

Seroepidemiological investigation of *Toxocara canis* in a female Greek pregnant population in the area of Athens

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Summary

The purpose of this study was to investigate the of areas where the soil is contaminated by *Toxocara* ova and also to assess seroepidemiological positivity in a Greek pregnant women population (ELISA IgG test). **Materials and Methods:** The authors carried out an examination of soil samples collected from different areas of Athens and Piraeus (Kazakos method). Blood serum was only collected from pregnant women living and conducting activities in places close to the places where the soil sample's were collected for at least a decade (ELISA IgG assay). **Results:** The authors suggest a correlation between the positive response in the ELISA assay IgG antibodies and the activities of people where soil was contaminated by *Toxocara* eggs. In conclusion, the prevalence of *Toxocara canis* infection in a population of Greek pregnant women was found to be a rate of 17.16% and the soil contamination rate of 17.08%.

Key words: *Toxocara canis*; Greek population; Pregnant women.

Introduction

Toxocariasis is a disease caused by a parasitic roundworm (*Toxocara canis*, *T. cati*, and related species). The infection is mainly due to oral ingestion of embryonated eggs originating from infected soil. It occurs either through intentional geophagy or when eating food with dirty hands, mainly in children's cases. The eggs become embryonated and thus infectious after remaining in the external environment for two or more weeks.

The disease clinical presentation includes a variety of symptoms which can render the differential diagnosis complicated. *Toxocara* roundworm can cause: *VLM* -visceral larva migrans the syndrome of migrating larva through viscera with eosinophilia greater than 30%, hepatosplenomegaly, and a high temperature. It can also include bronchopneumonia, pulmonary infiltration, cutaneous lesions, eosinophilic meningitis, and can lead to the formation of an eosinophilic granulation tissue.

OLM-ocular toxocariasis (ocular larva migrans) can occur during larvae migration to the eye which causes local reaction of the organism, resulting in retina lesions. The lesions are usually bilateral, appear after a few weeks, and cause sudden deterioration of vision, strabismus, iridocyclitis, and endophthalmitis.

Covert toxocariasis, in this case, serological tests give signs with either mild symptoms or without symptoms at all.

The purpose of the present study was the seroepidemiological investigation in a population of Greek pregnant women.

Materials and Methods

The purpose of the current study is the investigation of areas and places where soil is contaminated by ova (eggs) of the *Toxocara canis* parasite as well as the transmission of the aforementioned parasites to humans (both adults and minors). The study also includes a seroepidemiological investigation and some suggested measures to improve protection of public health against transmission of the above parasites.

The study was centered around two areas of investigation of which the first one focused on the detection of ova (eggs) of the *Toxocara canis* parasite in the environment (soil) and thus examined the infectious capacity of those places. The second study area focused on the determination of antibody level in pregnant women population. The aforementioned study areas were directly related with each other, as the study of the soil was combined with a simultaneous study of blood serum samples of people who are active in those places due to play and sports activities (e.g. soccer) and thus get in contact with soil (collection of plants, herbs, etc.)

Methodology

In order to work out the sero-epidemiological study of the current research, the authors carried out examination of soil samples collected from various areas of Attica and Piraeus. Those areas differ in terms of: A) social status of the inhabitants and B) population density, block of apartments or house availability, free spaces and parks, number of persons who visit open spaces or parks, and presence of stray or domestic dogs. They also differ in terms of personal hygiene habits (hand-washing), profession (e.g. jobs related with soil such as gardeners, land workers, etc) or hobbies related to plants, flowers or herb collection.

Blood serum was only collected from pregnant women living and acting in places close to those areas for at least a decade. The collection of blood samples was carried out in parallel with soil in-

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vestigation for *Toxocara ova* (through soil sample-taking) in order to allow to check potential correlation between specific geographical areas contaminated by the *Toxocara canis* roundworm and the serological response of individuals chosen for the sero-epidemiological study. After blood sample-taking, the tubes were transferred to the lab of the National School of Public Health where they underwent centrifuge, then the serum was either extracted for the test or stored at -20°C until the day of the test.

Patient's health records

For each participant's health record taken, special attention was paid in recording evidence of fever, cough, wheezing, hepatomegaly, and splenomegaly.

Following the declaration of the survey to the participants but prior to blood collection, their consent was requested assuring them that all data would only be used for research purposes and in strict compliance with medical ethics. Each participant gave her consent by signing an anonymous questionnaire that accompanied the blood serum to be examined.

The questionnaire included the number of each particular serum tube and the area of origin. It also included the following data: A) date of birth, sex, age, place of residence and approximate date of delivery, profession, and education level and B) personal hygiene habits that may raise the likeliness of infection by *Toxocara* parasite such as 1) not washing hands before meals; 2) indirect geofagia e.g. when cleaning herbs, vegetables etc; 3) the presence of dogs in the house or in the backyard/front yard.

