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Original Article

Seroprevalence and Risk Factors for *Toxoplasma gondii* Infection among Women of Reproductive Age in Northeast Iran

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Abstract

Background: We aimed to investigate the seroepidemiology of *Toxoplasma gondii* infection among women of childbearing age in Gonabad City, northeastern Iran, to assess the prevalence of infection and identify associated risk factors.

Methods: A cross-sectional study was conducted from March to April 2022 in Gonabad, Khorasan Razavi Province, northeastern Iran. A total of 320 blood samples were collected from consenting women of reproductive age. Each participant provided 5 mL of blood for serological analysis to detect anti-*Toxoplasma* IgG and IgM antibodies. Demographic and behavioral data were collected using a structured questionnaire.

Results: The mean age of participants was 29.74 ± 10.9 years. Of the 320 samples, 58 (18.12%) tested positive for anti-*Toxoplasma* IgG antibodies, while 1 (0.3%) tested positive for IgM antibodies. In addition, 81.9% of participants were seronegative. No significant associations were observed between anti-*Toxoplasma* antibodies and marital status, education level, vegetable washing practices, egg cooking methods, milk consumption, or fertility status. However, significant correlations were found between seropositivity and contact with cats ($P= 0.01$) and meat consumption ($P< 0.05$).

Conclusion: The majority of women of childbearing age in Gonabad remain susceptible to *T. gondii* infection, highlighting the risk of primary infection during pregnancy. To mitigate the risk of congenital toxoplasmosis, serological screening for *Toxoplasma* infection is recommended for women of childbearing age, coupled with targeted health education on transmission routes and preventive measures.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite with a global distribution. Warm-blooded animals, including humans, serve as intermediate hosts, while domestic cats and other felids act as definitive hosts, where the parasite undergoes sexual reproduction (1-3). Transmission of *T. gondii* occurs through several routes, including the ingestion of raw or undercooked meat containing tissue cysts, consumption of food or water contaminated with oocysts shed in feline feces, organ transplantation, blood transfusion, or vertical transmission from an infected mother to the fetus during pregnancy (4). In immunocompetent individuals, *T. gondii* infection is typically asymptomatic or manifests as mild, self-limiting symptoms such as flu-like illness and lymphadenopathy. However, in immunocompromised individuals and neonates with congenital toxoplasmosis, the infection can lead to severe and potentially life-threatening complications, including chorioretinitis, encephalitis, pneumonia, myocarditis, and death (2, 4).

Congenital toxoplasmosis, resulting from vertical transmission, occurs in approximately 1.5 cases per 1000 live births globally, with the highest incidence reported in South America, certain Middle Eastern countries, and low-income regions (4). The risk of fetal infection increases with gestational age, ranging from 10-15% in the first trimester to 60-90% in the third trimester. However, the severity of fetal complications is inversely related to the timing of infection. First-trimester infections, though less frequent, are associated with more severe outcomes, including spontaneous abortion, stillbirth, and congenital anomalies such as hydrocephalus, central nervous system abnormalities, and chorioretinitis (5). In contrast, third-trimester infections, while more common, often result in asymptomatic or subclinical disease at birth, though long-term sequelae may manifest later in life (1).

Early detection and timely treatment of acute *T. gondii* infections during pregnancy are critical for preventing congenital transmission and ensuring the health of both the mother and the fetus. Current evidence suggests that only untreated acute infections during pregnancy pose a risk of vertical transmission, whereas latent infections acquired prior to pregnancy are not associated with fetal transmission (6, 7). However, some studies suggest that latent toxoplasmosis may contribute to neurological and behavioral disorders in both mothers and children, highlighting the need for further research into its long-term implications (8). Laboratory diagnosis of *T. gondii* infection relies on the detection of specific Anti-*Toxoplasma* antibodies, IgG and IgM. IgM antibodies are detectable approximately one-week post-infection and may persist for several months, serving as a marker for acute infection. In contrast, IgG antibodies indicate past exposure but do not provide information on the timing of infection (9). Among the available serological tests, ELISA is widely used due to its high sensitivity and specificity (10, 11).

