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Prevalence of Food-Borne *Toxoplasma* in Pregnant Women Population of Urmia, Iran

A. Eshratkhah Mohammadnejad [1], G. Eslami [2*✉], F. Shamsi [3], A. Pirnejad [4], A. Samie [5], J. Safabakhsh [6], A. Sakhavand [6], M. Elloumi [7]

1. Department of Parasitology and Mycology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Research Center for Food Hygiene and Safety, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Biostatistics, School of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
4. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
5. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
6. Molecular Diagnostics Laboratories, Urmia healthcare centers, Urmia, Iran
7. Laboratory of Technologies of Information and Communication and Electrical Engineering, University of Tunis, Tunis, Tunisia

HIGHLIGHTS

- Out of 620 pregnant women, 114 (18.4%) and three (0.5%) had IgG and IgM to toxoplasmosis, respectively.
- The significant relation was shown between the IgG-toxoplasmosis level and age.
- *Toxoplasma gondii* isolated from three newborns of IgM-positive mothers were identified as type I.
- Screening of pregnant women and healthcare education are suggested to prevent toxoplasmosis in newborns.

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Acronyms and abbreviations
ELISA= Enzyme-Linked Immuno-Sorbent Assay
PCR=Polymerase Chain Reaction

ABSTRACT

Background: Toxoplasmosis is one of the most prevalent parasitic infections among humans and animals which caused by *Toxoplasma gondii*. This study was carried out to detection and identification of *T. gondii* in pregnant women population of Urmia, Iran.
Methods: This cross-sectional study was done from August 2015 to March 2016. Totally, 620 pregnant women referred to the urban and rural healthcare centers of Urmia were studied. IgG and IgM specific for *T. gondii* were assessed by enzyme-linked immunosorbent assay. The demographic and risk factors data were collected by questionnaires. All included women completed the consent letter of agreement. The identification of *T. gondii* was performed using nested Polymerase Chain Reaction. The statistical analysis was done using SPSS 16.0.
Results: Out of 620 pregnant women, 114 (18.4%) had specific IgG and three (0.5%) had specific IgM for *T. gondii*. Statistical analysis showed significant relation between the IgG level and age (p<0.05). We did not find any significant relation (p>0.05) between the level of *T. gondii* specific antibodies with career, education, and vegetable and meat consumption habits. Molecular identification of *T. gondii* showed type I in isolates obtained from three newborns of IgM-positive mothers.
Conclusion: In order to prevent of the disease in newborns, screening of pregnant women and healthcare education are suggested.

Introduction

Like some other members of Sarcocystidae family (e.g. *Sarcocystis*), *Toxoplasma gondii* is known as one of the most important food-borne agent (Hajimohammadi et al., 2014a, b; Ortega and Sterling, 2018). The definitive host

* Corresponding author. ✉ eslami_g2000@yahoo.com

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of this protozoon is cat and its intermediate host comprises all warm blooded vertebrates. The prevalence of toxoplasmosis is varied in the world ranged from 0.97 to 84% (Chaudhry et al., 2014; Razzak et al., 2005). The high prevalence of *T. gondii* was reported from different parts of Iran (Anvari Tafti and Ghafourzadeh, 2014; Sharbatkhori et al., 2014). Acute toxoplasmosis has been reported in 1 to 8 cases per 1000 pregnancy (Chaudhry et al., 2014). Incidence rates of congenital toxoplasmosis are 5 per 10000 for Europe, 6 per 10000 for North America, 18-34 per 10000 for South America, 20-24 per 10000 for Africa, as well as 8-13 per 10000 for South-East Asia (Maldonado and Read, 2017).

