect sociodemographic, behavioural and clinical data, a pretested questionnaire was used.

The questionnaire was written in English and then translated into Amharic (the local language) and then back to English to ensure consistency. The questionnaire was pretested in a nearby health centre on 5% of study participants.

Specimen collection technique

Five millilitres of venous blood was collected aseptically from each study participant using sterile vacuum tubes without anticoagulants and labelled with the blood sample's corresponding code. The sera were separated by centrifugation at 3000 rpm for 10 min at 4°C and put into Nunc tubes, which were packed and transported in an icebox with ice packs and maintained at −20°C until serological examination at the Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University, Addis Ababa, Ethiopia. Indirect enzyme-linked immunosorbent assay (ELISA) was used to detect anti-*T. gondii* antibody (immunoglobulin G [IgG]) in the serum.

Laboratory analysis method and procedures

Based on the manufacturer's instructions, serum samples were analysed for *T. gondii* IgG antibody using a commercially available indirect ELISA kit (HUMAN Gesellschaft für Biochemica und Diagnostica, Wiesbaden, Germany). Inactivated antigens were precoated on microtitre plates. The wells of these plates contained dilutions of the substances to be evaluated. Any *T. gondii* antibody binds to the antigens in the wells, forming an antigen–antibody complex on the plate well surface. Unbound items were washed out of the wells with a wash solution. Anti-*T. gondii* IgG conjugate with peroxidase labelling was added, which binds to antibodies complexed with *T. gondii* antigens. Washing was used to remove unbound conjugates and the substrate was introduced to the wells. The amount of *T. gondii*–specific antibody present in the sample was directly proportional to the degree of colour that developed. The result was achieved by comparing the sample well's optical density (OD) to the OD of the positive control. A 96-well microplate reader with a 450-nm filter was used to read the results.

Data quality control

To ensure the quality and representativeness of the data, all laboratory professionals who participated in sample collection, as well as clinicians who collected the clinical data, received onsite training on the project, interviewing study participants and sampling protocols. The completeness of the data was closely monitored by the lead investigator and regularly entered into Excel (Microsoft, Redmond, WA, USA).

Table 1. Sociodemographic characteristics and exposures of the study participants (N=340)

Variables	n (%)
Female	340 (100.0)
Age (years)	
18–24	71 (20.9)
25–34	124 (36.5)
35–44	145 (42.6)
Occupation	
Employed	169 (49.7)
Unemployed	39 (11.5)
Farmer	9 (2.6)
Other	123 (17.6)
Residence	
Urban	185 (54.4)
Rural	155 (45.6)
Education	
No formal education	97 (28.5)
Less than high school	56 (16.5)
Vocational training and diploma	112 (32.9)
Bachelor's degree and above	75 (22.1)
History of abortion	
No	211 (62.1)
Yes	129 (37.9)
History of blood transfusion	
No	283 (83.2)
Yes	57 (16.8)
History of needle stick injury/sharp cut	
No	81 (23.8)
Yes	259 (76.2)
Contact with cat	
No	171 (50.3)
Yes	169 (49.7)
Habit of eating raw/undercooked meat	
No	139 (40.9)
Yes	201 (59.1)
Habit of drinking raw milk	
No	154 (45.3)
Yes	186 (54.7)
Habit of eating unwashed vegetables	
No	99 (29.1)
Yes	241 (70.9)
Hand washing after contact with soil/animals	
No	47 (13.8)
Yes	293 (86.2)
History of contact with sheep/goat	
No	207 (60.9)
Yes	133 (39.1)
History of contact with the dog	
No	237 (69.7)
Yes	103 (30.3)
Source of drinking water	
Pipe	244 (71.8)
Other	96 (28.2)
Total *T. gondii* IgG results	
Positive	132 (38.8)
Negative	208 (61.2)

All participants were pregnant women recruited from the DMRH, Amhara Region, northwest Ethiopia.
Other occupation: housewives, daily workers and merchants; unemployed: students, retired participants; other water sources: wells, spring water and reservoir water.

Data processing and analysis

For statistical analysis, data were imported into Excel and then exported to SPSS version 20 (IBM, Armonk, NY, USA). All of the variables in the study were described using descriptive statistics such as frequencies and proportions and then the prevalence of toxoplasmosis and its associated factors was determined. To assess the association of study variables with *T. gondii* infection in pregnant mothers, χ^2 test and bivariable and multivariable logistic regression models were computed. The odds ratio (OR) and 95% confidence interval (CI) were estimated and a p-value <0.05 in the adjusted model was considered statistically significant.

