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Seroepidemiological Study for Toxocariasis among Children in Zanjan-Northwest of Iran

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Abstract: Determination of seroepidemiological survey of *Toxocara* infection in 810 sera from children inhabitants in rural and urban area of Zanjan (North-West of Iran) was adjusted for specific antibody level against of second stage larvae of *Toxocara* for excretory and secretory antigens based on ELISA technique. In contrast of the other studies, the overall seroprevalence was quite low, reaching to 2.7% out of 810 samples was positive to anti *Toxocara* species antibodies. Neither age nor gender seemed to be significant factor to positive serology. Seroprevalence of anti *Toxocara* antibody were detected in 8 out of 494 (1.6%) children from urban area, whereas 316 sample (4.4%) were the positive in the rural area. Positive children who had contact or ownership of cats and dog were 6.9 and 3.9%, respectively. Albeit, concerning to this study, seroprevalence of toxocariosis is lower than other countries and other studies in Iran. But protections of toxocariosis in children must be taking into account in public health program. Suggest that increase of hygiene education would be a crucial affective on population to reduce infection risk factor and zoonotic disease.

Key words: *Toxocara* infection, children, antibody, antigen, ELISA

INTRODUCTION

Toxocariosis is helminth infections predominantly caused by migrating of dog or cat round worm, *Toxocara canis* or *Toxocara cati* larvae to organs and tissues of human, respectively. The major clinical consequences of prolonged migration of *T. canis* larvae in humans are Visceral Larva Migrants (VLM) and Ocular Toxocariosis (OT) (Gilckman, 1993). Human can become infected either by ingestion of embryonated eggs (contaminated fully developed larva L3) from contaminated source or by means of the paratenic hosts of the parasite. Toxocariasis have a world wide distribution and young children are the main susceptible population to *Toxocara* infection due to dirt pica, poor hygiene, frequent contact with dogs and cats. Dogan *et al.* (2007) have shown that a strong correlation between frequent of *Toxocara* infection, life style and infection risk. Clinical manifestations are not specific in infected patient and most of the patients are asymptomatic (Alderete *et al.*, 2003). Upon immunological examination, the immunochemical composition of excretory and secretory antigens is well characterized, therefore determination of an antibody response to *T. canis* antigen in serum is sensitive and specific for diagnosis and serological survey based on ELISA

(Enzyme-Linked Immuno Sorbent Assay) 78% sensitivity and 92% specificity (Muradian *et al.*, 2005). Regarding to seroprevalance of toxocariasis among children in different countries has been reported to be within a rang of 4-86% based on using the TcES-ELISA (Alderete *et al.*, 2003; Fan *et al.*, 2004; Figueiredo *et al.*, 2005; Chan *et al.*, 2001). *Toxocara* infection in human in turkey (Dogan *et al.*, 2007) are relatively frequents as indicated by seroprevalance of 12.95% and various studies concerning toxocariasis have investigated in Iran, usually reporting relatively high frequencies, like Sadjjadi *et al.* (2000) reported 28.8%. Aim of the present study, determination of seroepidemiological survey of toxocariasis in children attended at the pediatric outpatient centers of the hospitals at Zanjan University.

MATERIALS AND METHODS

In total, 810 serum samples were obtained from children (421 male and 389 female), all randomly selected from children that attended to pediatric outpatient centers in Iran-Zanjan hospitals at 2007 involved in this survey. The mean ages were similar in both gender and ranged between 2-15 years old, for all children informed consent forms were obtained from the parents of all children included in this study. The questioner requested some

personal information like sex, age, origin contact site, present of dogs and cats, playing with soil and so on. As well as physical examination and clinical manifestation like respiratory problems, headaches, stomachache, anemia or eosinophilia was performed of target population and take into consideration. Blood samples were adjusted using ELISA immuno-enzymatic technique for detection of IgG specific antibodies, by secretory- excretory antigens from second stage larvae (L2) of *T. canis*. In the present study an ELISA kit (Ref.58721 and Lot TOCG025, IBL Germany) for the detection of anti- *T. canis* (E/S antigen) serum IgG was used. Each kit provided the material to perform 96 individual ELISAs on polystyrene.

