Seroprevalence of *Toxocara* Infection in School Children in Shiraz, Southern Iran

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Summary

The presence of anti-*Toxocara* antibodies in the sera of school children of Shiraz, southern Iran, was studied by means of an indirect enzyme linked immunosorbent assay with excretory/secretory antigen of infective stage larva. A total of 519 individuals of both sexes aged 6–13 years were analysed. The total prevalence was 25.6 per cent. A higher rate of infection was observed in urban (30.15 per cent) than rural (20.2 per cent) residents. Most potential risk factors were not related to *Toxocara* prevalence and no differences existed between socioeconomic classes except for parental education. Neither age or sex was found to be significantly associated with positive serology.

Introduction

Toxocariasis is an infection caused by the migration of the roundworm *Toxocara* larvae to organs and tissues.[1] *Toxocara cati* (roundworm of the cat) and *T. canis* (roundworm of the fox and dog) have been consistently implicated in the disease.[2] The disease manifests itself as two distinct forms: visceral larval migrans (VLM) and ocular larval migrans (OLM). The signs and symptoms of VLM vary from an asymptomatic state with mild eosinophilia to a severe and potentially fatal disorder, including hepatomegaly, hyperglobulinemia, pulmonary symptoms and fever. Pneumonitis and neurological disorders may also appear in visceral larval migrans.[3] In serious cases, the leukocyte count may reach 100 000/mm^3, while 80–90 per cent are eosinophils. The disease has a chronic state and the symptoms can even persist for more than a year. Patients with OLM also show variable clinical signs varying from asymptomatic states to acute lesions in the eye. The eye lesions include endophthalmitis, due to the penetration of larvae in the eyes, and accompanying loss of vision. In some patients a mass similar to retinoblastoma appears in the retina that leads to unnecessary eye enucleation. Distribution of the disease is worldwide and is more prevalent in children. There is no definitive method in diagnosing *Toxocara* infections. The diagnosis is further complicated by the fact that the antibody response varies widely depending on worm burden and the organ-tissue that the worms infect. However, numerous studies have shown that immunoassays using a purified excretory antigen from the larval stage significantly improve sensitivities and specificities compared to assays using crude antigens.[4,5] The most widely used test, because of its high sensitivity and specificity, is the enzyme-linked immunosorbent assay (ELISA), in which antibodies to *T. canis* or *T. cati* larval excretory secretory antigens,[6] or to larval extracts, are measured.[7]

Toxocara canis and *T. cati* has been reported from dogs and cats in several parts of Iran and there are some case reports of VLM in Iran.[8–11] No population study has been done in Iran, so far. The present study investigated the seroprevalence using ELISA with larval *Toxocara* excretory/secretory antigens in school children showing no clinical signs of toxocariasis, in Shiraz, southern Iran. The aim of this study was to measure the prevalence of toxocariasis in urban and rural populations of Shiraz, where the prevalence of *T. cati* and *T. canis* in cats and dogs is high.[12]

Materials and Methods

A total of 519 school children (274 males and 245 females) aged 6–13 years old were randomly selected from schools in Shiraz (286) and the suburbs (233). A questionnaire was filled for each child. All children were examined by a physician for any clinical signs and symptoms. Blood samples were collected using disposable syringes and sera were separated and stored at

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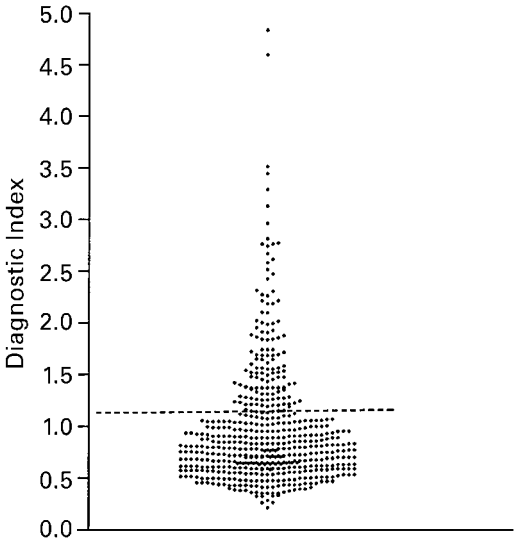

FIG. 1. Seroprevalence of *Toxocara* in the children from Shiraz, Iran according to Diagnostic Index. See the evaluation of the results in the text.