Results

Sociodemographic, behavioural and clinical characteristics of participants

In this study, data were collected from 340 pregnant women. The most frequent age groups among the study participants were 35–44 y (42.6%) and 25–34 y (36.5%). Nearly half of the study participants (49.7%) were working. The majority of study participants (71.8%) were utilizing pipe water for drinking and food preparation, which is dispensed from a reservoir that is chlorinated twice a year, while the remaining (28.2%) were using various water sources for drinking and food preparation, such as well, spring water and reservoir water. A large proportion of study participants (76.2%) had a history of needle stick injury or a sharp cut, and 49.7% of study participants were cat owners. Hand washing was common among study participants, with 86.2% doing so following contact with garden soil and household animals (Table 1).

Seroprevalence of *T. gondii* infection

Among pregnant women who attended ANC at the DMRH recruited in this study, 38.8% were seropositive for *T. gondii* IgG antibody. The majority of pregnant women (42.6% [n=145]) were 35–44 y of age, with 65 of them (44.8%) testing positive for *T. gondii* IgG antibody. The study participants were divided into four groups based on their occupations: employed, unemployed, farmer and other. *T. gondii* IgG antibodies were detected in 55 (44.7%) of the other participants, which is the highest prevalence in the group. *T. gondii* IgG-positive findings were also found in 61

Table 2. Sociodemographics and exposures of the study participants, stratified by *T. gondii* IgG status (N=340)

	Serologic status for *T. gondii* IgG		
Variables	Positive, n (%)	Negative, n (%)	p-Value
Age (years)			
18–24	27 (38.0)	44 (62.0)	0.107
25–34	40 (32.3)	84 (67.7)	
35–44	65 (44.8)	80 (55.2)	
Occupation			
Employed	61 (36.1)	108 (63.9)	0.319
Unemployed	14 (35.9)	25 (64.1)	
Farmer	2 (22.2)	7 (77.8)	
Other	55 (44.7)	68 (55.3)	
Residence			
Urban	62 (33.5)	123 (66.5)	0.306
Rural	70 (45.2)	85 (54.8)	
Education			
No formal education	55 (56.7)	42 (43.3)	0.534
Less than high school	20 (35.7)	36 (64.3)	
Vocational training and college diploma	36 (32.1)	76 (67.9)	
Bachelor’s degree and above	21 (28.0)	54 (72.0)	
Marital status			
Single (never married)	6 (33.3)	12 (66.7)	0.454
Married	118 (38.4)	189 (61.6)	
Separated/divorced	8 (53.3)	7 (46.7)	
History of abortion			
No	70 (33.2)	141 (66.8)	0.006
Yes	62 (48.1)	67 (51.9)	
History of blood transfusion			
No	112 (39.6)	171 (60.4)	0.526
Yes	20 (35.1)	37 (64.9)	
History of needle stick injury/sharp cut			
No	32 (39.5)	49 (60.5)	0.885
Yes	100 (38.6)	159 (61.4)	
Contact with cat			
No	46 (26.9)	125 (73.1)	<0.001
Yes	86 (50.9)	83 (49.1)	
Habit of eating raw/undercooked meat			
No	27 (19.4)	112 (80.6)	<0.001
Yes	105 (52.2)	96 (46.2)	
Habit of drinking raw milk			
No	53 (34.4)	101 (65.6)	0.129
Yes	79 (42.5)	107 (57.5)	
Habit of eating unwashed vegetables			
No	20 (20.2)	79 (79.8)	<0.001
Yes	112 (46.5)	129 (53.5)	
Hand washing after contact with soil/animals			
No	23 (48.9)	24 (51.1)	0.125
Yes	109 (37.2)	184 (62.8)	
History of contact with sheep/goats			
No	67 (32.4)	140 (67.6)	0.179
Yes	65 (48.9)	68 (51.1)	

Table 2. (Continued)

	Serologic status for *T. gondii* IgG		
Variables	Positive, n (%)	Negative, n (%)	p-Value
History of contact with the dogs			
No	98 (41.4)	139 (58.6)	0.147
Yes	34 (33.0)	69 (67.0)	
Source of drinking water			
Pipe	79 (32.4)	165 (67.6)	<0.001
Other	53 (55.2)	43 (44.8)	

All participants were pregnant women recruited from the DMRH, Amhara Region, northwest Ethiopia.
Other occupation: housewives, daily worker, and merchants; unemployed: students, retired participants; other water sources: wells, spring water and reservoir water.

(36.1%) of the employed members in the group. A total of 129 participants had a history of abortion and 62 (48.1%) of them tested positive for the *T. gondii* IgG antibody while 70 (33.2%) of the study participants who had never had an abortion tested positive for *T. gondii* IgG antibodies. *T. gondii* IgG antibodies were found in 86 (50.9%) of the study participants who kept cats and 46 (26.9%) of the study participants who had no direct interaction with cats. *T. gondii* was found in 112 (46.5%) persons who had eaten unclean vegetables and 20 (20.2%) participants with *T. gondii* IgG antibody who did not eat unwashed vegetables.

