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SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODIES AND ASSOCIATED RISK FACTORS AMONG CHILDREN IN LORESTAN PROVINCES, IRAN

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Abstract

Children due to their high hand-mouth activity, lack of hygiene standards in the consumption of food, water, fruits and vegetables and their immature immune systems are the most susceptible peoples to parasitic infections among all age groups. This cross-sectional study aims to determine the prevalence of IgM and IgG anti-*Toxoplasma gondii* antibodies and the associated risk factors among the Children (5–15 yr Old) Referred to health centres of Lorestan province, Iran from August 2016 to March 2017 on 316 children. To determine the anti-*T. gondii* antibodies, all the serum samples were tested using the commercially available ELISA kit (Dia.Pro, Milano, Italy). Analyses were carried out according to the manufacturer's instructions. Out of the 316 children, 31 (9.8%) tested seropositive for anti-*T. gondii* antibodies; 24 (7.6%) children tested seropositive for IgG antibody, 1 (0.3%) tested seropositive for both IgM and IgG, and 6 (1.9%) were positive for IgM antibody alone. The results showed that some risk factors were significantly correlated to *T. gondii* seropositivity included age (P= 0.005), living in rural areas (P=0.01) and consumption of undercooked or cured meat products (P<0.001). The findings of our study demonstrated a considerable seroprevalence of *T. gondii* infection in children in Lorestan province, Iran. Thus, proper strategies must be carried out to prevent and control *T. gondii* infection in children in this region.

Keywords: Toxoplasmosis, IgG, IgM, children, Iran.

1. Introduction

Toxoplasma gondii as a well-known protozoan parasite which uses domestic cats as its final host is prevalent among animals and humans around the world (Hill & Dubey, 2002). Naturally, Humans acquire toxoplasmosis chiefly during direct contact with the soil or fortuitously consumption of water or food contaminated with oocysts passed in cat feces (Hill & Dubey, 2002; Furtado, 2011). The disease in the immunocompetent adults and children are generally without symptoms or cover gentle and impulsively resolved signs; for example, fever, fatigue, lethargy and lymphadenopathy (Furtado, 2011; Mahmoudvand, 2016). On the other hand, toxoplasmosis in immunocompromised patients such as transplant recipients and patients with acquired immunodeficiency syndrome and the fetus can give rise to serious and fatal symptoms such as eye infection and brain involvements (Signorini et al., 2007; Torgerson & Mastroiacovo, 2013). The diagnosis of *T. gondii* infection is usually based on serological tests to find the anti-*T. gondii* antibodies; so that IgM and IgG anti-*T. gondii* antibodies are typically well-known as a indicator of acute and chronic infection respectively (Hill & Dubey, 2002).

2. Problem Statement

Reviews have shown that depending on social and cultural behaviour, geographic locations, weather, and the routes of transmission frequency of toxoplasmosis varies broadly between different countries from 10 to 80% worldwide (6). In Iran, studies demonstrated that generally seroprevalence rate of *T. gondii* infection among the general population was nearly 39.3% (95% CI = 33.0%–45.7%) (Daryani et al., 2014).

Children due to their high hand-mouth activity, lack of hygiene standards in the consumption of food, water, fruits and vegetables and their immature immune systems are the most susceptible peoples to parasitic infections among all age groups (Weatherhead & Hotez, 2015). So far, in numerous investigations the seroprevalence of *T. gondii* infection in children has been demonstrated in several countries around the world (Fu et al., 2014; Xin et al., 2015), however little is known about the seroprevalence of *T. gondii* infection in children in Iran.

3. Research Questions

Based on the concerns mentioned, we have two research questions:

1) What is the seroprevalence rate of toxoplasmosis among children referred to health centres in the Lorestan province, Iran using the ELISA method?

2) What is the seroprevalence rate of toxoplasmosis among children referred to the health centres of Lorestan province, Iran in terms of risk factors such as age, sex, place of residence, etc.?

4. Purpose of the Study

This cross-sectional study aims to determine the prevalence of IgM and IgG anti-*T. gondii* antibodies and the associated risk factors among children aged 5–15 years old referred to health centres of Lorestan province, Iran.

5.   Research Methods

5.1. Ethics

This study was approved by Ethics Committee of Lorestan University of Medical Sciences, Khorramabad, Iran. In addition, a written informed consent was obtained from all the participants before blood sampling. Parents/guardians provided informed consent on behalf of all child participants.

5.2. Questionnaire

Before collection of blood samples, a questionnaire based on demographic data including age, gender, and education was given to the children. Moreover, possible risk factors, such as animal contact (cat), raw/half-cooked meat consumption (lamb and beef), consumption of raw vegetables and residence were also evaluated.

