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A seroepidemiological survey for toxoplasmosis among schoolchildren of Sari, Northern Iran

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Abstract. A seroepidemiological survey for toxoplasmosis among schoolchildren of public schools of the urban areas of the Sari, Mazandaran province, Iran, was carried out from September 2006 to March 2007. A total of 1209 serum samples (686 males, 523 females) were examined for IgG antibodies by ELISA. Questionnaires were completed for all participants. In regard to criteria >10 IU/mL as positive, the infection coefficient (IC) was 266 (22%), of which 161 were males (IC=23.5) and 105 females (IC=20.1) (P=0.15). No age-antibody association was detected, although an increase in positivity with increasing age was observed, reaching 23.1% in the oldest group (P=0.84). A significant association was observed with the presence of cat, contact with soil, washing hands before meals and eating raw or undercooked meat. An improvement in personal hygienic conditions and behavioral characteristics such as eating cooked meat is important in reducing the rate of *Toxoplasma* infection.

INTRODUCTION

Toxoplasma gondii is a protozoan parasite widely distributed around the world (Hill *et al.*, 2005; Liesenfeld & Janitschke, 2005). *T. gondii* is horizontally transmitted to humans by accidental ingestion of oocysts in water, food or soil contaminated with cat's faeces, or by eating raw or undercooked meat containing cysts (Fayer *et al.*, 2004). Most infections in immunocompetent humans are asymptomatic and in up to 10% of infected individuals cervical lymphadenopathy or ocular disease occur (Montoya & Liesenfeld, 2004). *Toxoplasma gondii* can also cause severe encephalitis via acute infection or reactivation of latent infection among immune-suppressed persons, including those with acquired immunodeficiency syndrome, those with immunosuppressive cancer, and transplant recipients on immunosuppressive drugs. Toxoplasmosis is the most frequent severe neurologic infection among persons with acquired immunodeficiency syndrome. Newly acquired *T. gondii* infection in a pregnant woman can be transmitted to the fetus and may cause mental retardation, blindness, epilepsy, and death (Montoya & Liesenfeld, 2004; Fan *et al.*, 2006). While the seroprevalence of toxoplasmosis in adults, especially pregnant women, has been recorded in many studies, we have found no community based study of toxoplasma seroprevalence in schoolchildren in northern Iran. In this study we determined the seroprevalence of *T. gondii* infection and its association with epidemiological data in 1209 schoolchildren in this geographical area.

MATERIALS AND METHODS

Study design

The sample population was determined by mathematical model, in order to ensure the

representativity of schoolchildren at the public schools. In all, 1209 children participated in this study, with age ranging from seven to fourteen years and average of 10.62±2.34 selected at random, from September 2006 to March 2007.

Firstly a semi-structured questionnaire was completed by the parents or legal guardians, to identify and obtain socio-demographic data (sex, age, occupation and educational and socio-economic level of parents) environmental and behavioral data (presence of cats inside or outside the house, exposure to soil, hand washing after soil contact, hand washing before meals, raw or undercooked meat consumption). The population of children studied was stratified to socioeconomic parameters of their families. Three categories were created, based on the monthly household income. Persons that received less than US$ 200 per month in income as Lower socioeconomic population, Middle in which monthly household income ranged from US$ 200-800. Upper socioeconomic population, their households received more than US$ 800 per month. Education of parents was also grouped into three levels (none or elementary school, high school, college studies) for entry into all logistic regression models.

Collection and examination of blood samples

Blood samples were collected from each child, the serum was separated and maintained at negative 20ºC, until use. The presence of antibodies to *T. gondii* was determined by conventional ELISA technique, according to the manufacturer,s instructions (EUROIMMUN, D-23560 Lubeck. Seekamp 31. Germany). The optical density of IgG antibody titers were read at 490 nm using automatic ELISA reader (SPECTRA, Molecular Devices, USA). Sera with 10 IU/mL or above were considered positive for *T. gondii* immunoglobulin G antibodies.

Statistical analysis

SPSS software 16 was used for statistical analysis. A descriptive univariate analysis was performed to evaluate the frequency of the variable (risk factors) and *T. gondii* antibodies, estimated using an Odds ratio. The relative proportions were calculated with a confidence interval of 95%. Possible associations were identified using the Chi-Square and Fisher's exact statistical tests at a significant level of 5%.

Ethical approval

The research project was approved by the Research Ethics Committee of the Mazandaran University of Medical Sciences, Iran. The children's parents or legal guardians signed a free and informed term of consent prior to participation in the study.

