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

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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 Febr-uary 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 co-ntaminated 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 no-socomial 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 = 0.001) between *T. gondii* seropositivity and histories of pet cats and contact.

Housewives women were highly affected 16/32 (63.1%), followed by self-empoyment (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

Variants	Age group (year)			P value
	16-24	25-34	35-49	
Negative sera	64 (21.3%)	115 (38.3%)	89 (29.7%)	0.001
Positive sera	1 (0.3%)	8 (2.7%)	23 (7.7%)	

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

Variants	Contact with cat				P value
	No contact	Direct contact	Random contact	Live in close contact with cats	
Negative	164 (54.7%)	3 (1%)	32 (10.7%)	69 (23%)	
Positive	10 (3.3%)	3 (1%)	1 (0.3%)	18 (6%)	0.001

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 CO_2 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 56.1% 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).

Apart from cats, Morsy *et al.* (1994) reported anti-*Toxoplasma* antibodies in the domestic wild and rodents. Al Dakhil and Morsy (1996) in Saudi Arabia reported anti-*Toxoplasma* antibodies in the Indian grey mongoose (H. edwardi)

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* oocytes 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 undercooked poultry implicated as a potential risk factor for human toxoplasmosis and there was the probability of infection by consumption of raw eggs. But, Ahmadpour *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.

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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.