The prevalence of *T. gondii* infection varies significantly across regions and populations. A systematic review and meta-analysis conducted in Iran reported an overall prevalence of 32.9% in the general population, with the highest prevalence in Mazandaran Province (61%) and the lowest in Semnan Province (12.5%) (12). Similarly, a meta-analysis found that 10.3% to 77.2% of pregnant women in Iran tested positive for IgG antibodies, while 0% to 28.3% were positive for IgM antibodies, reflecting regional variability in exposure and infection rates (13). Globally, factors such as age, gender, ethnicity, health status, contact with soil, geographical location, and climatic conditions have been identified as determinants of susceptibility to *T. gondii* infection (14).

Despite the significant public health implications of toxoplasmosis, particularly for women of childbearing age, there is a low of epidemiological data in certain regions, including Gonabad City, Iran (15). However, because unpublished reports suggested frequent exposure of pregnant women to cats, this study needed to be repeated.

This study aimed to investigate the seroepidemiology of *T. gondii* infection among women of childbearing age in Gonabad City, Iran

Materials and Methods

This descriptive cross-sectional study was conducted in Gonabad, a city situated in the northeastern region of Khorasan Razavi Province, Iran, between March and April 2022. Gonabad covers an area of approximately 2,188.91 km² and is home to a population of over 36,367 residents. The city is characterized by a semi-arid climate, with elevations ranging from 894 to 2,774 meters above sea level, resulting in hot summers and cool winters. The average annual rainfall in the region varies between 149 and 155 mm, while temperatures typically range from 16.4 to 17.3°C (16, 17). The geographical and climatic characteristics of Gonabad, along with its unique hydrological infrastructure, make it an area of particular interest for epidemiological studies, including the investigation of infectious diseases such as toxoplasmosis (Fig. 1).

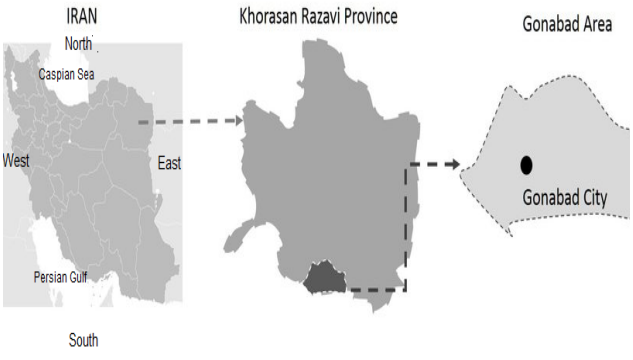

Fig. 1: The location of Gonabad city in the country (18)

Ethical Considerations and Participant Selection

To ensure adherence to ethical standards, this study obtained approval from the regional medical Ethics Committee (Code: Ir.gmu.rec.1399.123) prior to the initiation of participant recruitment. All participants were provided with comprehensive information regarding the study's objectives, procedures, and potential implications. Written informed consent was obtained from each participant prior to their inclusion in the study, ensuring voluntary participation and compliance with ethical guidelines.

Inclusion and Exclusion Criteria

Participants were selected based on predefined criteria to ensure the relevance and reliability of the study findings. Eligible individuals were women aged 15 to 45 years, reflecting the demographic of childbearing age, who expressed a willingness to participate actively in the study. Recruitment was conducted through systematic visits to diagnostic laboratories in Gonabad, where potential participants were identified and approached. Individuals who declined to collaborate or demonstrated a lack of interest in the study were excluded from participation. This approach ensured that the study consisted of engaged and motivated participants, which is essential for obtaining accurate and meaningful data. By adhering to these rigorous ethical and methodological standards, the study aimed to maintain scientific integrity while safeguarding the rights and well-being of all participants.

