Low prevalence of *Toxoplasma gondii* infection among children in a rural community in Fars province, Southern Iran

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SUMMARY

The current study aimed to determine the seroprevalence of *Toxoplasma gondii* infection and associated risk factors in children in a rural community in Fars province in southern Iran. Blood samples were collected from 671 children living in three rural areas, and sera and buffy coats were isolated from each sample. Anti-*T. gondii* antibodies were detected by ELISA, using a commercial kit. Also, buffy coats of seropositive children were examined by a PCR method, targeting a 529 bp gene of *T. gondii*. Of 671 children participating in the study, 319 (51.7%) were boys and 298 (48.3%) were girls. The mean age of the children was 9.7 (±10.7) years. Anti-*Toxoplasma* antibodies were detected in sera of 23 out of 671 children, corresponding to a seroprevalence rate of 3.8%. Gender and level of education had no significant influence on the risk of *Toxoplasma* infection (p>0.05). *Toxoplasma* DNA was not detected in buffy coats of any of the seropositive cases. Geographic location, as well as the low age of the participants, may contribute to the low rate of *Toxoplasma* infection in children of rural areas in southern Iran.

Keywords: *Toxoplasma gondii*, children, seroprevalence, Fars province, Iran.

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan with a worldwide distribution which can be transmitted through water or food contaminated with the parasite oocyst, ingestion of undercooked meat containing the parasite tissue cysts, or vertical transmission during pregnancy from mother to the fetus [1, 2].

Toxoplasmosis is usually asymptomatic in most of the immunocompetent individuals; however, it may result in toxoplasmic encephalitis, chorioretinitis, myocarditis and finally death due to the reactivation of latent infection in immunocompromised patients [3-5].

The seroprevalence rate of *T. gondii* varies considerably among the different communities of the world, depending on the geographical location, climate, exposure to risk-containing materials mainly contaminated soil, eating behaviors, and level of sanitation. *Toxoplasma* is a common infection in Iran where its seroprevalence rate among the general population was reported to be 39.3% [6]. The rate of infection in the defined population such as blood donors, pregnant women, and immunocompromised individuals are also considerable [7-12]. There is growing evidence that the rate of *Toxoplasma* infection is age-dependent and a significant increase in the rate of infection has been noted in older people in comparison with children or young people [3, 6, 13].

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Seroprevalence and risk factors of *Toxoplasma* infection in adults, especially pregnant women, immunocompromised patients and blood donor in different areas of the world, including Iran have been evaluated [6-8, 12, 14-16]. Whilst there is considerable data available about the seroprevalence of *Toxoplasma* in adults, such data are not available for children especially those who are living in rural communities. Lack of information about the rate of *Toxoplasma* infection in children living in rural areas of Iran justifies the current study which aimed to evaluate the prevalence of *T. gondii* infection and associated risk factors in children resident in three rural areas in Fars province, southern Iran.

MATERIALS AND METHODS

The study area

This cross-sectional study was carried out in May 2017 in three rural communities, Sar Mashhad, ToleSaman and HosseinAbad located in Kazeroon Township in Fars province, southern Iran (Figure 1). The study region has a tropical climate with hot summers and moderate winter. The resident population in these three communities, based on the National Population and Housing Census in Iran (2016) has been 5,000 inhabitants in 1000 families. Animal husbandry and gardening are common in this district. The area is considered as a focus of visceral leishmaniasis during the last decades [17-19]. The food habits of the local population related to possible risk factors for *Toxoplasma* infection are using undercooked meat, and also using not properly washed vegetables. Stray cats are abundantly seen in the area but keeping cats in the home by the local families is uncommon.

Sampling

Blood samples (about 5 mL) were collected from 617 children in three villages in the area. Ethical approval of the study was obtained from the Ethical Review Committee of Shiraz University of Medical Sciences (ethical code: IR.SUMS.Med.REC.1396. S322). Informed consent was obtained from the children parents, before sampling. A pre-designed questionnaire was used to collect the data about participant's age, sex, residence area, level of education and risk factors related to *Toxoplasma* infection including having contact with animals and using raw vegetables. Fresh venous blood was collected from each child in aplastic K_2EDTA tubes (Pishtaz Teb Zaman Diagnostics, Iran). Buffy coat and sera were separated

