



Maternal Serological Screening for Congenital Toxoplasmosis During Pregnancy and Evaluating the Pregnant Women's Knowledge on Toxoplasmosis in Qazvin, Iran

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Abstract

Background: The present study was designed to determine the prevalence of *Toxoplasma gondii* seronegative pregnant women, estimate the frequency of fetuses at risk of congenital toxoplasmosis in the study area, and also to investigate the awareness of the studied mothers about *T. gondii* disease.

Methods: A total of 740 pregnant women referred to two health centers in Qazvin province were surveyed voluntarily for anti-*T. gondii* IgG and IgM antibodies by enzyme-linked immunosorbent assay (ELISA). IgG avidity test was used as a confirmatory test for samples showing positive or borderline results for IgM antibodies. Data on socioeconomic, demographic, and knowledge of participants about toxoplasmosis were collected using questionnaires and through face-to-face interviews. Multivariable logistic regression modeling was used to identify the potential predictor variables for *T. gondii* infection.

Results: Overall, 21.2% (157/740) and 0.27% (2/740) of pregnant women were positive for *T. gondii* IgG and IgM antibodies, respectively. Only 1.7% (13/740) of expectant mothers had prior information on toxoplasmosis. Among socioeconomic and demographic variables, contact with soil was the merely cause of a significant association with *T. gondii* infection. Other variables including age, occupation, education level, residential area, source of drinking water, abortion history, number of delivery, consumption of raw/undercooked meat, having home cat and and consumption of raw vegetables failed to establish significant associations.

Conclusion: Our results clarified that the prevalence of *T. gondii* has remarkably reduced in the study area. This finding indicates a low risk of congenital toxoplasmosis in this region.

Keywords: *Toxoplasma gondii*, Prevalence, Pregnant women, Iran

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Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite found in humans and other warm-blooded animals, worldwide (1). There are many seroepidemiological studies in the literature focusing on the prevalence of *T. gondii* in pregnant women

due to the importance of congenital toxoplasmosis (2-7). Congenital infections with this parasite occur following primary infections during pregnancy in which tachyzoites are transplacentally transmitted to the fetus. Moreover, this parasite may be transmitted by blood transfusion and also organ transplantation



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(1). Most infections in immunocompetent subjects are asymptomatic, but in immunocompromised patients, especially in acquired immunodeficiency syndrome (AIDS), patients are susceptible to being infected by life-threatening opportunists. Toxoplasmic encephalitis is the most common clinical presentation of toxoplasmosis in these cases. Congenital toxoplasmosis can result in severe fetal injuries such as psychomotor or mental retardation, seizures, encephalitis, hydrocephalus, microcephaly, chorioretinitis blindness, or even death (8). There is a reverse relationship between the rate of congenital transmission of *T. gondii* tachyzoites and the severity of toxoplasmosis in the fetus based on the trimester of pregnancy. The risk of fetal transmission of the parasite is lower in the first trimester and increases to the highest level in the third trimester of pregnancy (1). Therefore, prenatal diagnosis of *T. gondii* infection during pregnancy is an essential measure for the prevention of congenital toxoplasmosis (9).

Maternal serological screening is considered an essential preventive measure for congenital toxoplasmosis. This process leads to a decrease in the incidence of congenital toxoplasmosis. In Iran, like many developed and developing countries, there is not a national mandatory program to prevent congenital infections, but prenatal care is a non-mandatory program requested by gynecologists. Pregnant women may be screened either merely for *T. gondii* or TORCH screen (such as *T. gondii*, *Rubella virus*, *Cytomegalovirus*, *Herpes simplex*, and other infectious diseases such as Chickenpox, Epstein-Bar virus, and measles). On the other hand, there is no national network for recording the positive cases of congenital toxoplasmosis in Iran and therefore, the importance of maternal serological screening of *T. gondii* in the country is obscure.

Another key measure to prevent congenital toxoplasmosis is the knowledge and awareness of pregnant women about *T. gondii* and its transmission routes. Despite numerous reports regarding the prevalence of this parasite during pregnancy in developed (10,11) and developing countries (3,12), there are only a few reports on pregnant women's knowledge about *T. gondii* (2,13-18). Based on the search results extracted from Data Bank, no evidence of toxoplasmosis knowledge among Iranian pregnant women has been documented.

Based on climatic status and epidemiological surveys, the prevalence of *T. gondii* is 20% to 50% in most areas of Iran (19-23). The high prevalence was reported in Guilan and Mazandaran provinces located on the southern coast of the Caspian Sea (20,24). This study aimed to serologically screen the pregnant women referred to health centers in Qazvin province to determine the current status of *T. gondii* prevalence, the probable frequency of fetuses at risk of toxoplasmosis, and also evaluate the knowledge and awareness of pregnant women about *Toxoplasma* and

toxoplasmosis in the study area.

