PREVALENCE AND RISK FACTORS OF TOXOPLASMOSIS AMONG WOMEN OF REPRODUCTIVE AGE, SOUTHWESTERN IRAN

By
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Abstract
Toxoplasma gondii is an obligate intracellular parasite that has a worldwide prevalence. This infection can lead to severe problems during pregnancy, including miscarriage, and fetal developmental retardation. This study assessed the seroprevalence of toxoplasmosis in women of reproductive age in Dehdasht, Iran.

Blood samples were collected from 300 women referred to Health Center between February and July 2020. Serum was separated and the anti-Toxoplasma IgG & IgM were assessed by using the ELISA. Also, all women were administered a questionnaire to record demographic and risk factors data such as age, residence, education level, marital status, contact with cats, consumption of raw vegetables, methods of cooking meat, occupation, raw liver consumption, contact with soil, and raw egg consumption. SPSS software v.23 and Chi-square test used for statistical analyses.

The results showed that 32/300 (10.6%) women were seropositive for anti-Toxoplasma IgG, but negative for anti-Toxoplasma IgM. There was a significant relationship between the prevalence of toxoplasmosis with age, accommodation, contact with cats, and methods of washing vegetables, number of serving vegetables per day, method of cooking meat, occupation, liver consumption, and contact with soil. There was no significant relationship between toxoplasmosis with marital status, consumption of raw vegetables, number of servings of meat, and consumption of eggs.

Keywords: Toxoplasmosis; Risk factors; ELISA; Women of reproductive age; Iran

Introduction
Toxoplasma gondii is an obligate intracellular zoonotic parasite of the phylum Apicomplexa that causes toxoplasmosis worldwide (Abbas et al, 2020). It infects a wide range of mammals, including humans, animals and birds (Cenci-Goga et al, 2011). Pinto-Ferreira et al. (2019) reported that the prevalence rate varied with differences in climate, culture, food habits, behavior, personal hygiene and cooking habits of different societies and ethnic groups. The definitive hosts of T. gondii are members of the cat’s family that sexual reproduction occurs in the intestine of these animals (Dubey et al, 2020). Humans can become infected by eating undercooked meat of animals harboring tissue cysts, consuming food or water contaminated with cat feces or by contaminated environmental samples (such as fecal-contaminated soil or changing the litter box of a pet cat), blood transfusion or organ transplantation, or transplacentally from mother to fetus (CDC, 2020) or nosocomial by needle-stick injury (Abdel-Motagaly et al, 2017). The remarkable resistance of the oocyst wall enables dissemination of T. gondii through watersheds and ecosystems, and long-term persistence in diverse foods such as shellfish and fresh produce (Shapiro et al, 2019). Domestic cats and wild felids can be infected by consuming of tissues of an infected intermediate host (bradyzoite cysts), ingesting oocysts, or via congenital transmission (Dubey, 2020). Infection with T. gondii in immune-competent individuals is asymptomatic, but can being dangerous in immunocompromised ones (Wang et al, 2017).
Most healthy people who are toxoplasmosis infected have no signs or symptoms and aren't aware that they're infected. Some people, however, develop signs and symptoms similar to those of the flu, including: Body aches, swollen lymph nodes, headache, fever and fatigue in patients on chemotherapy or have recently had an organ transplant, a previous infection reactivate risks including: Headache, confusion, poor coordination, seizures, lung problems and blurred vision caused by severe retinal inflammation. Baby is most at risk of contracting toxoplasmosis if you become infected in the third trimester and least at risk if he/she becomes infected during the first trimester. On earlier pregnancy infection occurs with baby more serious the outcome such as: Seizures, an enlarged liver and spleen, yellowing of skin and whites of eyes (jaundice), and severe eye infections (Madireddy et al, 2021). Abdallah et al. (1994) in Saudi Arabia reported Toxoplasma IgG ranged between 32.1% (IHAT), 46.2% (ELISA), but IgM (ELISA) was 23.1%. Elnahas et al. (2003) in Sudan reported that 65% women tested for anti-Toxoplasma IgG were negative, but they were at risk of sero-conversion during pregnancy Saleh et al. (2014) in Egypt reported that Toxoplasma (IHAT) antibodies were 22.2% among pregnant women, and 20% of non-pregnant ones. Mihu et al. (2020) in Romania reported Toxoplasma IgG in 1081 women was 41%. Anyhow, congenital toxoplasmosis can cause serious health problems, so women's awareness about its transmission and impacts in humans can greatly reduce the disease incidence (Prusa et al, 2017). Screening toxoplasmosis programs and treatment of pregnant women reduced severity of sequels (Lopes et al, 2009), and ELISA T. gondii-IgG/ IgM antibodies were more practical, cheap and sensitive (Liu et al, 2015).