Fifty-three (55.2%) pregnant mothers who had a practice of drinking and using other water sources (well, spring water and reservoir water) for food preparation were positive for *T. gondii* (Table 2).

Factors associated with seropositivity of *T. gondii* IgG antibody

Univariable analysis was performed to look at the relationship between each sociodemographic variable and the outcome among pregnant women. The relative influence of independent factors on the outcome variable was determined using a multivariable logistic regression model. The variables with a p-value <0.25 in the bivariable analysis were subjected to multivariable analysis.

The majority of sociodemographic variables such as age, occupation, place of residence, level of education, marital status, history of abortion, history of blood transfusion, history of needle stick injury/sharp cut, habit of drinking raw milk, history of contact with sheep/goats and history of contact with dogs did not show a statistically significant association with *T. gondii* IgG antibody seropositivity. However, contact with a domestic cat (presence of a cat in the house) (crude OR [COR] 2.8 [95% CI 1.8 to 4.4]), habit of eating raw/undercooked meat (COR 4.5 [95% CI 2.7 to 7.5]), habit of consuming unwashed vegetables (COR 3.4 [95% CI 2.0 to 6.0]), history of abortion (COR 1.9 [95% CI 1.2 to 2.9]) and the sources of drinking water (COR 2.6 [95% CI 1.6 to 4.2]) showed a statistically significant association with *T. gondii* antibody seropositivity (Table 2).

Based on the multivariable logistic regression model, explanatory variables such as a history of abortion (AOR 1.9 [95% CI 1.1 to 3.3]), contact with a cat (AOR 2.5 [95% CI 1.5 to 4.2]), habit of eating raw/undercooked meat (AOR 5.7 [95% CI 3.2 to 10.3]), habit of eating unwashed vegetables (AOR 4.1 [95% CI 2.1 to 8.0]) and sources of water (AOR 2.5 [95% CI 1.2 to 5.2]) were found to be independently associated with *T. gondii* infection in pregnant mothers (Table 3).

Discussion

In this study we found the seroprevalence of toxoplasmosis was 38.8%. This is almost twofold higher than the findings of a study conducted in the Felege Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia, which found a prevalence of 18.5%.[11] Other studies in Ethiopia found that the toxoplasmosis distribution based on serological evidence ranged from 18.5%[11] to 94.40%,[14] with the studies conducted in pregnant women and HIV-infected women 18–49 y of age, respectively.

There was a statistically substantial correlation between some characteristics and study participants. These factors included a history of abortion, contact with cats, admission of frequently ingesting undercooked or raw meat, regular use of non-pipe water sources (well, spring and reservoir water) and frequent consumption of unwashed vegetables. The prevalence of *T. gondii* antibody observed in this study was similar to studies that have been conducted among pregnant women in the Vhembe District of South Africa (31.7%),[15] Saudi Arabia (32.5%),[16] Egypt (33.8%),[17] city of Samambaia of the Federal District, Brazil (34.1%),[18] Adwa District, northern Ethiopia (35.6%),[13] northern Tanzania (40.2%)[19] and São Luís, in the Brazilian state of Maranhão (41.1%).[20]

However, our finding was lower than the findings of toxoplasmosis seroprevalence reported among the general population in Nazareth, Ethiopia (60%)[21] and among pregnant women in Debre Tabor, northwest Ethiopia (68.4%),[10] Bonga Hospital, southwest Ethiopia (75.5%),[22] Mizan Aman General Hospital, Bench Maji Zone, Ethiopia (85.3%)[12] and Addis Ababa (85.4%).[23]

Table 3. Multivariable analysis of *T. gondii* infection and associated factors by different predictor characteristics among pregnant women at the DMRH from March 2020 to May 2021

Variables	aOR (95% CI)	p-Value
Age (years)		
18–24	1.00	
25–34	0.8 (0.4 to 1.6)	0.579
35–44	0.6 (0.4 to 1.1)	0.088
Residence		
Urban	1.00	
Rural	0.7 (0.3 to 1.4)	0.332
Education		
No formal education	0.7 (0.3 to 1.7)	0.420
Less than high school	1.3 (0.5 to 3.5)	0.563
Vocational training and college diploma	0.5 (0.2 to 1.2)	0.109
Bachelor's degree and above	1.00	
Contact with cats		
No	1.00	
Yes	2.5 (1.5 to 4.2)	0.001
Habit of eating raw/undercooked meat		
No	1.00	
Yes	5.7 (3.2 to 10.3)	<0.001
Habit of drinking raw milk		
No	1.00	
Yes	0.9 (0.5 to 1.6)	0.749
Habit of eating unwashed vegetables		
No	1.00	
Yes	4.1 (2.1 to 8.0)	<0.001
Hand washing after contact with soil/animals		
No	1.5 (0.7 to 3.3)	0.285
Yes	1.00	
History of contact with dogs		
No	1.00	
Yes	0.9 (0.4 to 2.0)	0.882
History of abortion		
No	1.00	
Yes	1.9 (1.1 to 3.3)	0.031
Source of drinking water		
Pipe	1.00	
Other	2.5 (1.2 to 5.2)	0.010

Other occupation: housewives, daily workers and merchants; unemployed: students, retired participants; other water sources: wells, spring water and reservoir water.