The main transmission ways of toxoplasmosis are consumption of raw or semi-raw meat harboring tissue cyst; or water and vegetables contaminated by oocyst defected from cat feces (Ortega and Sterling, 2018). The transmission of infective agent of the parasite to pregnant woman for the first time makes a potential risk for the fetus. Toxoplasmosis in adolescence is often asymptomatic. Transmission of the infection from mother to fetus in the first three months of pregnancy make hazardous complications in fetus such as hydrocephaly, microcephaly, irreversible effects in neural systems, and even death (Chaudhry et al., 2014). Transmission of the protozoan agent in the last three months of pregnancy to the fetus may cause a hidden infection that turn into progressive central neural system damages, mental retardation, schizophrenia, decrease in eyesight, and chorioretinitis after several years (Brown et al., 2005). Congenital infections caused by *T. gondii* can be controlled if it is cured at the first days of birth. Therefore, screening and awareness of the pregnant women with negative specific antibodies against toxoplasmosis is too necessary.

European countries have been started the screening of the disease in pregnant women in order to preventing of serious disease in fetus and newborns (Lopes-Mori et al., 2011). Unfortunately, no regular screening is available in Iran. Awareness of toxoplasmosis prevalence and the related food-borne potential risk are necessary in each society in order to control the disease. Based on database, the most effective variables on toxoplasmosis prevalence are age, gender, food hygiene, soil interaction, geographical position, and environmental factors (Ortega and Sterling, 2018).

So, the main goal of this study was the detection of specific IgG and IgM levels against toxoplasmosis in pregnant women referred to the urban and rural healthcare centers in Urmia, Iran from August 2015 to March 2016. In addition, the relation of the effective variables with the incidence was assessed. Also, molecular identification of *T. gondii* was performed in isolates obtained from IgM-positive mothers and their newborns using nested PCR.

Materials and methods

Population study

This cross-sectional study included 620 women in third month of pregnancy referred to the urban and rural healthcare centers of Urmia, Iran were done from August 2015 to March 2016. The pregnant women were 14 to 47 years old. The age range of pregnant women was divided in three groups, including 14-23, 24-33, and 34-47 years old. All included women completed the informed consent letter of agreement, and the demographic and risk factors questionnaire, including age; career; abortion history; blood receiving history; eating habits of vegetables, meat, eggs, and milk; and contact with cats.

Sampling

Blood sample (5 ml) was obtained from each pregnant woman. The serum of each sample was separated using centrifuge in 3000 rpm for 5 min at the room temperature. The serum was transferred in a new sterile tube and stored at -20 °C for next steps.

Specific IgM and IgG detection by Enzyme-Linked Immuno-Sorbent Assay (ELISA)

Detection of specific IgM and IgG for *T. gondii* was done using *Toxoplasma* IgM ELISA kit (PishtazTeb, Tehran, Iran; #50314096) and *Toxoplasma* IgG ELISA kit (PishtazTeb, Tehran, Iran; #50313096), respectively according to the instructions. The results were analyzed with ELISA reader (Labsystems, Canada) alongside with the positive and negative samples.

Identification and typing

In order to molecular detection and identification of *T. gondii*, 2 ml blood sample was obtained from each woman and their newborns that had positive IgM. DNA extraction was done using DNA extraction kit (Exgene™ Blood SV, GeneAll, Korea). Extracted DNA was assessed using agarose gel electrophoresis and also spectrophotometry for quality and quantity analyses, respectively. Detection and identification of *T. gondii* was carried out using nested PCR by the specific primers of AK69Fex 5'-TTGAACATCTGGTGCGAG-3' and AK69Rex 5'-GTCTCCCAACCACCTCCA-3' with amplicon of 633 bp in length for the first run; and AK69F 5'-ACGAGCAACCATATCTTACC-3' and AK69R 5'-CGAACGGACAACAAGCTA-3' with the final fragment of 490 bp in length for the second run (Boughattas et al., 2011; Boughattas and Bouratbine, 2015). The amplification was done using thermocycler (QB-96, quants biotech, England) with the first denaturation of 94 °C for 5 min and then followed by 28 cycles of 94 °C for 60 s,

56.4 °C for 60 s, and 72 °C for 60 s. The final extension was done at 72 °C for 5 min. The negative as well as positive controls were run in each amplification reaction alongside with the samples. Amplification reaction was considered with 1x PCR buffer, 10 pmol of each primer, 1 U *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 0.2 mM dNTP, and 100 ng genomic DNA as the template. The amplification was assessed using 1% agarose gel electrophoresis alongside with 50 bp DNA ladder. All samples were assessed in triplicate. For verification and typing identification, the amplicons were sequenced and then analyzed using nBLAST software.

