Seroepidemiology of Toxocariasis in Children (5-15 yr Old) Referred to the Pediatric Clinic of Imam Hossein Hospital, Isfahan, Iran

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Abstract

Background: Human toxocariasis, a helminthozoanosis, is due to the migration of Toxocara species larvae into human organisms. Humans, especially children become infected by ingesting of embryonated eggs from soil, dirty hands, and raw vegetables. Seroprevalence of this infection is high in developed countries, especially in rural areas. The aim of this study was to investigate the seroepidemiology of Toxocariasis in children referred to the pediatric clinic of Imam Hossein hospital, Isfahan, Iran.

Methods: In this cross sectional study the sera of children aged 5 to 15 years old, admitted to Imam Hossein Pediatric Hospital were collected during 2013-14. Then the sera were examined for anti Toxocara canis antibodies using commercial ELISA kit.

Results: From 427 children, 196 (45.9%) were female and 231(54.1%) were male. 107(25.1%) were from rural and 320 (74.9%) were from urban area. Of them 129 (30.2%) were contacted with dog. One child (0.2%) had hypereosinophilia, 33 (7.7%) eosinophilia, and 6 (1.39%) were positive for T. canis IgG (two male and four female). Four of infected children with T. canis were from urban (1.25%) and two from rural areas (1.9%). There was no significant correlation between education of parents, gender, age, place of living and contact with dog with ELISA results test.

Conclusion: Toxocariasis is prevalent in the children of Isfahan region. Results suggest a low Toxocara exposure in children in this area. Therefore, more risk factors associated with Toxocara exposure should be identified in the further investigation.
Introduction

Toxocariasis is a parasitic infectious disease caused by *Toxocara cati* or *T. canis*. These two worms belong to roundworm family. This infection is transmitted from animal to human by ingestion of infective ova and then *Toxocara* larva will migrate to different tissues (1, 2). One of the major problems of toxocariasis is sever local reactions (1, 3). Toxocariasis has three kinds of clinical presentation: visceral larva migrants (VLM) syndrome, ocular larva migrants (OLM) syndrome and covert toxocariasis. High prevalence of toxocariasis was reported in developing countries especially in rural area (4).

On the other hand, the most involved organs are brain, liver, lung, and eye (1, 3, 5). Chronic cough in children is a relatively common clinical problem (6-8). Toxocariasis may be misdiagnosed as asthma and patients wrongly treated with corticosteroids, which may aggravate the patient. In the most cases, toxocariasis is not very serious and because of low larva burden, most of infected people do not show a special sign of disease. The sever form of disease is rare and commonly seen in children that play with soil contaminated with dog's feces (2, 4). Nervous toxocariasis is another form of toxocariasis, which has recently been recognized (9-11).

Systemic infection of *Toxocara* spp. is common in human and it seems that OLM is less common than VLM (2, 12). This parasitic infection is occurs worldwide, and for example, it is estimated that 10000 new cases occurs annually in USA (13). However, no data is available regarding the situation of this parasite in Isfahan.

Therefore, in this study, the seroprevalence of toxocariasis in children referred to the pediatric clinic of Imam Hossein Hospital of Isfahan, Iran using ELISA method was investigated.

Materials and Methods

Serum samples

In this cross – sectional study, 427 sera of children (196 girls and 231 boys) aged between 5 and 15 yr old referred to Imam Hossein hospital of Isfahan from March 2013 until March 2014 were collected. Serum samples were collected and kept at -20 °C for detection of anti toxocariasis antibody using commercial ELISA kit.

CBC (cell blood count) test

For every children CBC test was performed and hyper eosinophilia reported if blood eosinophil was >1000 cell/ml (14). For every individual a questionnaire form containing demographic data, location (urban/rural), education level of parents and contact with dog were filled.

Enzyme-linked immunosorbent assay

Human anti- *T. canis* IgG antibody was measured using a commercially available ELISA kit IBL (German company) according to the manufacturer’s guidelines. Based on the manufacturer’s instructions, the result was considered positive when a value higher than 11.0 U/ml was recorded.

Statistical analysis

Chi-square test, *t*-Student test and Spearman Correlation Coefficient were used for statistical analysis. Differences were considered significant if *P*-values were less than 0.05.

Results

From 427 children referred to the hospital, 196 children (45.9%) were female and 231 (54.1%) were male. 107(25.1%) were lived in rural area and 320(74.9%) in urban area. Overall, 129(30.2%) children were contact with dog.
One child (0.2%) had hypereosinophilia but 33 (7.7%) had eosinophilia. Six patients (1.39%) were anti-*T. canis* IgG positive, which two of them were male (33%) and four were female (67%). Furthermore, four were living in urban and two in rural area. Of these patients, three had eosinophilia and one had hypereosinophilia. Only 2 infected children were contacted with dog. There was no significant relationship between education of parents, gender, age, place of living and contact with dog with ELISA tests results (Table 1).