Sample Size Calculation and Methodology

The sample size for this study was determined using a statistical formula based on findings from a prior study by Ghadamgahi et al (19). With a confidence level of 95% ($\alpha = 0.05$), a margin of error (d) of 0.05, and an estimated prevalence (*P*) of 28% (0.28), the initial sample size was calculated as follows:

To account for a potential dropout rate of 10%, the final sample size was adjusted to 320 participants. This ensured an adequate number of participants for robust statistical analysis.

Data Collection and Laboratory Procedures

Data collection was conducted using a structured questionnaire designed to capture demographic information and relevant behavioral factors, including vegetable washing practices, cat ownership, and meat consumption patterns. The questionnaires were completed by either the participants or the researchers during the blood collection process.

Venous blood samples (5 mL) were collected from each participant under aseptic conditions. The samples were promptly transported to the immunology laboratory of the medical school, where they were centrifuged at 5,000 RPM for 10 minutes to separate the serum. The isolated sera were stored at -70 °C to preserve their integrity until further analysis.

Serological Analysis

The presence of IgG and IgM antibodies against *T. gondii* was assessed using ELISA kits (*Toxoplasma* IgG and IgM; pishtazteb, IRAN; Specificity: 91% and sensitivity:97%) following the manufacturer's instructions.

Following data collection, all data were entered into SPSS 27.0.1 (IBM Corp., Armonk, NY, USA) software for analysis to ensure accuracy and reliability. Qualitative variables were summarized using frequencies and percentages, while quantitative variables were expressed as means ± standard deviations (SD). The normality of quantitative variables was assessed using the Kolmogorov-Smirnov test. After confirming normality, the non-parametric Mann-Whitney test was applied for comparisons. Associations between qualitative variables were evaluated using the chi-square test. A *P*-value of <0.05 was considered statistically significant for all analyses.

Results

The study included 320 women of childbearing age, with a mean age of 29.74 ± 10.9 years (Table 1). Serological analysis revealed that 58 participants (18.1%) tested positive for specific IgG antibodies against *T. gondii*, indicating past exposure to the parasite. Only one participant (0.3%) tested positive for specific IgM antibodies, suggesting a recent or acute infection, Of course, these people were IgG negative. No significant associations were observed between anti-*Toxoplasma* antibodies and variables such as marital status, occupation, education level, vegetable washing methods, egg cooking methods, milk consumption, or fertility status. However, significant correlations were identified between seropositivity and place of residence, contact with cats, and meat consumption patterns ($P < 0.05$) (Table 2).

Table 1: Frequency distribution of variables in women reproductive age examined for serodiagnosis of *Toxoplasma gondii* in Gonabad northeast of Iran

Variable	Levels	Frequency	Percentage
Marital status	Single	121	37.8
	Married	199	62.2
Education	Pre-diploma	62	19.4
	Diploma	127	39.7
	Bachelor's degree or higher	131	40.9
Contact with cats	Yes	36	11.3
	No	284	88.7
Habit of eating raw vegetables	Yes	42	13.1
	No	278	86.9
Habit of eating raw egg	Yes	103	32.2
	No	217	67.8
Habit of eating raw milk	Yes	104	32.5
	No	216	67.5

Table 1: Continued…

Habit of undercooked meat	Yes	4	1.3
	No	316	98.8
Childbearing status	Have children	169	52.8
	Without children	151	47.2
Age group(yr)	15-20	58	18.1
	21-30	115	35.9
	31-40	106	33.1
	41-45	41	12.9
Place of residence	Urban	269	84.1
	Rural	51	15.9

Table 2: frequency distribution of variables in women reproductive age examined for serodiagnosis of *Toxoplasma gondii* in Gonabad northeast of Iran according to the studied variables