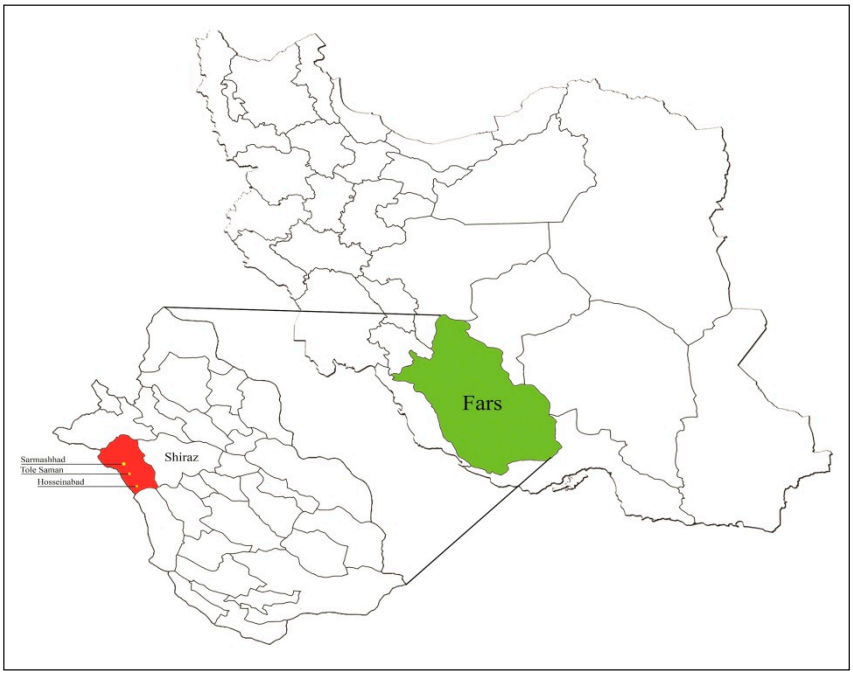

Figure 1 - Map of Iran showing the study area.

after clotting, by centrifugation for 10 minutes at 800 g. The samples were transferred, keeping the cold chain, to the immunoparasitology laboratory at Shiraz University of Medical Sciences (SUMS). Collected samples were stored at - 20° C until use.

Serological evaluation

Sera samples were examined for anti- *T. gondii* IgG antibodies, using an ELISA commercial kits (Acon Biotech, Hangzhou, China), according to the manufacturer's instructions. The wavelength absorbance of the samples was measured at 450 and 630 nm, using a plate reader (BioTek; Winooski, Vermont, USA). Index value >1.1 was determined as positive. A range between 0.9 and 1.1 was considered as equivocal and index value less than 0.9 was considered as negative.

DNA extraction and molecular method

Genomic DNA was extracted from the buffy coat of the seropositive cases, using a manual phenol/chloroform/isoamyl alcohol method [20]. Conventional PCR was performed on the extracted DNA, targeting a 529 base pair fragment of *Toxoplasma* as previously described [10].

Statistical analyses

Statistical analysis was performed using SPSS (Ver. 18). The association of various demographic characteristics and risk factors with *T. gondii* infection was examined using chi-square test. Seropositivity to *T. gondii* was considered as the response variable.

RESULTS

In total, 617 children were enrolled in the present study, including 475 (77.0%) cases from Sar Mashhad, 93 (15.1%) from ToleSaman and 49 (7.9%) cases from HosseinAbad. Girls constituted 298 (48.3%) and boys were 319 (51.7%) of the subjects. Mean age of the participants was 9.7 (±10.7) years. Most of the subjects (30.6%) were aged 5-9 years. The majority of the participants (55.4%) were in their elementary education level. Anti-*T. gondii* antibodies (IgG) were detected in the sera of 23 out of 617 (3.8%) recruited subjects. Seroprevalence of *T. gondii* antibodies among the resident children in Sar Mashhad ToleSaman and HosseinAbad were 2.5%, 6.5%, and 10.2%, respectively. The prevalence of antibodies varied significantly with the place of residence ($p<0.05$). Although

Table 1 - Demographic characteristics and relative seropositivity to *Toxoplasma* in children in rural areas in Fars Province, Southern Iran.

Characteristics	*Frequency (No.)*	*Percent (%)*	*Positive for anti-T. gondii IgG antibodies*		*P value*
			Frequency (No.)	*Percent (%)*	
Gender					
Male	319	51.7	15	4.7%	0.186
Female	298	48.3	8	2.7%	
Age group					
<5	229	37.4	7	3.1%	0.758
5-9	178	29.1	7	3.9%	
>9	205	33.5	9	4.4%	
Residence area					
Sar Mashhad	475	77.0	12	2.5%	0.008
ToleSaman	93	15.1	6	6.5%	
HosseinAbad	49	7.9	5	10.2%	
Educational levels					
Non-educated	260	42.1	8	3.8	0.128
Elementary level	342	55.4	13	3.8	
Secondary level	14	2.3	0	0	

the *Toxoplasma* seroprevalence in boys was higher than that of girls, this difference was not statistically significant (p>0.05).