Table 1: The relationship between age, gender, location, contact with dogs, patient parent’s education, with the ELISA test result in children aged 5-15 years old referred to Imam Hossein hospital in Isfahan, Iran

| Risk factors                  | No. (%) of analyzed samples | No. (%) of positive | No. (%) of negative | OR    | OR(95%CI) | P value |
|-------------------------------|-----------------------------|---------------------|---------------------|-------|-----------|---------|
| Age (yr)                      |                             |                     |                     |       |           |         |
| 5                             | 30 (7)                      | 0                   | 30 (100)            | --    | --        | 0.268   |
| 6                             | 74 (17.3)                   | 0                   | 74 (100)            |       |           |         |
| 7                             | 50 (11.7)                   | 0                   | 50 (100)            |       |           |         |
| 8                             | 48 (11.2)                   | 2 (4.16)            | 46 (95.84)          |       |           |         |
| 9                             | 40 (9.4)                    | 1 (2.5)             | 39 (97.5)           |       |           |         |
| 10                            | 52 (12.2)                   | 0                   | 52 (100)            |       |           |         |
| 11                            | 45 (10.5)                   | 2 (4.4)             | 43 (95.6)           |       |           |         |
| 12                            | 32 (7.5)                    | 0                   | 32 (100)            |       |           |         |
| 13                            | 21 (4.9)                    | 0                   | 21 (100)            |       |           |         |
| 14                            | 12 (2.8)                    | 0                   | 12 (100)            |       |           |         |
| 15                            | 23 (5.4)                    | 1 (4.34)            | 23 (95.66)          |       |           |         |
| Sex                           |                             |                     |                     |       |           |         |
| Male                          | 231 (54.1)                  | 2 (0.87)            | 229 (99.13)         | 0.419 | 0.76<OR<2.314 | 0.3     |
| Female                        | 196 (45.9)                  | 4 (2.05)            | 192 (97.95)         |       |           |         |
| Residency                     |                             |                     |                     |       |           |         |
| Rural                         | 107 (25.1)                  | 2 (1.8)             | 105 (98.2)          | 0.665 | 0.120<OR<3.68 | 0.6     |
| Urban                         | 320 (74.9)                  | 4 (1.25)            | 316 (98.75)         |       |           |         |
| Ownership and contact with dogs|                             |                     |                     | 1.157 | 0.209<OR<6.4 | 0.8     |
| Yes                           | 129 (30.2)                  | 2 (1.55)            | 127 (98.45)         |       |           |         |
| No                            | 298 (69.8)                  | 4 (1.34)            | 294 (98.66)         |       |           |         |
| Parents education             |                             |                     |                     | 0.62  | 0.112<OR<3.42 | 0.5     |
| High school education         | 237 (55.5)                  | 4 (1.68)            | 233 (98.32)         |       |           |         |
| University education          | 190 (44.5)                  | 2 (1.05)            | 188 (98.95)         |       |           |         |

The CBC tests showed a normal count of WBC in 406 (95.1%) children and 21 (4.9%) had high WBC counts. On the other hands, 138 (32.3%) children had abnormal RBC count and 289 (67.7%) had normal RBC count. 46 (10.8%) and 381 (89.2%) children had abnormal and normal platelet count respectively. Correlation between CBC test and ELISA test are presented in the Table 2.

Discussion

Seroprevalence surveys shows that toxocariasis exists worldwide. Worm infections are common in developing countries and some reports declared high frequency of human infection by *Toxocara larva* (15, 16).

Different prevalence of *Toxocara* infection was reported in various countries as 3%-86% (17). In this study, 6 (1.39%) of 427 children were seropositive for toxocariasis. 37.12% of individuals were seropositive in general population of the Amazonian City, and any information about contact with dog or cat was reported (18). In Hamadan City, out of 544 children (aged 1–9 yr), 5.3% were positive for toxocariasis.
Table 2: Relationship “between” the Cell blood count with the Elisa test result in children aged 5-15 years old referred to Imam Hossein hospital Isfahan, Iran