Materials and Methods

This cross-sectional study was conducted from May to September 2019 in Qazvin province located in the northern margin of central Iran. A total of 740 pregnant women referred to two health centers in Qazvin province participated in this study. Being in the first trimester of pregnancy was considered as inclusion criterion. The participants of this study were offered the opportunity to consciously and voluntarily enter the project. For this purpose, a notification sheet containing information about toxoplasmosis was provided. The information included transmission and prevention routes of *T. gondii* and its pathogenesis in the fetus. Also, the purpose and the procedures involved in the study were explained, and written informed consent was obtained from volunteers after 24 hours. Afterward, the participants responded to a pre-prepared questionnaire in a face-to-face interview. The questionnaire covered socio-demographic information, including age, level of education, occupation, residential location (urban/rural), source of drinking water, and abortion history. Also, the participants were asked about the behavioral risk factors including, consumption of raw/undercooked meat, consumption of raw vegetables, and contact with soil and domestic cats. Only the individuals, who stated that they had a previous awareness of *T. gondii* infection, answered the following questions about the parasite:

- What is the source of information?
- Were you aware that humans may be infected by contact with cat feces, eating raw/ undercooked meat or raw vegetables and contact with soil?
- Were you aware that only primary infections during pregnancy have the risk of congenital toxoplasmosis?
- Were you aware that it is one of the most important causes of congenital infections?
- Were you aware there is a higher chance of fetal infection in the third trimester of pregnancy and fetal infections in the first trimester?

The sample size was calculated using an expected IgG prevalence of 41% (20) and a 95% confidence interval (CI) with a 5% desired absolute precision. A systematic random sampling technique was used to obtain the required sample. Venous blood samples taken from the pregnant women were referred to the health center laboratory for routine prenatal tests. A total volume of 0.5 ml of a serum sample from each woman was used to perform toxoplasmosis serological tests. All samples were screened for IgG and IgM antibodies to *T. gondii* by enzyme-linked immunosorbent assay (ELISA). The positive samples for both antibodies or IgG positive and IgM borderline were tested by IgG avidity test as a confirmatory test to distinguish acute and chronic infections of *T. gondii*. Every sample was a candidate for

molecular identification of the *T. gondii* genome in blood samples if the IgG test was positive or IgM was borderline or positive with a low avidity index. The tests were free of charge for participants, and the results were delivered to the study population to be assessed by their gynecologists. If the test result for *T. gondii* IgG and IgM antibodies was negative, they were trained about the preventive measures for congenital toxoplasmosis during pregnancy.

Serological analysis

Indirect ELISA was applied for the detection of IgG and IgM antibodies against *T. gondii* by using commercial kits (Euroimmun, Germany). The test was conducted according to the manufacturer's instructions. The optical density (OD) of samples was measured by an ELISA microplate reader (BioTek Instruments, Epoch microplate spectrophotometer, USA) at wavelengths of 450 nm and 630 nm (as reference filter). The results were expressed quantitatively by calculating the international unit (IU) ml. Samples with OD < 8 IU/ml, OD ≥ 11 IU/mL, and OD ≥ 8 - < 11 mL were considered as negative, positive, and borderline, respectively.

IgG avidity test was performed by indirect ELISA using commercial kits (Euroimmun, Germany) according to the manufacturer's protocols. The percentage of relative avidity index (RAI%) was calculated by dividing the extinction of the urea-treated sample by the extinction of the untreated sample. The RAI < 40%, > 60%, and 40-60% were considered as low, high, and borderline antibodies, respectively.

Data analysis

The data obtained from the questionnaires and laboratory tests were analyzed using the statistical software SPSS version 23.0 (SPSS). Statistical analyses were performed using the chi-square test and the ANOVA test. A *P* value of less than 0.05 was considered significant, statistically.

Results

Socio-demographic characteristics

The age range of the participants was 14-48 years with a mean ± standard deviation (SD) of 28.1 ± 6 years. The majority of the participants were housewives (94.5%) and urban residents (86.8%). Except for three pregnant women, all individuals used tap water for drinking. The percentage of participants according to the level of educations were 3.1%, illiterate; 15.1%, elementary; 20.5%, secondary; 7.6%, high school; 42.2%, diploma, and 11.2%, college and above. From all, 34.9% of subjects had contact with soil and 76.6% of women consumed raw vegetables at least once a week. Only 48 women had a habit of eating raw or undercooked foods of animal origin. In terms of previous education, 28.9% and 29.7% were aware of the role of raw/undercooked meat, and also raw vegetables in the transmission of the parasitic infection, respectively.