Micro- titration strips sensitized with *T. canis* E/S antigen and a goat anti human IgG specific peroxidase conjugated and absorbance was read at 492 nm to stables optical density with State-Fax 2100 Awerness, USA). Optimal dilution of the antigen and antibodies were predetermined by check board titration (Jimenez *et al.*, 1997) optimally dilution test sera 1:100 and positive control which belongs to patients with *Toxocara* infection with positive results both clinical and laboratory diagnosis also negative control were included in each plate. For reading of the results for the test sera, the value of the no-serum blank had to be subtracted from all measured values. Samples with an absorbance lower than that of the weak positive control serum had a parasite-specific antibody concentration that was considered to be no significant for toxocariosis; interpretation was therefore negative. Samples with an absorbance higher than that of the weak positive control serum were regarded as positive; samples with absorbances higher than that of the positive control serum were regarded as strongly positive. Statistical analysis was performed with χ^2 -test ($p < 0.5$).

RESULTS AND DISCUSSION

Out of 810 blood samples, 22 sera (2.7%) have shown that positive reaction to TcES antigen based on ELISA test. A total of 494 children in urban areas and 316 children in rural areas were screened for toxocariosis. Crucial levels of anti- *Toxocara* antibodies were detected in 8 samples out of 810 children from urban area (1.6%), while the rate of anti *Toxocara* antibodies were positive in 14 children out of 810 samples in rural area (4.4%) (Fig. 1). Significantly, the levels of anti-*Toxocara* antibodies in rural area were much higher than in urban areas. In total positive rate (2.7%), 12 (2.9%) were from males and 10 (2.6%) from females, whose average age was between 2-15 years old. As well as 4 (3.9%) out of 22 positive children had a dog contact and 10 (6.9%) with cat

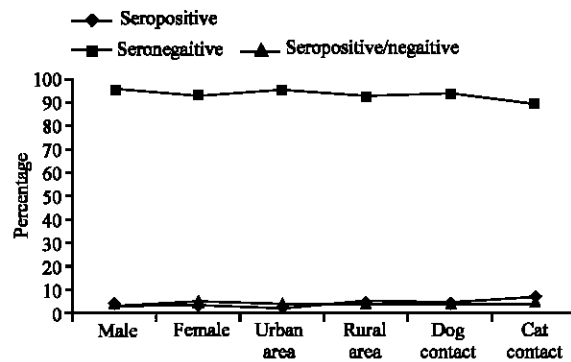


Fig. 1: Frequency and percentages of seropositive, seronegative and seropositive/negative toxocariosis in 810 children involving in this study

contact. Remarkably, anti *Toxocara* antibodies were crucially higher in children who have a cat contact in their site than the children who have a dog contact. According to statistical analysis no significant correlations have been shown between sex, age, origin, geography, dog contacts and seroprevalance ($p > 0.05$), while interesting correlation between children who have a cat contact and seropositive (anti *Toxocara* antibodies) ($p < 0.05$).

Nowadays, many researchers believed that TcES ELISA test is extremely recognized as having good specificity in the diagnosis of *Toxocara* infection, which is an important and common zoonotic disease from world wide (Despommier, 2003). Since the diseases frequently have seen in young children, usually does not cause sever problems; albeit it persists for a long time, therefore serological diagnosis one of the considerable importance in the detection of *Toxocara* infection, as the clinical manifestation of toxocariosis are limited value in the differential diagnosis (Schantz *et al.*, 1979). Since toxocariosis is usually a pediatric disease and generally asymptomatic and the seroprevalance varies from 3 to 86% in different countries (Alderete *et al.*, 2003). Present study regarding unknown information on serological survey for anti *Toxocara* species antibodies in Zanjan have done and seroprevalance level of anti *Toxocara* species antibodies was 2.7% and it is extremely different with other studies. Dogan *et al.* (2007) have announced that, seroprevalance of *Toxocara* antibodies is 12.9% which is 16.97% from rural area, while 0.71% from urban area. Fan *et al.* (2004) conducted a total seroprevalance of *Toxocara* infection among children (7-12 years old) living in Taiwan is 76.6%. Muradian *et al.* (2005) reported 26.9% out of 338 serum samples from children (1-15 years old) were positive based on ELISA technique. Figueiredo *et al.* (2005) have shown that *T. canis* infection must be