RESULTS

Out of 1209 children that were enrolled in the study, 686 (56.7%) were males and 523 (43.3%) females. When the children were grouped according to age, the distribution was: 7-10 years old, 589 (48.7%) and 11-14 years old, 620 (51.3%). The mean ages were similar for both gender (10.62±2.34 years) and ranged between 7 years and 14 years for all schoolchildren. It was found that 482 (39.9%) of the children were in contact with soil, 967 (80%) washed their hands before meals and 510 (42.2%) used undercooked meat. Six hundred and sixty four (54.9%) of children from those interviewed, reported the presence of cats in home or neighborhood and 143 (11.8%) presence of dog. The overall seroprevalence of *T. gondii*, reached 22% (266/1209). No significant gender difference in seroprevalence was found between boys (23.5%; 161/686) and girls (20.1%; 105/523) (χ^2=1.27, df=1, *P*=0.25) (Table 1).

The older age group of ≥11 years had higher seroprevalence (23.1%; 143/620); then 7-10 years (20.9%; 123/589). However, no differences were observed with regard to age (χ^2=0.4, df=2, *P*=0.81) (Table 1). Multivariate analysis showed that *T. gondii* infection in schoolchildren was positively associated with contact with soil (adjusted OR=1.75; 95% CI: 1.33-2.30,

Table 1. Seroprevalence of *Toxoplasma gondii* infection among schoolchildren in Sari, Iran, 2007

Variable	Group	No. tested	No. positive (%)	χ^2	*p*-value
Gender	Boys	686	161 (23.5)	1.27	0.25
	Girls	523	105 (20.1)		
Age group (years)	7-10	589	123 (20.9)	0.40	0.81
	11-14	620	143 (23.1)		
Total		1209	266 (22)		

P=0.000), presence of cat (adjusted OR=0.68; 95% CI: 0.48-0.94, *P*=0.02), eating undercooked meat (adjusted OR=0.73; 95% CI: 0.55-0.97, *P*=0.03) and negatively associated with presence of dogs at home or in neighborhood (adjusted OR=0.94; 95% CI: 0.62-1.43, *P*=0.79), family occupation (adjusted OR=0.85; 95% CI: 0.64-1.13, *P*=0.27) (Table 2).

DISCUSSION

In the present study 22% of schoolchildren showed anti *T. gondii* antibody by ELISA. The same seroprevalence rates of *T. gondii* in children were reported in Tehran, Iran (17.7%) (Soleimani *et al.*, 2003), and in our neighborhood country, United Arab Emirates (Abu-Zeid, 2002). However in some countries, children showed low prevalence rate, including those in Islamabad, Pakistan, 17.4% (Sadaruddin *et al.*, 1991), Dublin, Ireland, 12.8% (Taylor *et al.*, 1997), and Seoul, Korea, 12.6% (Kook *et al.*, 1999). Seroprevalence of *T. gondii* infection varies from 0% in Eskimos to 94% in Costa Rica and Guatemalan people (Gibson & Coleman, 1958; Feldman, 1982). This variation is presumably due to the presence or absence of cats, climatic factors and consumption of raw or improperly cooked meat. In Iran, the seroprevalence rate is about 51.8% (Assmar *et al.*, 1997); the highest infection rates have been reported from North provinces including Mazandaran and Guilan with a temperate and humid conditions, but in South of Iran where the climate is dry and warm, infection rate is low (Hatam *et al.*, 2005). It appears that the three factors mentioned above are there in this area. In our study since the children were not exposed to soil, water, or food at an early age, they were not very likely to seroconvert before adolescence. Therefore prevalence rate of anti-*Toxoplasma* antibodies was lower than other people in this area.

Although there was not a significant difference between age groups and prevalence rate of anti-*T. gondii* antibodies (20.9-23.1%), the positive rates increased with age. Seroprevalence of toxoplasmosis is known to increase with age (Dubey & Beattle, 1988). It seems that the increase is a reflection of increasing exposure years as the humans get older. Several minor infections might at first produce low antibody levels and later higher levels.