| CBC&diff       | No. (%) of analysed samples | No. (%) of positive | No. (%) of negative | OR       | OR(95%CI)     | P value |
|----------------|----------------------------|---------------------|---------------------|----------|---------------|---------|
| WBC            |                            |                     |                     | 4.01     | 0.447<OR<35.956 | 0.37   |
| Normal Normal  | 406(95.1)                  | 5(4.39)             | 401(95.61)          |          |               |         |
| Normal Normal  | 21(4.9)                   | 1(4.76)             | 20(95.24)           |          |               |         |
| Neutrophil     |                            |                     |                     | 0.095    | 0.016<OR<0.549 | 0.001  |
| Abnormal Normal| 21(4.9)                   | 2(9.5)              | 19(90.5)           | 4.02(99.02) |               |         |
| Lymphocyte     |                            |                     |                     | 0.047    | 0.009<OR<0.25  | 0.001  |
| Abnormal Normal| 22(5.2)                   | 3(13.63)            | 19(86.37)           | 4.02(99.26) |               |         |
| RBC            |                            |                     |                     | 0.233    | 0.042<OR<1.29  | 0.07   |
| Abnormal Normal| 138(32.3)                 | 4(2.8)              | 134(97.2)           |          |               |         |
| Normal Normal  | 289(67.7)                 | 2(0.69)             | 287(99.31)          |          |               |         |
| Platelet       |                            |                     |                     | 0.233    | 0.042<OR<1.311 | 0.135  |
| Abnormal Normal| 46(10.8)                  | 2(4.34)             | 44(95.66)           | 377(98.96) |               |         |
| Normal Normal  | 381(89.2)                 | 4(1.04)             | 377(98.96)          |          |               |         |
| Eosinophila    |                            |                     |                     | 26.06    | 4.587<OR<148.143 | 0.001  |
| Normal Eosinophila | 393(92.03)       | 2(0.5)              | 391(99.5)           |          |               |         |
| Normal Eosinophila | 34(7.97)                | 4(11.76)            | 30(88.24)           |          |               |         |

However, they did not investigate the correlation between contact with dog and cat and infection seropositivity (19). 20.2% of rural and 30.1% of urban children in Fars Province were infected with toxocariasis (20). Recently Sharif et al. reported that 25% of schoolchildren between 7-14 yr age in Northern Iran were seropositive. They reported a significant correlation between contact with dog and seroprevalence of toxocariasis (21).

Malla et al., reported 6.4% seropositivity in rural area among individuals with no clinical signs and 23.3% seropositivity in individuals with clinical signs. No significant correlation was reported between contact with dog and Toxocara infection (22). Fan et al. reported a prevalence of 76.6% for toxocariasis among 7-12 yr old students in Taiwan. They showed a significant correlation between histories of raising dogs and seropositivity (23). Ajayi et al. from Nigeria reported 30% seropositivity among individuals aged 2-21 yr old. They showed that dog’s ownership was not an important factor for Toxocara infection (24). In another study, 4.8% of healthy blood donors and 1.2% of children were infected (25).

Alavi et al. showed that 2% in children were seropositive (26). This prevalence is very similar to what we found in Isfahan. Based on the various studies, prevalence of toxocariasis is different in various societies and it may be related to difference in climate, culture and religion. In our study there was not a significant correlation between dog contact and seropositivity (P value=0.8). In addition, correlation between gender and seropositivity was not significant (P value = 0.3). In previous studies no statistically significant correlations between gender and age with prevalence of toxocariasis were seen (20, 23, 24, 26).

In our study two infected children were living in rural and four in urban and correlation between places of living with seropositivity was not statistically significant (P value = 0.6). In contrast to our finding, Sadjjadi et al. reported a significant correlation between places of living with seropositivity, however similar to our finding; Alavi et al. did not found statis-
ically significant correlation (20, 26). There is controversial reports regarding the relationship between keeping dog, prevalence of toxocariasis is in different investigation, and most of them found a similar finding to ours. Compatible to our findings, studies in Brazil and Ahvaz did not report any significant correlation between eosinophilia and toxocariasis (15, 26) but one study in Argentina reported a significant correlation in this regard (27).

In different studies in Iran such as our study, no correlation was seen between parents educational and prevalence of toxocariasis. Probably previous exposure to infection agents such as various species of *Toxocara* could be the reason for raised titer of antibody in these children. In this study beside eosinophil, other blood cell such as neutrophil and lymphocyte were surveyed and the correlation with sero-positive individuals was significant. Based on the information more study is recommended to understand the correlation of more factor with seropositivity.

**Conclusion**

Toxocariasis is prevalent in the children of Isfahan region. Results suggest a low *Toxocara* exposure in children in this area. Therefore, more risk factors associated with *Toxocara* exposure should be identified in the further investigation.

