Visceral larva migrans among children in Kütahya (Turkey) and an evaluation of playgrounds for *T. canis* eggs

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This research was undertaken to determine the seroprevalence of *Toxocara canis (T. canis)* in children aged 3-13 years in Kütahya, to determine the presence of infected larvae in playground sandboxes, and to obtain a view of the general situation regarding visceral larva migrans (VLM) in the city. The blood sera of 119 children (54 girls, 65 boys) were tested for *T. canis* IgG, and 9 (7.6%) were found positive. In sand samples collected from playgrounds in nine different residential areas across the city, only two of them, in Inköy and Zafertepe neighborhoods, had *T. canis* larvae; in total, 3 of the 30 sand samples (10%) contained larvae. It is concluded that the main factor regarding VLM infection in children is outdoor playgrounds or playing areas.

Key words: visceral larva migrans, Toxocara canis, parasite, children.

Visceral larva migrans (VLM) is a zoonosis caused by some nematode larvae mainly from *Toxocara canis* (*T. canis*), which enter extra-intestinal tissues and organs from non-natural hosts prior to the end of their life cycle for an extended life opportunity[1,2]. These parasitic larvae may invade the nervous system (cerebrospinal form), internal organs (visceral form), and the eye (ocular form)[3,4]; the ocular form can lead to some loss of vision or blindness through retinal degeneration [5,6]. In many cases, the course may take a covert form, while asymptomatic forms also occur[1,7].

The life cycle of *T. canis*[4,8,9], the primary agent of VLM, is completed with larvae inside the infective eggs in the Canidae -the actual hosts-maturing on return to the small intestine, following a full migratory period in the body (10). However, in paratenic intermediate hosts including humans, normal development does not take place by intake of infective eggs in the soil. Newly-hatched larvae in the small intestine penetrate the organ's wall and through lymphatic or blood circulation colonize organs or tissues such as the spleen, liver, lungs, central nervous system, eye, myocardium, and endocardium[1,3,4,8,11,12]. Humans may contract VLM by intake of infective eggs or ingestion of raw livers of some farm animals that are paratenic intermediate hosts[13,14].

Five different major *T. canis* excretory/secretory (TcES) antigens (macromolecules) with molecular weights ranging between 32 kDa and 400 kDa have been identified, which result in strong antibody responses in the host organisms. However, this immune reaction cannot seriously harm the larvae[15].

Visceral larva migrans is mostly observed in children aged 2-12 years, mainly due to the disorder pica-for-earth or geophagia, non-hygienic living conditions, and close proximity to dogs, with symptoms such as abdominal pain, loss of appetite, uneasiness, fever, cough, wheezing, and hepatosplenomegaly[1,16]. Serious clinical findings are usually reported in children aged 1-3 years[6]. Severity and longevity of this high-morbidity infection vary by the larvae count and the infected body part, though it is rarely fatal; diagnosis and medical treatment are costly[17]. It is remarkable that somatic larvae colonizing the body can sustain life for a period of 5-10 years[6,18].

T. canis larvae are identified through some imaging techniques that spot pathologic tissues or antibody responses. Tissue biopsy results provide the final diagnosis, though this process may be troublesome, as most cases involve children. Serologic diagnosis of VLM, the

determination of excretory-secretory (TcES) antigens of the second cycle of *T. canis* larvae, is the preferred method.

It has been reported that the more frequent VLM cases in children could be attributed to stray or uncontrolled dogs in parks and playgrounds where children spend time without proper hygienic measures and to the ability of Toxocara eggs to remain alive for a couple of years in nature[1,19]. The seroprevalence varies with the geographic properties (ecologic, climatic) of the surveyed location and the socioeconomic level of the people concerned [17,20]. The disorder has been stated to be a major public health problem in developing countries, while the case in developed countries is less serious[21]. High rates of presence of this parasite in stray dogs (including puppies and pregnant dogs) indicate this zoonosis is significant for public health[9,22].