Variable	**Levels**	**Anti-*Toxoplasma* IgG antibodies**		***P*-Value**
		Negative N (%)	**Positive (N%)**	
Marital status	Single	101 (38.5)	20 (34.5)	*0.56*
	Married	161 (61.5)	38 (65.5)	
Education	Pre-diploma	54 (20.6)	8 (13.8)	0.16
	Diploma	107 (40.8)	20 (34.5)	
	Bachelor's degree or higher	101 (38.5)	30 (51.7)	
Contact with cats	No	238 (90.8)	46 (79.3)	0.01
	Yes	24 (9.2)	12 (20.7)	
Habit of Eating raw vegetables	Yes	36 (13.7)	6 (10.3)	0.48
	No	226 (86.3)	52 (89.7)	
Habit of eating raw egg	Yes	83 (31.7)	20 (34.5)	0.67
	No	179 (68.3)	38 (65.5)	
Habit of eating raw milk	Yes	85 (32.4)	19 (32.8)	0.96
	No	177 (67.6)	39 (67.2)	
Childbearing status	Having children	125 (47.7)	26 (44.8)	0.69
	No children	137 (52.3)	32 (55.2)	
Age group(yr)	15-20	51 (19.5)	7 (12.1)	0.41
	21-30	92 (35.1)	23 (39.7)	
	31-40	88 (33.6)	18 (31)	
	41-45	31 (11.8)	10 (17.2)	
How to consume meat	Fully cooked	1 (4.0)	3 (5.2)	0.02
	Grilled	261 (99.6)	55 (94.8)	
Place of residence	Urban	225 (86.6)	44 (75.9)	0.03
	Rural	37 (13.4)	14 (24.)	

Discussion

The findings revealed that 18.1% of participants tested positive only for IgG antibodies, indicating past exposure, while only 0.3% had IgM antibodies, which is probably an acute infection. This implies that married women in this population remain susceptible to primary infection, posing a significant risk of congenital toxoplasmosis during pregnancy and are considered as high-risk group of congenital toxoplasmosis during pregnancy (20, 21).

The seroprevalence in Gonabad is lower than that reported in northern Iran, such as in Babol, likely due to the region's hot and dry climate, which is unfavorable for the development and survival and transmission of *T. gondii* oocysts (22, 23). Probably, the main way of *T. gondii* transmission in study area is ingestion of oocytes of the parasite. Therefore, the climatic influence may explain the lower-than-expected IgG and IgM antibody levels observed in this study. No significant association was found between age and *T. gondii* seropositivity (P= 0.41), contrasting with studies in Mazandaran (24), Kermanshah (25), and Mashhad (26), but aligning with findings from Babol (27). This discrepancy underscores the need for further research to elucidate the role of age in *T. gondii* epidemiology. Various studies have indicated that age is an influential variable in the prevalence of toxoplasmosis, with an increasing likelihood of infection as age rises (28).

No significant association was found between occupation and *T. gondii* infection in this study (P=0.56), a result consistent with studies in Jahrome (29), and Ramsar (30). Similarly, no significant association was observed between education level and the presence of anti-*Toxoplasma* antibodies in this study (P=0.16), which aligns with studies in Ardabil (31), northern Iran (32).

In general, the prevalence of antibodies in pregnant women in Iran is reported to be 41% (33). The prevalence of IgG and IgM in Iran stands at 38% and 4%, respectively. The prevalence of anti-*Toxoplasma* antibodies in women of childbearing age is 47% in Qom, 61% in Mazandaran, 16/8% in Yazd, 24% in Kermanshah, 19% in Birjand, 35% in Chaharmahal and Bakhtiari, 46% in East Azerbaijan, 24% in Alborz, 42% in Golestan, 26% in North Khorasan, 75.62% in Qazvin, 33.7% in Tehran, 20% in Bushehr, 14% in Fars, 34% in Hormozgan, 35% in Isfahan, 37% in Markazi, 33% in Hamedan, 29% in Ilam, 44% in Lorestan, 26% in Khuzestan, 23% in Kurdistan, 19% in South Khorasan, and 25% in Sistan and Baluchestan (12, 23, 34).

A significant relationship was observed in this study between contact with cats and the presence of anti-*Toxoplasma* antibodies (P=0.01). Among the 24 individuals who had contact with cats, 12 tested positives for IgG antibodies against *toxoplasma*. These findings align with studies from Hormozgan (35), Gonabad (15), Urmia (36), and but contrast with studies conducted in Ramsar (37) and Southwest Iran (38).