Statistical analysis

All data were collected and analyzed by SPSS software (version 16.0, SPSS Inc Chicago Ill, USA) using average percentage for descriptive data T-test and Fischer exact tests for analytical data. The significant differences were determined at $p<0.05$.

Results

Out of 620 pregnant women, 114 (18.4%) showed specific IgG for *T. gondii*. The average age of all pregnant women was estimated as 29.82 years old. As shown in Table 1, the most seropositive pregnant women were included in the group with the age range of 24-33 years old (57%). Statistical analysis showed significant association between age and toxoplasmosis incidence ($p<0.05$).

We did not find any association between the IgG level and eating habits (Table 2), including consumption of vegetable, meat, egg, and milk ($p>0.05$). Also, there is no association between the IgG level and abortion and blood receiving history ($p>0.05$). Cat contact and also toxoplasmosis had no correlation because none of the IgG seropositive pregnant women had any contact with cat. All women with IgG positive were housewife and therefore, we did not find any correlation with career and toxoplasmosis, too.

Out of 114 IgG positive found in the present investigation, three samples (0.5%) were IgM positive for *T. gondii*. As illustrated in Figure 1, *T. gondii* was detected in all the three pregnant women with anti-toxoplasmosis IgM and their newborns. Also, the sequence analyzing using nBLAST verified all isolates as type I.

Discussion

Toxoplasmosis is not considered as a serious disease in human with competent immune system but it can have irreversible complications in fetus of pregnant women that exposed to the parasite for the first time. Congenital toxoplasmosis is the most hazardous effect of *T. gondii* in fetus. In this study, we assessed the prevalence of specific IgG and IgM for *T. gondii* in 620 pregnant women in Urmia, Iran. As shown in results section, 18.4% women were IgG positive which is less than the ones in the other reports from Iran with 56-75.02% from the North of Iran (Firouz et al., 2014; Panah et al., 2013), 31.1% from Tehran (Ghasemloo et al., 2014), 41.8% from Gorgan (Sharbatkhori et al., 2014), and 32% from Yazd (Anvari Tafti and Ghafourzadeh, 2014). The prevalence of *T. gondii* varies in different countries, too. This data in United States is estimated as 30% (Brown et al., 2005). The IgG seropositive in Southern Brazil in pregnant women has been reported as 77.5% (Porto et al., 2008). Also, these data in Asian countries are varied; with the least (0.8%) in Korea (Buchy et al., 2003) and Vietnam (Song et al., 2005), and the highest (55%) in India (Khurana et al., 2010), and Nepal (Rai et al., 1998). The most important factors for making this differences are considered environmental condition, humidity level, heat, geographical circumstances, food habits, and hygienic behaviors (Anvari Tafti and Ghafourzadeh, 2014).

Our study showed that careers had no correlation with toxoplasmosis that differs with the findings of the other Iranian researchers such as Arbabi et al. (1997) in Kashan and Pashaie Naghadeh et al. (2015) in Tabas. They reported that housewives have higher exposure to the parasite. Probably the reason for this difference can cause to raising the level of public awareness and health of the housewives and increasing the level of education.