−20°C until used. An ELISA-IgG diagnostic kit was supplied as a *Toxocara* semi-quantitative test based on the principle of an enzyme immunoassay (Cypress Diagnostics, Schotelveldstraat3, B-3012 Leuven, Belgium). The assay system uses an inactivated purified specific excretory antigen. The specific antigen is coated on the microtiter well and an anti-human IgG + IgM antibody is contained in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test samples or controls were allowed to react with the solid phase specific antigen. If there were specific antibodies, these bound to the well surface. After 10 min of incubation at room temperature, the wells were washed with the washing solution to remove the unbound material. In a second step, anti-human IgG + IgM/HRPO conjugate was added to the wells resulting in the specific antibody being sandwiched between the solid phase antigen and enzyme conjugate antibody. After 5 min of incubation at room temperature, the wells were washed with the washing solution to remove the unbound material. A substrate/chromogen solution was added and incubated for 10 min, resulting in the development of a blue color. Color development was stopped by the addition of the stop reaction solution when the color turns yellow. The yellow color was measured spectrophotometrically at 450 nm. The concentration of specific IgG and/or IgM antibodies is directly proportional to the color intensity of the test sample.

Evaluation of results

For evaluation of the results the Diagnostic Index (DI) was used. We first calculated the average optical density (OD) of the negative control, then added 0.2 to the value obtained above. This value is the cut-off value of the assay. Division of the sample OD by the above value gives the DI.[13] According to the manufacturer's recommendations, values greater than 1.1 were considered positive.

Results

A total of 519 school children were studied; 52.8 per cent of the population was male and 47.2 per cent female. The results obtained from 519 sera based on the evaluation of the results indicating Diagnostic Index are illustrated in the Fig. 1. According to this figure a total of 133 children out of 519 were considered seropositive with the Diagnostic Index criteria, giving a global seropositivity of 25.6 per cent. The distribution of the global seroprevalence among age, sex, geographical region and risk factors are detailed in Table 1. The results showed no significant association between age or sex and infection with toxocariasis. However, there was a significant association between parental education and seropositivity (Table 2).

Discussion

Serological surveys conducted in different parts of the world demonstrate a high variation in *Toxocara*

TABLE 1
Epidemiologic analyses for Toxocara *seroprevalence in the children of Shiraz, Iran*

	No. of analysed samples	No. (%) of positive	No. (%) of negative	Odds Ratio (OR)	95% CI	*p* value
Age classes (years)				0.94	0.64 < OR < 1.42	0.76
6–9	275	69 (25.1)	206 (74.9)			
10–13	244	64 (26.2)	180 (73.8)			
Sex				0.99	0.66 < OR < 1.50	0.96
Male	274	70 (25.5)	204 (74.5)			
Female	245	63 (25.7)	182 (74.3)			
Residency				0.59	0.38 < OR < 0.90	0.01
Rural	233	47 (20.2)	186 (79.8)			
Urban	286	86 (30.1)	200 (69.9)			

TABLE 2
Relationship between Toxocara *seroprevalence and assumed risk factors in school children of Shiraz, Iran*