This study aimed to identify ELISA IgG/ IgM against Toxoplasma gondii among reproductive aged women in Dehdasht, Iran and to clarify the risk factors and consequence during pregnancy.

Subjects and Methods

Ethical statement: All experiments and study protocols were reviewed and ethically approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Approval ID: IR. SSU.SPH. REC.1399.113) and written informed consent was obtained from all the participants.

Study area: This study was carried out in Dehdasht, the Capital of Kohgiluyeh and Boyer-Ahmad Province, Iran with geographical character of 30°47’42”N 50° 33’52”E. The mean annual temperature is 23°C (https://en.wikipedia.org/wiki/Dehdasht).

Study design: This descriptive-analytical was a cross-sectional study, carried out on reproductive aged women referred to Dehdasht Comprehensive Health Service, maternity facilities Centers from February to July 2020. The sample size was 300 based on the previous studies with a 95% confidence interval and 6% accuracy, using a standard statistical formula. All the participated women were given a questionnaire to record demographic and risk factors data, which provided information about their age, type of accommodation, occupation, education, marital status, history of cat ownership and contact, exposure to soil, consumption of raw vegetables, number of servings of fruits and vegetables per day, methods of washing fresh plant materials before eating, ways to cook meat, and consumption of raw egg & liver.

Sample collection: A total of 300 samples were obtained from reproductive aged women (15 to 49 years). After gathering blood sample (5 ml), serum was separated and stored at -20°C. Anti-Toxoplasma IgG & IgM was assessed using commercial ELISA kits (Pishtaz Teb, Iran) strictly following the manufacturer's instructions. The Index of ≥1.1 IU/ML indicated positive; but an index of <0.9 IU/ML denoted negative anti-Toxoplasma IgG & IgM.

Statistical analysis: Data entered in an excel sheet and analyzed using the SPSS software v.23 (SPSS Inc., Chicago, IL, USA). Association between T. gondii sero-prevale-
nce and each risk factor was analyzed using Chi-square test (bivariate). P-value < 0.05 was considered significant.

Results
Of 300 samples, 32 ones (10.6%) were seropositive for anti-Toxoplasma IgG, but none was negative for anti-Toxoplasma IgM. Most of the healthy participant aged 25-34 years were 115/300 (38.3%) and most of the infected ones aged 35-49 years were 23/300 (7.7%), followed by 8/300 (2.7%) aged 25-34 years. Significant associations were between T. gondii IgG positive and ages (P= 0.001).

Risk factors were in T. gondii seropositivity women 9.37% (3/32) had pet cats, with significant correlations (P-en = 0.01) between T. gondii seropositivity and histories of pet cats and contact.

Housewives women were highly affected 16/32 (63.1%), followed by self-employment (house-wife) patients 13/32, and the least were students 3/32. Chi-square tests showed significant correlations between T. gondii seropositivity and residence (P=0.01), occupation (P =0.001), number of servings of fruits and vegetables per day (P =0.01), methods used for washing fresh materials for eating (P = 0.001), ways to cook meat (P =0.001), consumption of raw liver, and contact with soil (P = 0.001). There was no significance between T. gondii seropositivity and education level, consumption of raw vegetables, number of servings meat per day, and consumption of egg.

Details were given in tables (1 & 2) and figures (1 & 2).