In the current investigation, consumption of vegetable did not show any association with toxoplasmosis that does not agree with the study by Fallah et al. (2006) in Hamedan, Iran. But our results agreed with the study by Saeedi et al. (2002) in Gorgan, Iran. This difference may be due to the habits of washing and disinfecting raw vegetables in different regions. Consumption of raw or semi-raw meat has been considered as one of the important ways for infecting human. Despite of this, our survey showed no significant differences between having raw/semi-raw meat and toxoplasmosis that is in agreement with the results of Ertug et al. (2005). Chickens may have critical role in transmission of *T. gondii* by either consumption of raw/semi-raw chicken meat or rarely having raw eggs (Ortega and Sterling, 2018). Our study did not show any relationships between *T. gondii* prevalence and egg usage because all people in region we studied were using cooked egg. We did not also find any association between *T. gondii* prevalence and using milk that is agreed with the study by Fallah et al. (2008) in Hamedan, Iran. In the present study, the women had not the habit of using raw milk. Therefore, transmission of the protozoon by milk is not considered as a key role for

Table 1: The anti-toxoplasmosis IgG level among pregnant women and its correlation with age

Age range	IgG positive No. (%)	IgG negative No. (%)
14-23	19 (16.7)	151 (29.8)
24-33	65 (57)	261 (51.6)
34-47	30 (26.3)	94 (18.6)

Table 2: The anti-toxoplasmosis IgG level among pregnant women and its correlation with eating habits/ abortion and blood receiving history

Risk Factors	*Toxoplasma* IgG		*P* value
	Positive No. (%)	Negative No. (%)	
Vegetable eating habits			
Wash with water	0 (0)	4 (0.8)	0.496
Raw + cooked + water	111 (97.4)	495 (97.8)	
Raw + cooked + Perchlorin	3 (2.6)	7 (1.4)	
Meat			
Well done	3 (2.6)	11 (2.2)	0.729
Well done + barbecue	111 (97.4)	495 (97.8)	
Egg			
Raw	0 (0)	2 (0.4)	1.00
Boiled	114 (100)	504 (99.6)	
Milk			
Raw	0 (0)	2 (0.4)	1.00
Boiled	114 (100)	504 (99.6)	
Abortion history			
Yes	13 (11.4)	69 (13.6)	0.525
No	101 (88.6)	437 (86.4)	
Blood receiving history			
Yes	2 (1.8)	10 (2)	1.00
No	112 (98.2)	496 (98)	

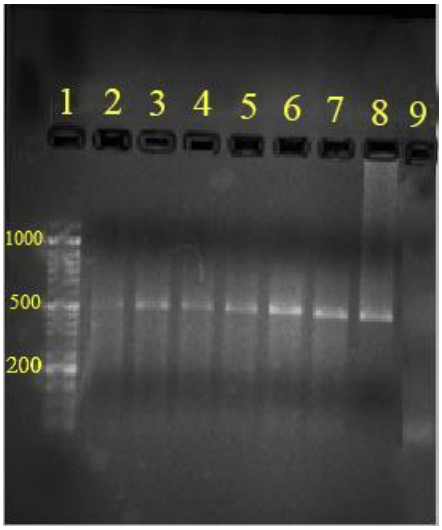

Figure 1: Agarose gel electrophoresis (1%) for analyzing the amplification of the target gene of *AK69*; the amplicon size of 490 bp showed *T. gondii*. Lane 1: 50 bp DNA ladder; lanes 2, 3, 6: The pregnant women samples with IgM seropositive; lanes 4, 5, 7: newborns samples from women with IgM seropositive; lane 8: positive control; lane 9: negative control

the prevalence of toxoplasmosis in the area that was assessed in present study.

It has been stated that blood transmission is considered as the other important way for infecting human if the donor has acute toxoplasmosis (Ortega and Sterling, 2018). Especially this way of transmission is very dangerous in immunocompromised patients. In this study, two pregnant women with IgG seropositivity had blood receiving history that statistical analysis showed no correlation between toxoplasmosis and blood receiving. We did not find any meaningful correlation between toxoplasmosis and abortion history that agreed with Akhlaghi et al. (2014). This issue shows probably that the most reason of infection *T. gondii* in our studied area is eating habits and hygienic behaviors.