Among the participants, 21.9% had a history of abortion. Also, 1.5% of the women kept a cat as a domestic pet in their homes.

Seroprevalence of *T. gondii* infection

Overall, the prevalence of specific anti-*T. gondii* IgG antibodies was 21.2% (157/740) (Table 1). Only, 0.27% (2/740) of women were seropositive for IgM antibodies against this parasite. The test results of four cases were positive for IgG and borderline for IgM with similar results on repetition of the tests. These six samples were tested by the IgG avidity test in which a high avidity index was observed for all six specimens.

T. gondii infection and risk factors

Statistical analysis showed no significant association between *T. gondii* seropositivity and the demographic characteristics of the individuals, including age groups, education level, occupation, residence, number of delivery, occupation and history of abortion (Table 1). Also, among the behavioral characteristics of the individuals, only, "contact with soil" showed a significant association with *T. gondii* infection (*P* < 0.05) (Table 2, Figure 1).

In terms of previous knowledge and awareness about toxoplasmosis, most participants (n=727, 98.2%) had never heard about toxoplasmosis or knew how to prevent the infection by *T. gondii*. Thirteen (1.7%) pregnant women claimed to partially know about the transmission routes of *T. gondii* and also clinical syndromes of toxoplasmosis. The sources of information were scientific book (n=3), internet sites (n=2), academic education (n=4), TV (n=2), and others (n=2).

Discussion

In the present study, 21.2% of the studied pregnant women were positive for *T. gondii* infection which is about two times less than the prevalence rate of 41%, described for the same population in a systematic review and meta-analysis reported from Iran (20). Also, the prevalence of the parasite in our study was approximately 1.6 times lower than a previous study conducted on women referred for pre-marriage examinations in Qazvin province (25). In the current study, 6.5% of participants had a history of raw/undercooked meat consumption which is much less than the value of 58% reported in a systematic review, and meta-analysis reported from Iran (26). This difference is probably related to variations in eating habits of meat foods in different regions of the country, and also may be due to the lack of a unique definition of undercooked meat in various studies. One of the favorite meat dishes among Iranians is kebab which is prepared using pieces of livestock or poultry meat heated directly on charcoal or gas grill. Some people like it to be juicy which can be a risk factor for toxoplasmosis. In our study, the kebab was considered as cooked meat. In recent

Table 1. The anti-*T. gondii* IgG antibodies among 740 pregnant women referred to health care centers in Qazvin province and its association with demographic characteristics of the individuals, 2019

	Positive No. (%)	Negative No. (%)	Borderline No. (%)	Total	P value
Age (y)					
≤25	48 (19.2)	199 (79.6)	3 (1.2)	250 (33.8)	0.569
>25	109 (22.3)	373 (76.1)	8 (1.6)	490 (66.2)	
Education level					
Illiterate	3 (13)	19 (82.6)	1 (4.3)	23 (3.1)	0.561
Elementary	27 (24.1)	83 (74.1)	2 (1.8)	112 (15.1)	
Primary	39 (25.7)	110 (72.4)	3 (2)	152 (20.5)	
High school	15 (26.8)	41 (73.2)	0 (0)	56 (7.6)	
Diploma	54 (17.3)	253 (81.1)	5 (1.6)	312 (42.2)	
College and above	19 (22.4)	66 (77.6)	0 (0)	85 (11.5)	
Occupation					
housewife	151 (21.6)	538 (77)	10 (1.4)	699 (94.5)	0.955
Non-housewife	6 (14.7)	34 (82.9)	1 (2.4)	41 (0.5)	
Residence location					
Urban	131 (20.4)	502 (78.2)	9 (1.4)	642 (86.8)	0.326
Rural	26 (26.5)	70 (71.4)	2 (2)	98 (13.2)	
Number of delivery					
1	45 (20.3)	175 (78.8)	2 (0.9)	222 (30)	0.768
2	69 (20.4)	265 (78.2)	5 (1.5)	339 (45.8)	
>2	43 (24)	132 (73.8)	4 (2.2)	179 (0.2)	
History of abortion					
Yes	29 (17.9)	128 (79)	5 (3.1)	162 (21.9)	0.94
No	128 (22.1)	444 (76.8)	6 (1)	578 (78.1)	
Total	157 (21.2 %)	572 (77.3 %)	11 (1.5 %)	740 (100)	