Table 1: Frequency and Distributions of anti-Toxoplasma IgG by age group using ELISA

<table>
<thead>
<tr>
<th>Variants</th>
<th>Age group (year)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16-24</td>
<td>25-34</td>
</tr>
<tr>
<td>Negative sera</td>
<td>64 (21.3%)</td>
<td>115 (38.3%)</td>
</tr>
<tr>
<td>Positive sera</td>
<td>1 (0.3%)</td>
<td>8 (2.7%)</td>
</tr>
</tbody>
</table>

Table 2: Distribution of anti-Toxoplasma IgG by contact with cat as a risk factor

<table>
<thead>
<tr>
<th>Variants</th>
<th>Contact with cat</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No contact</td>
<td>Direct contact</td>
</tr>
<tr>
<td>Negative</td>
<td>164 (54.7%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Positive</td>
<td>10 (3.3%)</td>
<td>3 (1%)</td>
</tr>
</tbody>
</table>

Discussion
Toxoplasma infection is a risky health problem in pregnant women, immunocompromised individuals and other immune-suppressed individuals such as with cancer or organ transplant (Wang et al, 2017). Primary T. gondii infection during pregnancy may be rare, but poses challenges in establishing the diagnosis. The important consequence of primary infection is vertical transmission to the fetus, resulting in congenital toxoplasmosis. Vertical transmission and its effects on the fetus are dependent upon the gestational age at which the primary infection is acquired (Chaudhry et al, 2014). Maternal infections can cause serious medical conditions during pregnancy and have severe squeals in the infant (Kadhim et al, 2013). If acute toxoplasmosis is acquired during pregnancy, the infant is at the risk of developing congenital toxoplasmosis. The classic triad of signs associated with congenital toxoplasma infection is chorioretinitis, cerebral calcifications, and hydrocephalus, but only 10% to 15% of congenitally infected infants manifest signs of a congenital infection (Van Kessell and Eschenbach, 2008). In the present study, the prevalence rate of anti-Toxoplasma IgM & IgG was 0% and 10.6%, respectively. This agreed with Jahantigh et al. (2020) in Sistan Region who reported that the IgG & IgM among 90 pregnant women were IgG 14%. This disagreed with Panah et al. (2013) in Amol, Northern Iran who reported among 739/1057 pregnant women anti-Toxoplasma IgG & IgM (69.91% & 5.39%) respectively. The difference might be related to environmental and/or climatic conditions. Dehdasht City temperate climate annual averaged mean was 23°C. Its low T. gondii prevalence was compared with other in Iranian areas, especially the northern reg-
ions that could possibly affect the survival of *T. gondii* infective stages (Hajimohammadi et al., 2022). The climatic variations globally have the direct effect on the maintenance of oocysts excreted from cats. Meerburg and Kijlstra (2009) in North-Western Europe reported that global warming resulting from greenhouse emissions led to a changing climate in temperature, rainfall, and atmospheric CO₂ concentrations, which affected multiple biophysical processes, including the pathogens life cycle. Besides, this climate change influences the animal habitats as a consequence, range shifts would occur (Parmesan and Yohe, 2003). Thus, the species that are incapable of adaptation to these changes would become extinct (Thomas et al., 2004). Yan et al. (2016) in China reported that the environmental factors and human activities affected on the *T. gondii* occurrence, transmission and distribution. Tutuncu et al. (2003) in Turkey reported high toxoplasmosis prevalence in hot-humid climates. Shoura et al. (1974) in Riyadh found 22.1% positive toxoplasmin tests among pregnant women. El Harthi et al. (2006) in Saudi Arabia reported IgG-positivity varied from 9.13% in Hail area to 39.43% in the Eastern Region. Picone et al. (2020) in France reported the fetal transmission, outcome was live birth in 95% of cases, with latent congenital toxoplasmosis in 90% of cases and symptomatic forms in 10% of cases, of which 1/3 were severe and 2/3 moderate. Apart from climatic conditions, the frequency of stray cats in a humid rainy climate favoring the survival of oocysts contributed to the high *Toxoplasma* prevalence in Central America (Remington et al., 2001). Besides, Wilking et al. (2016) in Germany reported that the *T. gondii* prevalent was due to eating habits (consuming raw meat) appeared to be of high epidemiological relevance. Thus, there are many risk factors, such as cat contact, handling or eating raw or undercooked meat and drinking unfiltered water have an influence on *Toxoplasma* transmission worldwide.

In the present study, there are risk factors between prevalence of *T. gondii* and with increased with human’s ages. This agreed with Zemene et al. (2012) and Shamsinia et al. (2019) who suggested that aging caused possibility of the more exposure to infection. In the current study, no significant relationship was found between prevalence of *T. gondii* seropositive and marital status, which agreed with Dehnavi et al. (2018). But, this disagreed with Salih et al. (2020) who found a higher rate of seropositivity among married females (39.93%).