The rate of acute toxoplasmosis in pregnant women of this study was estimated as 4.8 per every 1000 pregnancy (3 people). Mean incidence rate of acute infection in the world is about 1-8 per every 1000 pregnancy (Chaudhry et al., 2014). The amount of the acute infection of *T. gondii* in Kuwait among pregnant women is 13.8% (Iqbal and Khalid, 2007). This data in the other countries are different such as South America with 2.8% (Rosso et al., 2008), Iraq with 0.97% (Razzak et al., 2005), Spain with 0.01% (Roc et al., 2010), and Poland with 0.5% (Nowakowska et al., 2006). In Iran, the acute toxoplasmosis prevalence during the pregnancy has been reported 1.4% in Zanjan (Hajsoleimani et al., 2012), 4.8% in Isfahan (Alameh and Tavangar, 2002), and 0.6% in Kashan (Rasti et al., 2012). Our results showed that 0.5% pregnant women from Urmia had acute toxoplasmosis which is less than the ones in Zanjan and Isfahan but agreed with the one in Kashan, Iran. Also, in comparison with the other countries, the prevalence of acute toxoplasmosis in Urmia, Iran is less than the countries such as Kuwait (Iqbal and Khalid, 2007) and Colombia in South America (Rosso et al., 2008), but the same as the one in Iraq (Razzak et al., 2005) and Poland (Nowakowska et al., 2006). Based on the literature, Spain (Roc et al., 2010) has the least prevalence rate of toxoplasmosis. The cause of these various rates of infection in different parts of Iran and the world can be related to the socio-cultural status, contact with the cat, and the observance of health points (Chaudhry et al., 2014).

Molecular detection and identification of *T. gondii* obtained from three pregnant women and their newborns with acute toxoplasmosis was done using nested PCR with the target gene of *AK69*. The *AK69* gene is used as a marker to detect and identify *T. gondii* in order to genotyping (Boughattas and Bouratbine, 2015). In this study, all three *T. gondii* isolates obtained from pregnant women were type I, also all isolates from their newborns were type I. Totally, *T. gondii* are classified into three types, including I, II, III (Fazaeli et al., 2000). Although these types have little difference in their sequences but their pathogenesis are different in hosts. Type I is the most severe type, type II usually had slightly longer period and a milder course of disease, and finally type III resulted in mostly lacking clinical symptoms (Fazaeli et al., 2000). The dispersal of these types depends on the region where the hosts are located. As mentioned previously, all isolates obtained from patients in this research were type I. In a study that conducted by Tavasoli et al. (2012) in Urmia, the *T. gondii* isolates from men and women was found as type I. Another survey in Tabriz reported toxoplasmosis in animals with *T. gondii* type I (Tavassoli et al., 2010). The mentioned results reveal that ecological and geographical conditions may lead to a different prevalence of the types of parasite in different parts of the world.

Conclusion

It was showed that the prevalence of toxoplasmosis in Urmia had no meaningful association with some risk factors such as eating habits, including consumption of raw vegetable, meat, egg usage, and milk. Also, we did not find any correlation between abortion history, blood receiving history, and career with toxoplasmosis. In this population, age had significant association with IgG seropositivity of *T. gondii*. Our study showed acute toxoplasmosis in three pregnant women. Molecular detection and identification of *T. gondii* isolates obtained from pregnant women and their newborns verified *T. gondii* type I. Type I is the most severe type and lethal for the animals; also, it is known as the most virulent type for human that may resulted in serious symptoms in newborn such as miscarriage, progressive visual, hearing, and the other problems. Because of the importance of acute toxoplasmosis infection in pregnant women and its transmission to fetus, it is necessary to aware people especially pregnant women about the current ways of transmission. The screening test for toxoplasmosis before marriage or pregnancy should be considered as a routine test by healthcare officials.

Conflicts of interest

The authors declare that there is no conflict of interest.

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