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Seroprevalence of Toxocariasis in Schoolchildren in Northern Iran

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Abstract: This cross-sectional study was carried out on 1210 randomly selected schoolchildren, attending sixteen primary and secondary schools, during the period between November 2005 and June 2006. Parents who accepted to include their children were requested to be present at sampling time and to fill in a simple questionnaire with personal and epidemiological data. Three milliliter of venous blood were taken by vein puncture under sterile conditions from each subject for detection and titration of antibodies to *T. canis* and eosinophil counts. Total IgG anti-*Toxocara* antibodies was evaluated by *T. canis* IgG ELISA kit. According to the manufacture recommendations, an index positivity >11 U was considered positive. Of the 1210 serum specimens tested, an overall seroprevalence for *Toxocara* antibodies of 25% was obtained. There was no association between positive seroprevalence and age ($p = 0.34$). Boys and girls differed significantly with regard to *Toxocra* titre ($p = 0.003$). Eosinophilia in peripheral blood (≥5%) was detected in 24.5% (297/1210) of the population studied, 97/297 (32.8%) of whom were seropositive for toxocariasis. The findings of this study confirmed that infection with *Toxocara* is quite high and widespread in children in Northern Iran. Therefore, health promotion efforts must be directed at increasing the awareness of the population about the potential zoonotic hazards associated with the disease and how to minimize them.

Key words: Seroprevalence, toxocariasis, schoolchildren, Iran

INTRODUCTION

Toxocariasis is an important zoonosis caused by the infection of larvae with *Toxocara canis* and *T. cati* (Glickman and Schantz, 1981). The eggs of *Toxocara* sp. passed by feces of dogs and cats into the environment under optimal temperatures and humidity develop into embryonated eggs (Barriga, 1988; Glickman, 1993). In humans, when *Toxocara* eggs containing infective larvae are accidentally ingested, the larvae hatch in the small intestine and migrate through somatic organs, especially the liver and eyes (Glickman *et al*., 1987; Taylor *et al*., 1988). The major clinical consequences of prolonged migration of *Toxocara* sp. larvae in humans are visceral larva migrans and ocular toxocariasis (Glickman, 1993). Young children playing in areas contaminated with dog and cat feces are the main population at risk for *Toxocara* infection due to geography, poor hygiene, or frequent contact with dogs and cats (Glickman, 1993; Roldán *et al*., 2006). Since, the larvae migrate in tissue and do not develop further in human, a definitive diagnosis by stool examination is impossible. The laboratory diagnosis of human *Toxocara* infection relies mainly on the detection of antibodies against *Toxocara* larval Excretory-Secretory (TES) antigen-based Enzyme-Linked Immunosorbent Assay (ELISA) (Glickman *et al*., 1978).

Human infection by *Toxocara* sp. is distributed worldwide and appears in variable frequencies, depending on local factors such as close contact with soil contaminated by dog and cat feces and low income levels of the exposed community (Aguiar-Santos *et al*., 2004; Moreira-Silva *et al*., 1998). Seropositivity has been reported in the world ranging from 1% in children in Madrid (Guerra *et al*., 1995) to 86% in rural children in the Caribbean (Thompson *et al*., 1986).

In Iran there have been few epidemiological studies on the seroprevalences of toxocariasis. Fallah *et al*. (2007), found 5.3% in children aged 0-9 years in Hamadan and Sadjjadi *et al*. (2000) reported 25.6% in schoolchildren in Shiraz.

Since, data on the epidemiological and clinical aspects of toxocariasis in the Mazandaran province are not available, we determined the seroprevalence of *T. canis* infection and its association with epidemiological

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data in a population of schoolchildren to identify probable risk factors for *Toxocara* infection in this population.

MATERIALS AND METHODS

Study area: The area of study, Sari City lies in the centre of the Mazandaran Province in Northern Iran with a human population around 196,000 and lies between the parallels 35°58' and 36°50' of the Northern latitude and between 52°56' and 53°59' of the Eastern longitude. The mean yearly relative humidity is 85.83% with rainfall occurrence in all seasons of the year and an average temperature of 17°C.

Study population and human serum samples: This cross-sectional study was carried out on 1210 randomly selected schoolchildren, male and female (age range = 7-14 years), attending sixteen primary and secondary schools, during the period between November 2005 and June 2006. The study protocol was considered and approved by the Research and Ethics Committee of Mazandaran University of Medical Sciences. After official permissions were taken from school administration, information and consent forms were prepared and given to parents of each subject. Thus, parents were informed and their consent was taken. Those parents who accepted to include their children were requested to be present at sampling time and to fill in a simple questionnaire with personal and epidemiological data (e.g., sex, age, father's job, socio-economic levels, washing hands before eating food, contact with sand, presence or absence of dogs within the home and close contact with dogs). Then, 3 mL of venous blood were taken by vein puncture under sterile conditions from each subject for detection and titration of antibodies to *T. canis* and eosinophil counts.