In the present study, a significant association was between toxoplasmosis and residence, and most of the patients were urban city residents. However, Soltani et al. (2018) detected more seroprevalence in rural inhabitants. In this cross-sectional study, 496 subjects from Abadan City participated and 36.1% of them were from rural communities with positive anti-*Toxoplasma* IgG. Daryani et al. (2014) found high IgG seropositive cases among Iranian living in rural areas. The present study didn’t find significant relationship between the toxoplasmosis prevalence and education. This agreed with Mojadadi et al. (2016) who reported that increased knowledge through the expansion of social media and its consequence on patterns of life may lead to increase awareness among the population. Eshratkhah et al. (2018) reported no significant relation between anti-*Toxoplasma* IgG and education. No doubt, frequency of eating outdoors fast food led to the increase in *Toxoplasma* prevalence among them (Pinto-Ferreira et al., 2020).

The present study showed that the contact with cats was a potential risk factor for women to acquire toxoplasmosis. Cats are the definitive host of *T. gondii*, and contact with them is a potential risk factor for human infection (Negero et al., 2017). The only definitive hosts for *Toxoplasma gondii* are members of family Felidae; domestic cats and their relatives (Rifaat et al., 1981). The relation between *T. gondii* and contact with pet cats or living in neighborhood to them was documented (Abamecha et al., 2016). Others
found no link between *Toxoplasma* infection and the pet cats (Mwambe *et al*, 2013).


In the present study, there was a significant relationship between the number of servings of fruits and vegetables per day, methods used for washing fresh produce before eating, and contact with soil. This agreed with Hatam *et al.* (2005) in Iran who reported that water, soil, and vegetables were among the major pathways for *T. gondii* oocysts to its hosts. Although in the present study, as in the study carried out by Namaei *et al.* (2010) there were no significant differences between the prevalence of *T. gondii* and the consumption of raw vegetables. But abroad, toxoplasmosis transmission occurred by consumption of raw or undercooked infected meat (Kerman *et al.*, 2014; Belluco *et al.*, 2018; Abdelbaset *et al.*, 2020; Ducrocq *et al.*, 2021; Silva *et al.*, 2021). The current results revealed a significant relationship between seropositive cases and the way of cooking liver and meat, but no significant relationship was observed with number of servings of meat. These agreed with Fallah *et al.* (2005).

In the current study, there was no significant relationship between toxoplasmosis prevalence and consumption of raw eggs. Ahmadi *et al.* (2020) reported that consumption of raw or uncooked poultry implicated as a potential risk factor for human toxoplasmosis and there was the probability of infection by consumption of raw eggs. But, Ahmadvandpour *et al.* (2017) showed statistical difference between *Toxoplasma* infection and eating raw or uncooked eggs.

Meanwhile, Mohamed *et al.* (2021) correlated between hepatic cell carcinoma (HCC) and toxoplasmosis, which led dramatic changes in the clinical pictures of both diseases to the risk of fatality.

## Conclusion

Many risk factors, such as cat contact, handling or eating raw or undercooked meat and drinking unfiltered water have an influence on *T. gondii* transmission worldwide. The present data showed that women were (89.4%) sero-negative, but being susceptible to infection. Accumulated evidence showed that changes of environmental and climatic factors as well as human habitats and behaviors influence the prevalence and incidence of toxoplasmosis. The pregnant women must avoid cats, raw vegetables, undercooked meat and other risk factors. Also, they must be routinely screened for toxoplasmosis by her midwife or doctor to check for infection.

The awareness of toxoplasmosis in public health programs as to *T. gondii* transmission control is therefore strongly advocated.

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**Author’s contribution:** All authors equally contributed in the field and laboratory work.

## References


Abdel-Motagaly, AME, Ibrahim, AMA, Morsy, TA, 2017: An intervention program on blood protozoa acquired by needle stick injury and


CDC, 2020: Parasites - Toxoplasmosis (Toxoplasma infection), https://


Madireddy, S, Rivas Chacon, ED, Mangat,
R, 2021: Toxoplasmosis. StatPearls [Internet].

Explanation of figures
Fig. 1: Distribution of anti-Toxoplasma IgG according to accommodation as a demographic characteristic of women of reproductive age (15-49 years old) population referred to Dehdasht Health Centers in 2019.
Fig. 2: Distribution of anti-Toxoplasma IgG according to education in women of reproductive age (15-49 years old) population referred to Dehdasht Health Centers in 2019.