5.3. Study design and sample collection

In this cross-sectional study, a total of 316 children aged 2-15 years, referred to health centres of Lorestan province, Iran were studied from July 2016 to February 2017. Five mL of blood was obtained from each child by means of venipuncture, under sterile conditions. The samples were centrifuged at 1000 r.p.m. and the sera were stored at 20°C until serological examination.

5.4. Enzyme-linked immunosorbent (ELISA) test

To determine the anti-*T. gondii* antibodies, all the serum samples were tested using the commercially available ELISA kit (Dia.Pro, Milano, Italy). Analyses were carried out according to the manufacturer's instructions. Based on the ELISA kit, positive results for IgG and IgM were defined as values of ≥ 50 international units (IU)/mL and index values of ≥ 0.6, respectively. Also, negative results were defined as <25 IU/mL and index values of <0.5 were considered for IgG and IgM, respectively.

5.5. Statistical analysis

In this study, analytical and descriptive statistics were carried out using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were showed in terms of percent (for categorical) and mean (SD) (for continuous) variables. The Chi-square test was applied to assess the univariate association between independent variables and outcome. All the variables in univariate analysis (Chi-square test) that had a p value less than 0.25 were entered into multivariate analysis (Logistic regression). Multifactorial Logistic regression models were used to evaluate association between *T. gondii* seropositivity and the potential risk factors. P < 0.05 was considered to be statistically significant.

6. Findings

6.1. Participants

A total of 316 children (aged 2-15 years), referred to the health centres of Lorestan province, Iran were included in the present study. The mean age of the participants was 7.9 ± 2.5 years and the majority were boys (176, 55.7%). In total, 240 (75.9%) of the participants lived in urban regions, the remaining (24.1%) lived in rural areas. Among the participants, 66 (20.8%) children had contact with cats, whereas 250 (79.1%) children did not have any contact with cats. Among the participants, only 32 (10.1%) children consumed raw or unwashed vegetables and fruits; whereas 78 (24.7%) children showed the consumption of undercooked or cured meat products. (See Table 01)

6.2. Seroprevalence of anti-T. gondii antibodies

Out of the 316 children, 31 (9.8%) tested seropositive for anti-*T. gondii* antibodies; 24 (7.6%) children tested seropositive for IgG antibody, 1 (0.3%) tested seropositive for both IgM and IgG, and 6 (1.9%) were positive for IgM antibody alone. In term of gender, 14 (7.9%) boys were positive for IgG anti-*T. gondii* antibodies compared to 10 (7.1%) girls children (P=0.87); moreover, there was no significant difference in the prevalence of IgM anti-*T. gondii* antibodies among the girls (2.14%) and boys (2.72%) (P=0.93). However, statistical analysis showed that seroprevalence of anti-*T. gondii* antibodies increased with age (P=0.002) (Table 01).

From the 240 participants living in urban regions, 12 (5.0%) tested seropositive for anti-*T. gondii* antibodies; whereas from 76 participants living in rural areas, 12 (15.8%) tested seropositive for anti-*T. gondii* antibodies (P=0.002). From 66 children who had contact with cats, 5 (7.6%) tested seropositive for anti-*T. gondii* antibodies; whereas from 250 children who no contact with cats, 19 (7.6%) tested seropositive for anti-*T. gondii* antibodies (P=0.99). From 32 children who consumed raw or unwashed vegetables and fruits, 6 (18.8%) were seropositive for anti-*T. gondii* antibodies; while from 284 children who did not eat raw or unwashed vegetables and fruits, 18 (6.3%) tested seropositive for anti-*T. gondii* antibodies. There was a significant difference in the prevalence of anti-*T. gondii* antibodies among the children who ate raw or unwashed vegetables and those had no eat raw or unwashed vegetables and fruits (P=0.024). The statistical analysis also showed that among children who eat undercooked or cured meat products, seroprevalence of anti-*T. gondii* antibodies was significantly higher (17.9%) than those had no eat undercooked or cured meat products (4.2%) (P < 0.001).

Table 02 shows the associations between risk factors, status of anti-*T. gondii* antibodies in the Logistic regression analysis. The results showed that some risk factors were significantly correlated to *T. gondii* seropositivity included age (P= 0.005), living in rural areas (P=0.01) and consumption of undercooked or cured meat products (P<0.001).

Table 01. Demographic characteristics and seroprevalence of anti-*T. gondii* antibodies among children (aged 2-15 years), referred to health centres of Lorestan province, Iran.