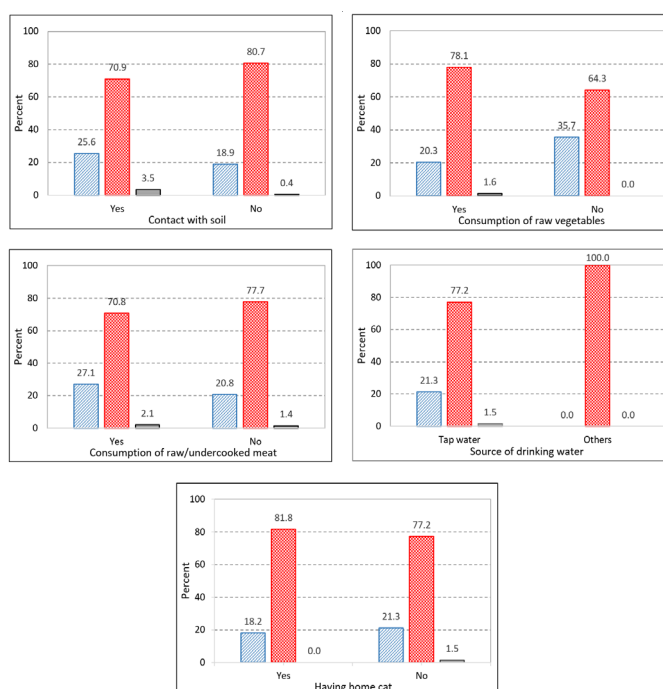


Figure 1. Distribution of results of anti-*T. gondii* IgG antibodies among 740 pregnant women referred to health care centers in Qazvin province and its association with behavioral characteristics of the individuals, 2019 (1st Column positive test, 2nd negative and 3rd borderline, The P value respectively is 0.03, 0.15, 0.39, 0.93, 0.89).

Table 2. The anti-*T. gondii* IgG antibodies among 740 pregnant women referred to health care centers in Qazvin province and its association with behavioral characteristics of the individuals, 2019

	Positive No. (%)	Negative No. (%)	Borderline No. (%)	Total	P value
Contact with soil					
Yes	66 (25.6)	183 (70.9)	9 (3.5)	258 (34.9)	0.03
No	91 (18.9)	389 (80.7)	2 (0.4)	482 (65.1)	
Consumption of raw vegetables					
Yes	142 (20.3)	545 (78.1)	11 (1.6)	698 (94.3)	0.151
No	15 (35.7)	27 (64.3)	0 (0)	42 (5.7)	
Consumption of raw/undercooked meat					
Yes	13 (27.1)	34 (70.8)	1 (2.1)	48 (6.5)	0.393
No	144 (20.8)	538 (77.7)	10 (1.4)	692 (93.5)	
Source of drinking water					
Tap water	157 (21.3)	569 (77.2)	11 (1.5)	737 (99.6)	0.927
Others	0	3 (100)	0	3 (0.4)	
Having home cat					
Yes	2 (18.2)	9 (81.8)	0 (0)	11 (1.5)	0.885
No	155 (21.3)	563 (77.2)	11 (1.5)	729 (98.5)	

years, due to economic difficulties, the purchase of meat has been greatly reduced in the food basket of Iranians, especially among low-income people. Consequently, these individuals usually use the meat for the preparation of stew foods in which the components are completely cooked. It seems that meat-based transmission is not the main route for infection to *T. gondii* in the study area. The parasite is usually more prevalent among the people who eat undercooked meat as a habit. For example, the percentage of raw/undercooked meat consumers and the prevalence of *T. gondii* were 47% and 85.3% in Ethiopia (27), 44.3% and 44.5% in Tanzania (28), 66.7% and 67.9% in Brazil (29), 47.94% and 48% in Afghanistan (30), 24.5% and 29.9% in Sri Lanka (5), and 32.6% and 31.1% in Burkina Faso (3), respectively.

Considering the risk factors related to *T. gondii* oocysts including, an abundance of cats, the habit of consumption of raw vegetables, climatic conditions, and contact with soil, it seems that oocyst-based transmission is the primary source of the infection among Iranians compared with tissue cyst-based transmission. Cats freely enter the agricultural lands and home gardens so that the defecation of these animals on the fields can lead to contamination of the soil and vegetables with oocysts of the parasite. Then, humans may be infected by ingesting the sporulated oocysts. The oocysts in soil buried at the depth of 3-9 cm can remain infective for 18 months (1). The high prevalence of *T. gondii* in Iran has been reported in the Northern provinces (Guilan and Mazandaran) with a humid subtropical climate which is the most suitable region for the development and survival of oocysts (20,24,26). However, there is evidence that indicates oocysts-based transmission of *T. gondii* has

probably declined in Iran. This transmission route of the parasite is similar to many human intestinal parasites, such as *Ascaris lumbricoides*, Hook worm and *Trichuris trichiura* for which the prevalence rates have dramatically decreased in the country within recent years (31,32). The current status of these parasites is probably related to the synergistic effect of several essential factors, especially the promotion of health awareness, development of public health services, and improved socio-economic and health facilities for Iranians in recent years.