Risk factors	Total no. analysed	% Positive	% Negative	Odds Ratio (OR)	95% CI	*p* value
Pet in house				1.42	0.74 < OR < 2.74	0.25
Yes	53	32.1	67.9			
No	466	24.9	75.1			
Expose to dog or cat				1.24	0.80 < OR < 1.91	0.31
Yes	169	28.4	71.6			
No	350	24.3	75.7			
Exposed to soil				1.05	0.69 < OR < 1.55	0.81
Yes	227	25.1	74.9			
No	292	26.0	74.0			
Pica				4.54	1.02 < OR < 2.81	0.025
Yes	27	7.4	92.6			
No	492	26.6	73.4			
Thumb suck				1.06	0.58 < OR < 1.96	0.83
Yes	73	24.7	75.3			
No	446	25.8	74.2			
Father's education				1.07	1.14 < OR < 3.06	< 0.05
Yes	416	23.1	76.9			
No	103	35.9	64.1			
Mother's education				0.82	0.52 < OR < 1.30	0.37
Yes	369	24.1	75.9			
No	150	29.3	70.7			
No. of brothers or sisters				1.31	0.77 < OR < 2.19	0.26
0–5	419	26.6	73.4			
> 5	100	30.0	70.0			
Birth order				1.42	0.85 < OR < 2.36	0.14
1–4	420	24.3	75.7			
> 4	99	31.3	68.7			

seroprevalence ranging from 2.6 per cent among adult blood donors in Britain[6] to 86 per cent among the rural children of St. Lucia.[14] However, the present study in Iran showed a higher rate of infection (25.6 per cent) than that of Iraq (7.3 per cent), Sudan (6.5 per cent) and Jordan (10.9 per cent).[15–17] Systematic serological surveys in the developing countries have not been conducted.[18] Glickman and Schantz[19] and Barriga[20] studied the dependence of the larva of *Toxocara* on age and reported higher occurrences of toxocaral antibodies in children, and developing infections as a result of geophagia. In our study, no significant difference in seroprevalence was detected between different age groups. The present study showed no difference in the infection rate between the two sexes as the studies of Uhlikova and Hubner[21] showed. However, some workers reported higher seroprevalence in males than females.[19,20] The result of the present serological tests, evaluated by the place of residence, revealed higher prevalence in urban than rural people—a result which is different to that in other reports.[21] Considering the degree of infection in dogs and cats in different countries, as well as a high prevalence of *T. cati* and *T. canis* in cats and dogs in Shiraz,[12] the incidence of human toxocariasis should be high. However, most cases probably go undiagnosed because most carriers are asymptomatic. If VLM is suspected, a tissue biopsy can be taken and examined for migrating larvae, but finding the larvae is like 'searching for a needle in a haystack'. The best way of VLM identification is by immunological tests. In epidemiologic studies, several risk factors have been reported to be important such as pica, exposure to dogs or cats, thumb sucking and rural residency. However, the relative importance of these risk factors may differ between different countries and between geographic regions within a country. In our study, the children whose fathers were illiterate, were more seropositive; indicating the effect of economic situation on seropositivity. The differences in risk factors reflect the concept that a larger dose of larvae is required to produce clinical signs of VLM.[21] This explains why geophagia, poor hygiene, and contact with puppies predisposed to the ingestion of large numbers of *Toxocara* eggs in contaminated soils, are more prominent in children with VLM than OLM.[22]

The medical profession is only starting to recognize visceral larva migrans as a relatively frequent syndrome in children and adults. Population surveys in many countries amongst healthy people have definitely shown that subclinical toxocariasis is common.[4,5,14–17,21]

The high prevalence of toxocariasis in school children and the possible complications due to *Toxocara* infections represent a potential public health problem in this region. It is necessary to pay attention to the education of

both the veterinarian and medical profession, as well as the general public.