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**References**

1. Gillespie S, Pearson RD. Principle and practice of clinical parasitology. 1st ed. West sussex: John Wiley & Sons; 2001.
2. Afzal F. Medical helminthology. 7th ed. Tehran: Nashredivaj; 2010.
3. Overgaauw PA, Nederland V. Aspects of *Toxocara* epidemiology: human toxocarosis. Crit Rev Microbiol. 1997;23(3):215-31.
4. Muller R. Worms and human disease. 2nd ed. Wallingford: CABI publishing; 2002.
5. MacLean J, Graeme-Cook F. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 12-2002. A 50-year-old man with eosinophilia and fluctuating hepatic lesions. N Engl J Med. 2002;346(16):1232-9.
6. Aguiar-Santos AM, Andrade LD, Medeiros Z, Chieffi PP, Lescano SZ, Perez EP. Human toxocariasis: frequency of anti-*Toxocara* antibodies in children and adolescents from an outpatient clinic for lymphatic filariasis in Recife, Northeast Brazil. Rev Inst Med Trop Sao Paulo. 2004;46(2):81-5.
7. Alavi SM, Sefidgaran G. Frequency of anti *Toxocara* antibodies in school children with chronic cough and eosinophilia in Ahvaz, Iran. Pak J Med Sci. 2008;6(8):300-3.
8. Azizi F, Hatami H, Janghorbani M. Epidemiology and control of common Diseases in Iran. 1st ed. Tehran: Ministry of Health Publications; 1998.
9. Fenoy S, Cuellar C, Guil len J. Seroprevalence of toxocariasis in children and adults in Madrid and Tenerife, Spain. J Helminthol. 1996;70(2):109-14.
10. LS G. Diagnostic Medical Parasitology. 4 th ed. Washington: ASM Press; 2001.
11. Moore T, McCarthy J. Toxocariasis and larva migrans syndromes. 2nd ed. Philadelphia: Elsevier Inc; 2006.
12. Magnaval J-F, Glickman LT, Dorchies P, Morassin B. Highlights of human toxocariasis. Korean J Parasitol. 2001;39(1):1-11.
13. Quattrocchi G, Nicoletti A, Marin B, Bruno E, Druet-Cabanac M, Preux P-M. Toxocariasis and epilepsy: systematic review and meta-analysis. PLoS Negl Trop Dis. 2012;6(8):1-10.
14. Weller P. *Trichinella* and other tissue nematodes. Harrisons Principles Int Med. 2005;16(1):1253.
15. Anaruma Filho F, Chieffi PP, Correa CRS, Camargo ED, Silveira EP, ARANHA JJB, et al. Human toxocariasis: a seroepidemiological survey in the municipality of Campinas (SP), Brazil. Rev Inst Med Trop Sao Paulo. 2002;44(6):303-7.
16. Rubinsky-Elefant G, Hirata C, Yamamoto J, Ferreira M. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. Ann Trop Med Parasitol. 2010;104(1):3-23.
17. Alderete J, Jacob C, Pastorino AC, Elefant GR, Castro AP, Fomin AB, et al. Prevalence of *Toxocara* infection in schoolchildren from the Butantã region, São Paulo, Brazil. Mem Inst. Oswaldo Cruz. 2003;98(5):593-7.
18. Roldán WH, Caveró YA, Espinoza YA, Jiménez S, Gutiérrez CA. Human toxocariasis: a seroepidemiological survey in the Amazonian city of Yurimaguas, Peru. Rev Inst Med Trop Sao Paulo. 2010;52(1):37-42.
19. Fallah M, Azimi A, Taherkhani H. Seroprevalence of toxocariasis in children aged 1-9 years in western Islamic Republic of Iran, 2003. East Mediterr Health J. 2007;13(5):1073-7.
20. Sadjjadi S, Khooravi M, Mehrabani D, Oryan A. Seroprevalence of *Toxocara* infection in school children in Shiraz, Southern Iran. J Trop Pediatr. 2000;46(6):327-30.
21. Sharif M, Daryani A, Barzegar G, Nasrolahei M, Khalilian A. Seroprevalence of toxocariasis in schoolchildren in Northern Iran. Pak J Biol Sci. 2010;13(4):180-4.
22. Malla N, Aggarwal A, Mahajan R. A serological study of human toxocariasis in north India. Natl Med J India. 2002;15(3):145-7.
23. Fan CK, Hung CC, Du WY, Liao CW, Su KE. Seroepidemiology of *Toxocara canis* infection among mountain aboriginal schoolchildren living in contaminated districts in eastern Taiwan. Trop Med Int Health. 2004;9(12):1312-8.
24. Ajayi O, Duhlinska D, Agwale SM, Njoku M. Frequency of human toxocariasis in Jos, Plateau state, Nigeria. Mem Inst. Oswaldo Cruz. 2000;95(2):147-9.
25. Kimmig P, Naser K, Frank W. Seroepidemiologic studies of human toxocariasis. Int J Hyg Environ Health. 1991;191(4):406-22.
26. Alavi SM, Hosseini SA, Rahdar M, Salmanzadeh SH, Nikkhuy AR. Determination of Seroprevalence Rate of *Toxocara canis* in 6-15 years Aged Rural and Urban School Children in Ahvaz, Iran. Sci Med J. 2011;10:406-22.
27. Alonso JM, Bojanich MV, Chamorro M, Gorodner JO. *Toxocara* seroprevalence in children from a subtropical city in Argentina. Rev Inst Med Trop Sao Paulo. 2000;42(4):235-7.