Variables		No. (%)	IgG positive	*P* value
Gender				
	Male	176 (55.7)	14 (7.9)	0.87
	Female	140 (44.3)	10 (7.1)	-
Age groups				
	<7yrs	105 (33.2)	6 (5.7)	-
	≥7yrs	211 (66.8)	18 (27.0)	0.002[*]
Residential place				
	Urban	240 (75.9)	12 (5.0)	-
	Rural	76 (20.1)	12 (15.8)	0.002[*]
Being in contact with cats				
	No	250 (79.1)	5 (7.6)	0.99
	Yes	66 (20.8)	5 (7.6)	-
Unwashed vegetables/fruit consumption				
	No	284 (89.9)	18 (6.3)	-
	Yes	32 (10.1)	6 (18.8)	0.024[*]
Undercooked or cured meat products				
	No	238 (75.3)	10 (4.2)	-
	Yes	78 (24.7)	14 (17.9)	0.000[*]

* $P<0.05$, difference is statistically significant

Table 02. Logistic regression analysis of the potential factors associated with *T. gondii* antibodies among children (aged 2-15 years), referred to health centres of Lorestan province, Iran.

Variables		OR (95% CI)	*P* value
Age groups			
	<7yrs	1	-
	≥7yrs	5.8 (1.7-20.1)	0.005[*]
Residential place			
	Urban	1	-
	Rural	3.3 (1.2-8.9)	0.014[*]
Undercooked or cured meat products			
	No	1	-
	Yes	6.8 (2.5-18.0)	0.000[*]

* $P < 0.05$ was statistically significant.

7. Conclusion

Toxoplasmosis has been explained as the most prevalent zoonotic infection of humans as well as animals (Sukthana, 2006).The disease in immunocompetent adults and children is generally without any symptoms or cover gentle and spontaneously treated signs for instance, fever, discomfort, and lymphadenopathy. But toxoplasmosis in immunocompromised individuals and the fetus can cause serious and fatal symptoms (Signorini et al., 2007; Torgerson & Mastroiacovo, 2013). Nowadays, the diagnosis of human toxoplasmosis is usually based on the detection of the anti *T.- gondii* antibodies by serological methods including ELISA, latex agglutination (LA), and indirect fluorescent antibody (IFAT) (Robert-Gangneux & Darde, 2012). In serological tests, anti-*T. gondii* IgM antibodies are typically recognized as an indicator of acute toxoplasmosis that show earlier and reduce sooner than IgG antibodies. However, *T.*

gondii specific IgM may occasionally continue for more than one year post-infection, which indicated the false positive diagnoses of acute toxoplasmosis whereas no supplementary examinations for anti-*T. gondii*-IgG antibodies were performed (Lim & Othman, 2014; Balfour et al., 1982). As a result, the diagnosis of newly acquired toxoplasmosis is commonly based on the recognition of specific IgM antibodies, followed by detecting the anti-*T. gondii*-IgG 1 to 3 weeks later (Lim & Othman, 2014; Balfour et al., 1982).

Children due to their high hand-mouth activity, lack of hygiene standards in the consumption of food, water, fruits and vegetables and their immature immune systems are the most susceptible peoples to parasitic infections among all age groups (Weatherhead & Hotez, 2015). So far, in numerous investigations the seroprevalence of *T. gondii* infection in children has been demonstrated in several countries around the world (Fu et al., 2014; Xin et al., 2015), however little is known about the seroprevalence of *T. gondii* infection in children from Iran. In the present investigation, totally 316 children (aged 2-15 years), referring to health centres of Lorestan province, Iran were studied to determine the anti-*T. gondii* antibodies. The obtained results showed that 31 (9.8%) children tested seropositive for anti-*T. gondii* antibodies; 24 (7.6%) children tested seropositive for IgG antibody, 1 (0.3%) tested seropositive for both IgM and IgG, and 6 (1.9%) were positive for IgM antibody alone. This seroprevalence of toxoplasmosis was higher than the values reported among children of southern Brazil (7.4%), Swaziland (8%); while it was significantly lower than São Tome and Principe (63.1%), Madagascar (36.3%), and China (15.13%) (Fan, 2012; Xin et al., 2015; Lopes, 2008; Dromigny, 1996). This difference is most probably because of variations in sample size, experiments method, nutritional habit and climatic conditions.

In line with studies conucted by Ali et al (2007) and Gyang et al (2015), we found that the seroprevalence of anti-*T. gondii* antibodies among children referring to health centres of Lorestan province, Iran increased with age; which is most likely due to increased opportunity for exposure. Furthermore, in this survey, consistent with the some previous studies, we reported that some risk factors such as consumption of raw or unwashed vegetables and fruits and consumption of undercooked or cured meat products are related to *T. gondii* seropositivity; indicating that among children in the present study, both infection ways, the ingestion of oocysts and consumption of tissue cyst in meat were present (Montoya & Liesenfeld, 2004; Alvarado-Esquivel, Estrada-Martínez, Liesenfeld, 2011).

The findings of our study demonstrated a considerable seroprevalence of *T. gondii* infection in children in Lorestan province, Iran. Thus, proper strategies must be carried out to prevent and control *T. gondii* infection in children in this region.

Acknowledgments

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