In the laboratory, the blood films were treated with Giemsa stain and examined by microscopy for the numbers of eosinophils per 100 White Blood Cells (WBC). Eosinophil count was grouped to: normal eosinophils (0-3%), mild (4-10%), moderate (11-15%) and severe (16->%) eosinophilia. The rest of the blood was centrifuged and the serum removed and stored at -20°C until used.

Total IgG anti-*Toxocara* antibodies was evaluated by *T. canis* IgG ELISA kit; (Prod No: TOCG0450IH; Hamburg- Germany), with a reported high specificity of >98% and sensitivity of >98%. The ELISA procedure was carried out according to the manufacture's instructions. Results were considered valid if the absorbance of the positive control was ≥0.965 and of the negative control was ≤0.200 and the mean absorbance of cut-off was between 0.25-0.900. According to the manufacture recommendations, an index positivity >11 U was considered positive, 9 to 11 U as borderline and <9 U as negative for *T. canis* infection.

Statistical analysis: The SPSS (Statistical Programme Social Science) Software version 15 for Windows package programme was used to evaluate data. The relationships between seroprevalence and host factors such as age, sex, socio-economic status and risk factors such as contact with dogs, were explored using Pearson Chi square analysis. The test level of significance was set at 5% and p-values are stated in the text. The relative proportions were calculated with a confidence interval of 95%.

RESULTS AND DISCUSSION

A total of 1210 blood specimens were collected from 16 primary and secondary schools. Of these, 687 (56.8%) were collected from boys while, 523 (43.2%) were from girls. They were between 7-14 years of age (10±2.3).

Of the 1210 serum specimens tested, an overall seroprevalence for *Toxocara* antibodies of 25% was obtained. There was no association between positive seroprevalence and age (p = 0.34); however, boys (28.2%) and girls (20.7%) differed significantly with regard to *Toxocra* titre (p = 0.003) with boys exhibiting higher seroprevalence than girls (Table 1).

The percentage of children who had dog in and around their homes was 44.1%. There was a highly significant association between *Toxocara* seropositivity and contact with dog (p<0.001).

However, there was a significant association between all risk factor and seropositivity (Table 2).

Eosinophilia in peripheral blood (≥5%) was detected in 24.5% (297/1210) of the population studied, 97/297 (32.8%) of whom were seropositive for toxocariasis and 200/913 (21.9%) were seronegative (p = 0.004).

Seroepidemiological studies are an important research tool, especially in public health. Serologic tests are of considerable importance in the detection of human infection with *T. canis* because, the clinical symptoms of toxocariasis are of limited value in differential diagnosis of toxocariasis (Glickman and Schantz, 1981).

Table 1: Epidemiologic analysis for *Toxocara canis* seroprevalence in the schoolchildren of Sari, Iran

Variable	No. of analysed samples	No. of positives	Odds ratio (OR)	95% CI	p-value
Age group (years)					
7-10	588	141 (24)	0.93	0.72-1.21	0.34
11-14	620	156 (25.2)			
Sex					
Male	687	194 (28.2)	0.66	0.50-0.86	0.003
Female	523	108 (20.7)			

Values in brackets indicate percentage

Table 2: Relationship between *Toxocara canis* seroprevalence and assumed risk factors in the schoolchildren of Sari, Iran

Risk factors	Total No. analysed	No. of positives	Odds ratio (OR)	95% CI	p-value
Father's job					
Employee	487	113 (23.2)	1.17	0.89-1.53	0.24
Free jobs	722	189 (26.2)			
Economic status					
Good	117	24 (20.5)	1.02	0.90-1.15	0.72
Medium	922	241 (24.8)			
Weak	121	37 (30.6)			
Contact with sand					
Yes	482	226 (46.9)	0.13	0.09-0.17	0.000
No	728	76 (10.4)			
Contact with dog					
Yes	143	63 (44.1)	0.36	0.25-0.52	0.000
No	1067	239 (22.4)			
Washing hands before eating food					
Yes	968	219 (22.6)	1.78	1.31-2.42	0.000
No	242	83 (34.3)			

Values in brackets indicate percentage

In this current study in Iran, the overall seroprevalence level for infection with *T. canis* was 25%. Our results are similar to that reported by Sadjjadi *et al.* (2000) in Shiraz, Southern Iran (25.6%). Although, Khazan *et al.* (2005) reported frequency of 38.5 in patients with eye disorders, Fallah *et al.* (2007) found lower seropositivity for anti-*Toxocara* antibodies (5.3%) in children aged 1-9 years in Western Iran.