References

1. Beaver PC, Jung RC, Cupp EW (eds). Clinical Parasitology. Lea & Febiger Philadelphia, 1984.
2. Petithrory JC, Chaumeil C, Liotet S, Rosseau M, Bisognani AC. Immunological studies on ocular larva migrans. In: Lewis JW, Maizel RM (eds), *Toxocara* and Toxocariasis, Clinical, Epidemiological and Molecular Perspectives. British Society of Parasitology, London 1993; 81–8.
3. Gillespie SH. The clinical spectrum of human toxocariasis. In: Lewis JW and Maizel RM (eds), *Toxocara* and Toxocariasis, Clinical, Epidemiological and Molecular Perspectives. British Society of Parasitology, London 1993; 55–61.
4. Kenny JV, MacCabe RJ, Smith HV, Holland C. Serological evidence for the presence of toxocariasis in the Turkana District of Kenya. Trans Roy Soc Trop Med Hyg 1995; 89: 377–78.
5. Fenoy S, Cuellar C, Guillen JL. Seroprevalence of toxocariasis in children and adults in Madrid and Tenerife, Spain. J Helm 1996; 70: 109–13.
6. deSavigny DH, Voller A, Woodruff AW. Toxocariasis: serological diagnosis by enzyme immunoassay. J Clin Pathol 1979; 32: 284–88.
7. Glickman LT, Grieve RB, Lauria SS, Jones DL. Serodiagnosis of ocular toxocariasis: a comparison of two antigens. J Clin Pathol 1985; 38: 103–07.
8. Eslami A, Mohebali M. Helminths infection of sheep dogs and their implications in Iran. Arch Faculty Vet Med Tehran Univ 1994; 6: 116–19.
9. Sharifi I, Tasbiti A. The prevalence of intestinal helminths in stray dogs in Kerman city. Iranian J Pub Hlth, 1994; 23: 13–23.
10. Farivar K, Rafat S. A case report of visceral larva migrans from Zabol (in Persian). J Medical Assoc 1991; 11: 94–7.
11. Kabiri M, Shariat S, Mirbod P, Behravan N. A case of visceral larva migrans. Medical J Nat Univ Iran 1991; 7: 136–40.
12. Sadjjadi SM, Oryan A, Jalali AR, Mehrabani D. Study on the prevalence of *Toxocara cati* of stray cats in Shiraz, Iran. Parasitol Int 1998; Suppl. 47: 128.
13. Cuellar C, Aguila C, Fenoy S, Guillen JL. Comparative study of ELISA Diagnostic Index and larval micro-precipitation test in human toxocariosis. VII International Congress of Parasitology, Paris, France, 1990; 952.
14. Thompson DE, Bundy DA, Cooper ES, Schantz PM. Epidemiological characteristics of *Toxocara canis* zoonotic infection of children in a Caribbean community. Bull WHO 1986; 64: 283–90.
15. Woodruff AW, Watson J, Shikara I, *et al.* Toxocara ova in soil in the Mosul District, Iraq, and their relevance to public health measures in the Middle East. Annals Trop Med Parasitol 1981; 75: 555–57.
16. Woodruff AW, Salih SY, de Savigny D, Baya EI, Shah AI, Dafalla AA. Toxocariasis in The Sudan. Annals Trop Med Parasitol 1981; 75: 559–61.
17. Aboshehada MN, Sharif L, Elsukhon SN, Abuharfeil N, Atmeh RF. Seroprevalence of *Toxocara canis* antibodies in humans in northern Jordan. J Helm 1992; 66: 75–8.
18. Glickman LT. The epidemiology of human toxocariasis. In: Lewis JW, Maizel RM (eds), *Toxocara* and Toxocariasis, Clinical, Epidemiological and Molecular Perspectives. British Society of Parasitology, London 1993; 3–10.
19. Glickman LT, Schantz PM. Epidemiology and pathogenesis of zoonotic toxocariasis. Epi Rev 1981; 3: 230–50.
20. Barriga OO. A critical look at the importance, prevalence and control of toxocariasis and the possibilities of immunological control. Vet Parasitol 1988; 29: 195–234.
21. Uhlikova M, Hubner J. Seroprevalence of *Toxocara canis* infection in Czech Republic. Cent Eur J Pub Hlth 1998; 6: 195–98.
22. Schantz PM, Weis PE, Pollard ZF, White MC. Risk factors for toxocaral ocular larva migrans: A case control study. Am J Pub Hlth 1980; 70: 1269–72.