In the present study, contact with soil was the only risk factor that showed a significant association with infection to *T. gondii* (Figure 1). This variable has been demonstrated as the essential risk factor for toxoplasmosis in some previous studies (33-35). It seems that oocyst-based transmission through contact with soil has a more important role in comparison with eating raw vegetables in the transmission of this parasite in the study area, even though the consumption of raw vegetables is a common habit among Iranians. The frequency of vegetable consumers was 94.3% in our study which is higher than 39%, a figure presented in a systematic review and meta-analysis of *T. gondii* seroprevalence among Iranian pregnant women (22).

In the present study, only 2 participants were positive for IgM antibodies to *T. gondii*. The specific IgM antibodies are considered as the primary criterion for acute (recent) toxoplasmosis in individuals whose specific IgG antibodies are simultaneously positive for the parasitic infection. However, IgM positivity along with IgG is not a definitive criterion for acute toxoplasmosis as some individuals have persistent IgM positivity for many months after acquired infection. In these cases,

the IgG avidity test is used as a complementary test to differentiate between acute and chronic toxoplasmosis. In our study, the avidity index was high in 2 participants who had IgM positive results. This finding is interpreted as the occurrence of infections before pregnancy. These results demonstrate a low incidence of *T. gondii* infections during pregnancy in the study area, despite the fact that 78.8% of the pregnant women were potentially at risk for congenital toxoplasmosis.

In our study, similar to some others, there was no significant association between the prevalence of *T. gondii* and age (3,5,6,36), occupation (27,37,38), and education level (2,7,38), and residence location (5,27) of the participants. However, in several other reports, the association was statistically significant in terms of age (7,27,34,38) occupation (2,3,7), education level (3,27,35), and residence (3,7,33-35) of the participants. The variations may reflect the differences in demographic characteristics and socio-economic status among different geographical areas. The results of the present study confirm the previous study in which no significant association between abortion and *T. gondii* infections was established (2).

The feces of the cat is the main source of environmental contaminations with *T. gondii* oocysts, but the animals usually do not carry the parasite on their fur. In our present study, no significant association between the seropositivity for this parasite and contact with cats was found, a finding consistent with the results claimed in other reports (2,7,33). However, some studies reported a significant association between *T. gondii* infection and contact with domestic cats (12,27,34,35,37). It seems that in areas where contact with cats has shown a significant relationship, other risk factors including an abundance of cats, consumption of raw vegetables, high-level contamination of surface soils, and higher frequency of soil contact have the main role in the oocysts-based transmission of the parasite.

In the present study, most participants had never heard about *T. gondii* and toxoplasmosis; however, only 1.7% (n=13) of the pregnant women had previous knowledge about this parasite and the disease caused by this organism. Our data showed that *Toxoplasma*-related information was significantly lower than the values observed in other countries where the figures reported were 94.4% in Poland (39), 48% in the United States (14), 58% in Japanese (17), 27.8% in Brazil (16), and 17.19% in China (15). The results of the present study showed that despite the low level awareness of participants about toxoplasmosis, *T. gondii* prevalence was lower than what we expected. Probably, in addition to the reasons mentioned above, public health care promotion and increased general knowledge about preventive measures against parasitic infections played essential roles in reducing this parasitic infection in the recent years within the study area.

Conclusion

Our results clarified that the prevalence and incidence of *T. gondii* have remarkably reduced in pregnant women in the study area; however, it can be associated with an increased at-risk population for congenital toxoplasmosis. Considering a very high frequency of unaware pregnant women about toxoplasmosis, *toxoplasma*-related education must be provided in pregnancy care training programs to prevent Iranian pregnant women from congenital toxoplasmosis.

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Authors' Contribution

SAG and AB; data collection. PH and FS participated in the writing. EH and MS; study designed and supervised the research. NV and AJ; data analysis. All authors contributed to helpful discussions, read and approved the final manuscript.

Conflict of Interests

The authors declared that there is no conflict of interest.

Ethical Approval

The protocol of this study was approved by the Ethics Committee of Qazvin University of Medical Sciences, Iran (Ref. No. IR.QUMS.REC.1397.375).

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