As regards other countries, our results are similar to those found in Argentina (23%- Chiodo *et al.*, 2006), Nigeria (29.8%- Ajayi *et al.*, 2000) and Brazil (21.8%- Campos *et al.*, 2003).

Different studies have reported varying rates of infection ranging from 2.6% in adults of Britain (De Savigny *et al.*, 1979) to 86% in rural children in St Lucia, West Indies (Thompson *et al.*, 1986).

The differences in prevalences reported in different surveys may be due to several factors, such as: seroepidemiological studies based on a small number of children; heterogeny of individuals studied (i.e., blood donors, hospitalized patients or high risk subgroups for toxocariasis); non-standardized laboratory procedures; differences in methodologies, including antigen preparation and assay procedures and different cut-off titers used by the various investigators.

In this study, significant difference was found between the males and females. Such as in several studies, a higher frequency was observed in male individuals, which may be due to differences in the play and social behaviors of boys, resulting in increased exposure to the eggs of *Toxocara* sp. (Glickman and Schantz, 1981; Overgaauw, 1997) also the fact that older girls are restricted from outdoor play since, they are needed indoors to perform household chores. Contrariwise, Magnaval and Baixench (1993) indicated that females are more frequently infected than males. Of course some workers reported no significant difference between two sexes (Sadjjadi *et al.*, 2000; Chiodo *et al.*, 2006; Teixeira *et al.*, 2006).

Observations from this study suggest the lack of a significant statistical difference between age and anti-*Toxocara* antibodies. Similar findings have been reported by other workers in Iran (Sadjjadi *et al.*, 2000; Fallah *et al.*, 2007), Taiwan (Fan *et al.*, 2004), Argentina (Alonso *et al.*, 2000; Chiodo *et al.*, 2006), Nigeria (Ajayi *et al.*, 2000), Venezuela (Garcia-Pedrique *et al.*, 2004) and Brazil (Figueiredo *et al.*, 2005). Of course toxocariasis is seen more frequently among children than among adults due to such reason as frequent contact with contaminated soil, poor hygiene and consuming contaminated food (Radman *et al.*, 2000; Garcia, 2001).

In this study, contact with soil contributed to the risk of infection by toxocariasis and the association was statistically significant (OR = 0.132; CI = 0.098-0.178). In addition, in a study in this area, we found that 60% of stray dogs were infected with *T. canis* (Daryani *et al.*, 2009) and it is reported that the risk factor for toxocariasis is contact with soil contaminated with cat-dog feces, rather than owning a cat or dog at home (Overgaauw, 1997; Ajayi *et al.*, 2000; Garcia, 2001).

Some environmental and behavioral variables were significantly associated with the positive anti-*Toxocara* antibody responses. In the current study, close contact with dogs significantly increased the risk of infection. Some workers reported a higher frequency of infection in individuals who had contact with dogs (Schantz *et al.*, 1980; Matsumura and Endo, 1983; Khazan *et al.*, 2005; Chiodo *et al.*, 2006). Other studies found no association between ownership or professional contact with dogs and *Toxocara* infection (Ajayi *et al.*, 2000; Woodruff *et al.*, 1978).

We found a significant association between washing hands before eating food and frequency of anti-*Toxocara* antibodies. Personal hygiene and behavioral factors e.g., geographia, nail-biting, sucking fingers and frequency of daily hand washes could also lead to increase *Toxocara* seroprevalence (Ajayi *et al.*, 2000; Sadjjadi *et al.*, 2000; Garcia, 2001; Baboolal and Rawlins, 2002). If the soil is contaminated and hands are not properly washed, infection can occur.

A significant association was observed between low family incomes and infection by *Toxocara*. Precarious sanitarian and hygienic conditions and probably other factors associated to low income, facilitate the transmission of *Toxocara* infection as well as of many other diseases (Alderete *et al.*, 2003). While, some studies reported that *Toxocara* seroprevalence increase with low socio-economic status (Matos *et al.*, 1997; Alonso *et al.*,

2000; Coelho *et al*., 2004), some workers claim that it does not change (Buijs *et al*., 1994; Sadjjadi *et al*., 2000; Kaplan *et al*., 2004).

The findings of this study confirmed that infection with *Toxocara* is quite high and widespread in children in Northern Iran. Different studies have been shown that a single risk factor alone is not sufficient to produce infection or disease and a complex of risk factors (e.g., duration of exposure to dogs, thumb sucking is necessary for successful transmission).

As most cases of human toxocariasis are preventable by simple measures such as careful personal hygiene, elimination of the parasite from dogs and cats and not allowing children to play in environments contaminated with dog and cat feces, therefore health promotion efforts must be directed at increasing the awareness of the population about the potential zoonotic hazards associated with the disease and how to minimize them.

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