In this study, no significant correlation was found between the method of washing vegetables and the presence of *Toxoplasma* antibodies (P=0.48). Among 226 individuals who did not use vegetable disinfectants, 52 had antibodies, while among 30 individuals who washed vegetables with disinfectants, 6 were found to have antibodies. This finding contrasts with studies conducted in northern Iran (36) and Shiraz (39), but aligns with research in Qazvin (37), Ramsar (30), and West Azerbaijan (40). There was no significant association between the consumption of raw eggs and the presence of *Toxoplasma* antibodies (P=0.67). This result diverges from studies in western Iran (41) and Kerman (42) but corresponds with findings in Urmia (43) and southern Iran (44). No significant correlation was found between the type of milk consumed (pasteurized or local) and the presence of *Tox-*

oplasma antibodies (*P*=0.96), contradicting the study by Ahmadpour in western Iran(41), but aligning with study in Urmia(43).The results of this study aligned with the findings of Mizani et al.(45), which indicated that 45% of women consuming raw milk had *Toxoplasma* antibodies, and no significant correlation was found between milk consumption and the presence of these antibodies. This study also demonstrated that no significant association existed between having before pregnancy (having children) and the presence of *Toxoplasma* antibodies (*P*=0.69). Mizani's study indicated that the prevalence of *Toxoplasma* antibodies among childless women was 41%, while mothers with more than one child had a prevalence of 53% (45). Khademi's study showed that mothers with more pregnancies had a higher level of *Toxoplasma* antibodies, indicating a significant correlation between the number of pregnancies and the presence of these antibodies. Additionally, Maani found a significant relationship between *Toxoplasma* infection and the number of children (29).

In this study, a significant correlation was observed between place of residence and the presence of *Toxoplasma* infection (*P*=0.039). Hassanzadeh found a significant association between *Toxoplasma* infection and place of residence in western Iran (46). Ghoravi in northern Iran (32) indicated that this correlation was not statistically significant. Moreover, no significant association was observed between marital status and the presence of *Toxoplasma* antibodies This means that, based on the data collected and analyzed in that study, a person's marital status (whether they are married, single, etc.) did not appear to be a factor that influenced whether or not they had antibodies indicating a past or present *Toxoplasma* infection (*P*=0.56). Among 161 married individuals, 38 tested positives for antibodies, contradicting the findings of previous studies conducted by Hassanzadeh in western Iran (46) and Yilmaz in Turkey (47). In this study, we found a significant association between the method of meat consumption and the presence of *Toxoplasma* antibodies. This finding is consistent with a meta-analysis conducted in Iran, which reported that individuals who consume grilled meat exhibit higher levels of *Toxoplasma* antibodies. These results collectively underscore the potential risks associated with grilled meat consumption in relation to *Toxoplasma* exposure (48).

The findings of this study highlight a seroprevalence of 18.1% for IgG antibodies among women of childbearing age in Gonabad City, which is consistent with regional epidemiological patterns. The low prevalence of IgM positivity (0.3%) suggests a relatively low incidence of acute infections in this population. The lack of significant associations between seropositivity and variables such as education level or hygiene practices may reflect the complex and multifactorial nature of *T. gondii* transmission.

The significant correlations between seropositivity and place of residence, contact with cats, and meat consumption align with established risk factors for *T. gondii* infection. These findings underscore the importance of targeted public health interventions, such as promoting safe food handling practices, reducing exposure to cat feces, and raising awareness about the risks associated with consuming undercooked meat.

Conclusion

To prevent congenital toxoplasmosis, it is recommended to implementation educational programs pre-pregnancy screen protocols. In addition, further studies are needed to assess temporal trends and congenital transmission. Serum in women of reproductive age and to provide essential health education regarding the transmission of the parasite and methods for its prevention to this group.

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Conflict of interest

The authors declare no conflict of interest.

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