Stray dogs in the city of Kütahya multiply uncontrolled, as in most other towns in Turkey, and they are seen frequently in children's playgrounds, as in almost all parts of the city. This research was made to determine the seroprevalence of *T. canis* in children aged between 3-13 years in Kütahya, to determine the presence of infected eggs in playground sandboxes, and to obtain a view of the general situation regarding VLM in the city.

Material and Methods

Toxocara canis IgG antibody was investigated in the blood sera of 119 children admitted to Dumlupınar University, Faculty of Medicine Hospital, Pediatric Clinic for various medical reasons between October 2005 and May 2006 and whose parents completed a detailed questionnaire. The collected sera were put in 1.5 ml Eppendorf capped tubes and kept in a -70 °C deep freezer until the tests were executed after defrosting and centrifuging.

The test, ELISA Rida Screen™, R-Biofarm®, was executed as instructed by the company. Stool tests for 73 of the children were also carried out to detect probable helminth infections.

A total of 30 sand samples were taken from 10 empty lots frequented by children for playing and children's playgrounds in six different neighborhoods in the city of Kütahya. Samples were collected from three separate spots with no vegetation in the same location. For each playground, three samples were taken, each of which was about 50 grams from up to 5 cm depth. Samples were floated by centrifuging for 1 hour (h) in a flotation solution added while on magnetic mixer before leaving to rest for a minimum of 3 h and inspected under 10x and 40x magnifying microscope after removing all lamellae. When the first tests resulted in a negative outcome, the tests were conducted a second time with the same samples. All findings of the study were statistically assessed by chi-square method with the help of the SPSS (ver. 13) program.

Results

The blood sera of 119 children (54 girls, 65 boys) were tested for *T. canis* IgG, and 9 (7.6%) were found positive, while 5 (4.2%) others were at the threshold values (Table I). These children were aged 3 to 13 years with an average age of 7.14 (±2.86). Of 73 stool tests carried out to detect possible helminth infections, *Ascaris lumbricoides*, *Trichuris trichiura* and *Hymenolepis nana* eggs were found in 4 samples (5.5%).

Table I. Visceral Larva Migrans Infection Cases and Age Groups

		Infection						
		Positive		Negative		Uncertain		Total
Age		M	F	M	F	M	F	
3-5	(n)	1	–	18	15	–	1	35
6-9	(n)	2	1	27	26	1	1	58
10-13	(n)	4	1	16	3	1	1	26
Total	(n)	7	2	61	44	2	3	119

Antibody against *T. canis* was detected in only one of the children found to carry helminth eggs, while other seropositive children's stool tests resulted in no helminth eggs.

In sand samples collected from playgrounds in nine different residential areas across the city, only two of them, İnköy and Zafertepe neighborhoods, had *T. canis* larvae; in total, 3 of the 30 sand samples (10%) contained larvae.

Discussion

In studies on *T. canis* seroepidemiology, positivities were found as: 36-76.6% in Taiwan [16,24,25], 5.1-37.8% in South Korea[13,26], 45.2-57.8% in Malaysia[27,28], 49.2% in P.R. of China [29], 8.8% in Iran[30], 28% in Slovenia[31], 40.35-58.2% in Poland[32,33], 5.4-14% in the United States[34,35], 29.7% in Brazil[17], 10.6-31.6% in Argentina[36,37], and 1.65% in New Zealand[38]. This study found the rate for the city of Kütahya as 7.6%. If the data with threshold values were included, the rate reaches 11.8%.

In examinations of park or garden samples for *T. canis* eggs, positivities were found as 35.1% in Argentina[37], 29.7% in Brazil[17], 63.3% in Japan[44], 2.8% in Spain[43], 55% in Venezuela[39] and 15-30.6% in Turkey[40-42]. The rate for the Kütahya study was 10%. Positive samples in the city were collected from two neighborhoods, and all samples from one particular neighborhood, Inköy, proved positive. It may be assumed that this result is attributable to the close proximity of the location to a garbage dump site where dogs find food. It was reported that sand and sandy soil parts of the ground were more homogeneous than other soil types, and therefore it was more likely to find larvae in such samples[23,43]. In line with this finding, samples in the study were gathered from sandy playgrounds.