The variation in *T. gondii* prevalence distributions across the country was thought to be due to differences in the density of definitive hosts (wild and domestic Felidae), which affects the prevalence of toxoplasmosis in intermediate hosts such as chickens, cattle, sheep, goats and others, including humans.[24,25]

Ethiopia has a high frequency of *T. gondii* in cats. Ethiopian cats live outside, hunt and eat leftovers and garbage, thus they are more likely to be infected with the parasite (87.7%).[26]

According to the Sholla and Akaki-Kality Veterinary Clinics in Addis Ababa, the prevalence of *T. gondii* is reported as 36.6%, 37.5%, 25%, 47.5%, 35% and 33.3% in ovine, caprine, bovine, swine, equine and camels, respectively.[27] Seroprevalence of toxoplasmosis is reported as 56% and 25.9% in sheep and goats, respectively.[28] The other difference could be due to differences in raw/undercooked meat consumption, type of meat, environmental sanitation and how cat faeces is disposed of.

These significant variations in toxoplasmosis infection prevalence could be linked to differences in social, economic and cultural behaviours, as well as geographic considerations, meteorological circumstances and transmission routes of the parasite.

According to reports, the incidence is higher in hot and humid climates.[24,29,30,31] Cat interaction, feeding habits, inadequate hygiene conditions and favourable climatic parameters for sporulation and survival of oocysts in the environment are all linked to the varied distribution and high seroprevalence.[29] Variation is also caused by differences in the immunological condition of study participants, the technique of testing and the sample size for each study participant.[30]

Even though there are contradictory reports, contact with domestic cats or cat ownership is often mentioned as an associated factor for toxoplasmosis in humans.[8,10–12,20,30–33] Our findings agreed with these and other factors such as consuming raw/undercooked meat,[8,11,12,22,24,30,34] consuming unwashed vegetables/fruits,[8,9,13,22,33] sources of drinking water,[13,22] a history of abortion[11,22] and a lack of consistent hand washing after contact with garden soil/domestic animals.[13,22]

Conclusions

In this study, toxoplasmosis, as determined by seroprevalence, was found in a reasonably high percentage of pregnant women (38.8%). Toxoplasmosis has been linked to contact with cats/cat ownership, consuming raw/undercooked meat, consuming unwashed vegetables/fruits, sources of drinking water, a history of abortion and a lack of consistent hand washing habits. Based on the findings of this study, we can conclude that preventive measures such as raising awareness about toxoplasmosis, understanding how *T. gondii* is transmitted from mother to child and methods of prevention and control, such as proper disposal of cat faeces, avoiding raw/undercooked meat, maintaining hand hygiene and following environmental sanitation protocols, are required to prevent *T. gondii* infection.

Since this study design was cross-sectional, we were unable to conclusively determine the cause of the previous abortions and their relationship to *Toxoplasma* infection. Even though there is a statistically significant association between the history of abortion and the *T. gondii* IgG antibody, the ultimate health status of study participants is also unknown. Therefore, additional cohort or case–control studies investigating all risk factors for toxoplasmosis at the time of conception are required to better understand the link between *T. gondii* infection and a history of abortion.

Authors' contributions: EK assisted with the study's planning and execution, statistical analysis and writing. NK and TK spearheaded the research and contributed significantly to its design and statistical analysis. AG, MG and YA were all involved in the laboratory work. The manuscript was reviewed by AA, SD and MT. All authors contributed to the manuscript's writing and gave their approval to the final version.

Acknowledgements: We would like to thank the Aklilu Lemma Institute of Pathobiology at Addis Ababa University for their support. The National Animal Health Diagnostic and Investigation Center is also thanked for supplying the materials. We want to express our gratitude to the study participants as well as the staff at the DMRH's Antiretroviral Therapy and ANC divisions for their support with sample collection.

Funding: This study was supported by Addis Ababa University but this research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Competing interests: None declared.

Ethical approval: The study was evaluated and authorized by the Aklilu Lemma Pathobiology Institute's Institutional Review Board at Addis Ababa University. The DMRH was asked for authorization to perform this research. After getting written informed consent or assent from each participant or guardian, data and specimens were gathered. Patients' privacy was protected and the study participants were invited as volunteers to participate in the study. Participants were also able to withdraw at any point from the study.

Data availability: All original raw data are available from the corresponding author.

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