Toxoplasma seroprevalence was highest in the age group >9 years (4.4%) followed by the age group 5-9 years (3.9%), and 1-5 years (3.1%). There were no associations between seropositivity to *T. gondii* and age groups (p>0.05). Furthermore, no significant association was found between *T. gondii* seropositivity and the education level (p>0.05). As almost all of the children had a positive answer to the questions concerning the contact with animals and using raw vegetables, no association could be found between these variables and seropositivity to toxoplasmosis.

DNA of *T. gondii* was not detected in the buffy coat of any of the seropositive samples. Table 1 shows the demographic characteristics and relative seropositivity to *Toxoplasma* in children of rural areas in this study.

DISCUSSION

The current report is one of the few studies carried out regarding the seroprevalence and molecular evaluation of *T. gondii* infection in children of rural areas in a tropical region in Fars province, south of Iran with a relatively large sample size.

The seroprevalence rate of 3.8% for anti-*T. gondii* antibodies in the current study demonstrated a low rate of infection in comparison with a study conducted on schoolchildren in Sari (22%) in the north of Iran [21]. Differences in climate condition in different regions of Iran could affect the rate of seroprevalence of toxoplasmosis. North region of Iran, the littoral plain of the Caspian Sea and forest areas with a high humidity, provide an ambient environment for the viability and survival of *Toxoplasma* oocyte excreted in cats' feces. On the other hand, in the south of the country, where the current study has been undertaken, hot and dry climate make an unfavorable condition for maintaining the *T. gondii* oocysts and decreases the chance of *T. gondii* infection both for animals as well as human [22-25]. Moreover, in general, human toxoplasmosis in children is lower compared with the adult people. Seroprevalence of *T. gondii* in adults' populations in different areas of Iran is higher than the rate of infection in children in the current study [11, 12, 14, 15, 26, 27]. The differences might be due to the influence of the age on infection rate, as adult populations have more chances of acquiring the infection through their lifetime. In the current study, a higher seroprevalence rate of *Toxoplasma* infection was seen in children aged >9. This again supports the concept that *Toxoplasma* seropositivity increases with age. The rate of seropositivity to *T. gondii* in the current study was also low in comparison with rates reported in children from other countries such as Nigerian (23.8%), Ghana (58%), Brazil (7.4%), China (14.5%), and West Africa (63.1%) [26-32]. These disparities in seropositivity of *T. gondii* could be attributed to several factors such as presence or absence of cats, pet-keeping and management issues, exposure to soil, or food-related factors.

Among the demographic variables, place of residence was identified as a risk factor related to *T. gondii* seropositivity in the current study. The relatively small differences in humid, temperature and altitude among the study sites might contribute to the differences in the prevalence rate, although differences in the sample size may fade the significance of such variations in the seropositivity.

Several studies have indicated that lower levels of education might increase the chance of *T. gondii* infection. However, in our study, no significant association was found between *T. gondii* seropositivity and level of education. This can be justified by the fact that most of the recruited children were uneducated since they were below the school age. Hence, no conclusion can be drawn from this association.

The overall seroprevalence of *T. gondii* infection in boys was higher than the girls but the difference was not statistically significant. These findings were consistent with other studies about seroprevalence of *T. gondii* infection among children [33]. It is mainly because boys and girls in rural communities are equally exposed to environmental risk factors for acquiring *T. gondii* infection.

Our study indicated a low prevalence rate of *T. gondii* infection in children in comparison with seroprevalence rates reported in children from other areas of Iran and some of the other countries. This seemingly low seroprevalence rate in the current study in children may be possibly connected to the age of the participants, although the hot and dry climate of the area may contribute to the low prevalence of *T. gondii* infection.

CONCLUSION

Findings of the current study revealed a low rate of *Toxoplasma* infection among children in a rural area in Fars province in the south of Iran. Low age of the participants and also the geographic location are likely to contribute to the low rate of *Toxoplasma* infection in children of this area. Further studies are needed to find out the genotype of *Toxoplasma* circulating in human and also in animals in the area.

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Conflict of interests
None.

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