Fan et al.[16] reported that toxocariasis seroprevalence is not related to dog raising, but it was also stated that this condition is mostly relevant in societies with a low socioeconomic level[24,25]. Martinez-Moreno[43] reported that imaginal prevalence of parasites in dogs is high in semi-rural and rural areas, while it is low in urban areas, and pointed out that, in line with this data, children living in urban areas have low seroprevalence rates.

In a similar manner, Luo et al.[29] and Fan et al.[24] observed high prevalences in children living in rural areas and pointed to the relevance of this finding with the keeping of dogs. Contrary to these findings, of the children studied in Kütahya, there was no recorded presence of a dog in the home, while the figure for the presence of a dog kept in the garden was very low compared to the 7.6% seropositivity, indicating VLM infection has no direct link to the keeping of dogs. However, a much lower seroprevalence than that reported[24] points to the keeping of dogs as a primary causation in high seroprevalence.

Despite the conclusion that there is no relation between increasing age and VLM infection [16,17,26,35], Lee et al.[13] and Minvielle at al. [36] pointed to the significance of age in this disorder. Fan et al.[25] stated that age was a significant factor in rural areas. Marmor et al. [35] reported VLM prevalence as 12% in the 10-15 age group, 8.6% in the 6-9 age group, and 5.6% in the 3-5 age group. The same study found *T. canis* seropositivity as 19.2% in the 10-13 age group, 5.2% in the 6-9 age group, and 2.9% in the 3-5 age group. All five threshold values found in the study were within the 6-14 age group.

The intensity of VLM positivity in children between 6-14 years of age points to the fact that these children usually play outdoors and sometimes fail to fulfill proper hygiene practices. It was found that 54.5% of children play in home gardens, 34.1% in nearby playgrounds, and 9.6% occasionally have contact with dogs, but no statistical direct relation was established (p>0.05).

Avcioglu and Burgu[41] reported that they found Toxocara eggs in 15% of the soil samples studied, and they drew attention to seasonal variations in their presence. In our study, this rate was found to be 10%; no seasonal variations could be observed since the samples were collected within a matter of days. Oge and Oge[40] found the rate as 30.6% in their study, in which samples were collected from public parks. Sample collection was limited to children's playgrounds in this work. The disparity between the rate of egg presence in this study and the rest might be attributed to the sampling choices.

Fan et al.[16] found a meaningful relation between the habit of hand-washing before eating and toxocariasis epidemiology in children, while refuting any link with raw food ingestion. On the other hand, Lee et al.[13] reported high prevalence in those who ingest raw liver. Some studies[25,30,36] reported no direct relation between sex and VLM infection, whereas Lee et al.[13] found sex a significant factor.

In our study, gender distribution of *T. canis*-seropositive children was 22.2% girls and 77.8% boys, with no statistical variation (p>0.05). No relation between the habit of hand-washing before eating and the infection could be found (p>0.05). No rural area data were included in the study as all children resided within the city of Kütahya.

Researchers have argued that higher infection prevalence with increasing age could result from raw liver ingestion and habitation with dogs in rural areas[13,25,26]. The low prevalence in our study is in line with non-existent raw liver ingestion in the country. While we observed some higher prevalence with increasing age, no statistical difference could be found (p=0.092).

Stool tests for 73 children (61.3%) were carried out, exposing *A. lumbricoides* and *T. trichiura* eggs in four children (5.5%) with only one infection. No other parasitosis was detected in the remaining children. Similarly, Magnaval and Glickman (1), reported no cross-reaction among *A. lumbricoides*, hookworm, *Escherichia coli*, and Giardia using TcES ELISA.

In conclusion, the etiologic source of VLM in children in the city of Kütahya was determined to be *T. canis*-contaminated earth, as the parasite eggs were found in sandy playgrounds in two neighborhoods. It was considered that the children in our study group, aged 3 to 13 years, with 7.6% serologic prevalence, most likely contracted *T. canis* via eggs in the playground soil, since no keeping of dogs or pica-for-earth history was reported in the survey, and there was compliance with hygiene standards.

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