It has been reported that the seroprevalence of toxoplasmosis is not significantly different according to gender (Abu-Zeid, 2002; Montaya & Liesenfeld, 2004), which the present study also confirms. Because about 80% of schoolchildren in this study were sero negative against *T. gondii*, therefore these people are susceptible to acute *Toxoplasma* infection during the childbearing years, and their infants are susceptible to congenital toxoplasmosis. The infected babies suffer from symptoms such as chorioretinitis, convulsion, jaundice, hydrocephalus, fever, pneumonitis, hepatosplenomegaly, lymphadenopathy, microcephalus, cataract, hypothermia, and rash (Sever *et al.*, 1988). Most toxoplasmic children develop clinical

Table 2. Potential risk factors for *Toxoplasma gondii* IgG antibody seropositivity in schoolchildren in urban areas of Sari, Iran, 2007

Characteristic	No. persons	No. positive (%)	Odds Ratio	95% CI	*P*
Educational level					
None or Elementary school	196	53 (27)	1.08	0.69–1.69	0.72
High school	808	206 (25.5)	0.72	0.50–1.04	0.08
College studies	205	41 (20)	Ref		
Occupation					
Non Laborer	487	99 (20.3)	0.85	0.64–1.13	0.27
Laborer	722	167 (23.1)	Ref		
Socioeconomic					
Lower	121	30 (24.8)	Ref		
Middle	922	240 (26)	0.82	0.53–1.27	0.38
Upper	117	25 (21.3)	1.00	0.55–1.80	0.99
Cats at home or in the neighborhood					
Yes	664	164 (24.7)	0.68	0.48–0.94	0.02
No	539	102 (18.9)			
Dogs at home or in the neighborhood					
Yes	143	32 (22.3)	0.94	0.62–1.43	0.79
No	1066	234 (22)			
Raw or undercooked meat					
Yes	510	97 (19)	0.73	0.55–0.97	0.03
No	698	164 (24.2)			
Hand washing before meals					
Yes	967	189 (19.5)	0.68	0.48–0.94	0.01
No	242	77 (31.8)			
Contact with soil					
Yes	482	136 (28.2)	1.75	1.33–2.30	0.000
No	727	130 (17.9)			

manifestations several years after birth (Desmontz & Thulliez, 1985). For this reason, it is important that women of childbearing age, especially pregnant women, be educated about not eating raw or undercooked meat and soil related hygiene.

This study showed that *T. gondii* infection in school children was positively associated with contact with soil. The higher proportions of infections in these children may be related to their play activity, which covers larger extensive areas and so increases the opportunities ~~of~~ for contamination with soil. Oocyst contamination of the environment was held responsible for recent outbreaks of toxoplasmosis in the Americas and future epidemiological studies of *T. gondii* infections should consider the role of oocysts as potential source of infection for humans (Tenter *et al.*, 2000).

In this study 54.9% of people showed contact with cats. The authors in the previous study revealed that 40% of cats in northern Iran are infected by *T. gondii* (Sharif *et al.*, 2009). Infected cats play an important role in contaminating soil. Risk of *T. gondii* infection in humans derives from exposure to the faeces of a cat that is shedding oocysts. Cats shed oocysts for only a few weeks during their lives. Domestic cats that are not fed raw meat are not likely to acquire *T. gondii* infection and

pose little risk to humans. Otherwise feral cats that defecate in gardens may pose the greatest risk of *T. gondii* infection for some people.

Although in most previous studies cat ownership has been associated with either increased risk for toxoplasmosis or no change in risk, a study found that possession of cats decreased the risk for seropositivity. The lack of association with cat ownership suggest that either most infection is acquired through the food chain or other routes of ingestion or that environmental contamination by cats is so widespread that owning a cat has little effect on the level of exposure to *Toxoplasma* infection (Taylor *et al.*, 1997).

In the study of Frenkel *et al.* (1995) some of the highest relative risks of *T. gondii* transmission to children were predicted by contact with dogs, suggesting the possibility that dogs, by eating and rolling in cat faeces, were instrumental in mechanically transmitting *T. gondii* infection. We did not find significant association between dog ownership and *T. gondii* infection.

Although Assmar *et al.* (1997) reported that the main infection route of toxoplasmosis in Iran is through soil and water, concerning the nutritional habits of the studied population, intake of poorly cooked meat (42.2%) were very common and meat consumption was a significant factor for *T. gondii* seropositivity in our analysis. Of course, in this area two main modes of *Toxoplasma* transmission, by infective cat faeces or tissue cysts from undercooked meat, play a predominant role in the spread of human infection in this population. Huldt *et al.* (1979) found no association between antibody titres and the presence of cats in the family or the consumption of raw meat by family members.

In regard to the high prevalence of infection in these age groups and significant variables, it seems that performance of control and preventative measures of *T. gondii* including prevention of soil contamination by cat faeces in areas where children play and health education such as hand washing before meals and consumption of cooked meat in north part of Iran is necessary.

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