considered in at-risk children, such as those with puppies at home, who have had contact with soil. Surprisingly, *Toxocara* species was found in the soil samples from 21 out of 22 playgrounds (95.5%) in Italy (Giacometti *et al.*, 2000). Muradian *et al.* (2005) reported that 29.7% of soil samples were positive for the presence of *Toxocara* species Eggs. Gurel *et al.* (2005) also showed that 18.9% of 111 soil samples were contaminated with *Toxocara* species. Eggs in Aydin province and they highlighted the high Toxocariasis risk. Children who admitted living in a household where dogs were kept or playing in soil appeared at increased risk of seropositivity for *Toxocara* infection (Fan *et al.*, 2004). Iddawela *et al.* (2003) have shown that dog ownership, especially puppies and geophagia-pica, were significant risk factors. Coelho *et al.* (2004) performed a seroepidemiological survey for Toxocariasis, among 180 schoolchildren of the public schools in Brazil; the infection risk was higher among the children living in the city outskirts where the socioeconomic conditions were worse than in the central region of the city. Interpretation of seroprevalance data is still problematic problem owing to different cut off titers used by the various researchers and difficulty in evaluating the relationship between antibody titers, infection and clinical findings for the disease (Aguiar-Santo *et al.*, 2004; Teixeira *et al.*, 2006). Although, many studies in our country in different area have been done and the results of seroprevalance of anti *Toxocara* antibodies were different, results were much higher than in this study. Sadjjadi *et al.* (2000) reported 25.6% seropositive of Anti *Toxocara* antibodies out of 519 (6-13 years old) children living in Shiraz, Iran and Falah *et al.* (2005) have announced 5.3% of seropositive of toxocariasis in children under the 10 years old at Hamadan-Iran, whereas crucial level of anti-*Toxocara* antibodies in this survey were detected in 1.6% from urban area and 4.4% from rural area. Significantly in positive samples 2.7% out of 810 children 3.9 and 6.9% seropositive children who have a dog and cat in their house, respectively. However, many studies have shown that no relationship between ownership or professional contact with dogs and toxocariasis (Glickman and Cypress, 1977). Nevertheless, Fan *et al.* (2004) have found high relationship between high seropositive rate in 79.4% of dog owners and 67.9% in non-owners and suggestion that these two groups are equally at risk of infection. Figueiredo *et al.* (2005) have reported toxocariasis must be significant in pediatric such as those with poor sanitary hygiene, who had soil contact because of *Toxocara* species was found in the soil samples from 21 out of 22 play grounds 95.5% in Italy (Giacometti *et al.*, 2000). Gurel *et al.* (2005) announced that 111 (18.9%) soil

samples were contaminated with *Toxocara* species. Muradian *et al.*, (2005) have shown that 29.7% of soil samples were positive from *Toxocara* species eggs point of view. As well as Coelho *et al.* (2004) in seroepidemiological survey for toxocariosis in Brazil among 180 schoolchildren of the public school have shown that the risk of infection in too high in children living in the city outskirts, where the socioeconomic conditions were poor than in the central region of the city. In the current study, we have not seen any correlation between age group, gender, differences dog contact and seropositive toxocariasis. Fan *et al.* (2004) also mentioned that neither age nor gender seemed to be significant factors related to a positive serology same in present study. Nevertheless females were extremely more infected than male and children over the age of 10 years old are more seropositive (Theodoridis *et al.*, 2001). In the present survey, crucial association was observed between the presences of anti *Toxocara* antibodies with children who had a cat contacts, living in rural area, low family income and poor hygiene. Therefore seropositivity rate of toxocariosis in healthy children living in rural area was much higher than children living in urban areas. In addition serological cross reaction is too low between toxocariosis and other helminth infection. To sum up, ELISA technique based on TcES IgG antibodies would be a significant measurement of toxocariosis and we suggest that increase of hygiene education would be a crucial affective on population to reduce infection risk factor and zoonotic disease as well as It has also emphasized to improve measures for the sanitary control of pets and stray dogs and cats.

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