Serological investigation

Serum examination for detection of antibodies against *Toxocara canis* (IgC) was carried out through the use of Ridascreen testing kit which according to the manufacturer, has a sensitivity of 100% and a special sensitivity of 98.4%. The test was performed through the binding of clear antigens on a micro-tilt plate. The result is obtained by dividing the absorption value of the sample by the cut-off value. It has to be less than 0.9 (negative) and less than 1.1 (positive). Any result between 0.9 and 1.1 is considered unreliable. In the present measurements no boundary-values were found and no hemolytic samples were detected which would have been rejected anyway.

Method for collection of ova

In order to calculate the soil contamination degree, soil samples were collected from several areas in which the residents of Athens and Piraeus used to frequent for athletic and/or children's activities. Places where the ground was covered by gravel, cement or asphalt-based materials were excluded from sample taking. The number of samples taken was related to the dimensions of each place. Sample takers carried out a rough calculation of the place dimensions by measuring its diagonal with steps (one step = approximately one meter).

With a small garden shovel marked down with colour lines to a depth of three cm, sample-takers dug square-shaped holes three cm deep, then collected the soil sample and placed it into plastic bags [1, 2]. Where sample-takers noted that soil was less dense, they dug below three cm to get a sample. They used air-tight plastic bags to store the samples. The soil volume kept in each bag was about 70 cm³ and weighed slightly over 50 grams. Both the name of the place and the sample-taking date were marked on each bag which was then stored in a dry and cool place at about 25°C until soil examination (which was planned to be carried out soon after collection).

Detection of ova in soil samples (Kazacos technique, 1983)

The method implemented was that of flotation in a saturated and hypertonic solution NaNO₃ (sodium nitrate) with specific gravity

1.35. This technique is considered the most suitable for the detection of ova of various roundworms. The use of non-ionic detergents i.e. polysorbate 40, 60, or 80 may further increase efficiency [3,4]. Besides, the use of saturated NaNO₃ is now efficient for detecting ova of toxocara in the soil with a success rate of 65% to 82.5% [2].

For microscopy the authors used a microscope carrying x10 and x40 magnification lenses.

The criteria used for recognition of the ova were the following: 1) oval or round shape; 2) cortex (thickness); 3) colour (light-coloured shell compared with the egg internal); 4) existence of pronymph; 5) size between 65-90 µm (measured by the microscope graduated scale). A single egg detected in the sample would automatically rendered it positive.

Statistical analysis

The statistical correlation attempted was that between the frequency rate of *Toxocara* contamination in soil and the frequency rate of *Toxocara* seropositivity in blood. For the statistical analysis of the two aforementioned sets of data, the authors used the *Pearson's correlation coefficient*. The statistical analysis method applied is the correlation between two variables. The correlation coefficient was found to be 0.72 and considering that the value is always between -1 and 1, the calculated value (0.72) suggests a fairly strong correlation between the infectious factors in the soil (*Toxocara* eggs) and a positive serological test in people.

Results

In the central area of Athens there were detected in a total number of 270 soil samples, 44 positive samples which gave a rate of 16.3%. In the same area blood samples from pregnant women were gathered and an ELISA assay was performed to determine levels of specific *Toxocara* IgG antibodies. From the 25 blood samples that were collected, the authors had four positives which is a rate of 16%.

In the west Attica area, in a total number of 390 soil samples that were tested, 89 were found positive, which gave a rate of 22.8%. The blood samples from pregnant women from the same area gave a result of ten positives in a total of 40 blood samples. This is a 25% rate of seropositive samples tested for specific *Toxocara* IgG antibodies.

In the North suburbs area the authors examined 510 soil samples which resulted in 89 positives, which gave a rate of 17.45%. The total number of blood samples from pregnant women that were tested for specific *Toxocara* IgG antibodies was 20 and which resulted in five positive results, giving a rate of 10%.

In the Southern suburbs area the authors were able to examine 90 soil samples with a result of 11 positive, which gave a rate of 12.2%. From the blood samples from pregnant women of the Southern suburbs area the authors had one seropositive sample for specific *Toxocara* IgG antibodies in a total of ten tested samples, giving a rate of 10%.

In the Piraeus area, in a total number of 150 soil samples, the authors had 15 positive results, giving a rate of 10%. The positive blood samples from this area were three in a total of 30 blood samples, resulting in a 10% rate.

Finally in the Elefsina area, in a total number of 100 soil samples that the authors tested, ten were found positive

Table 1. — Prevalence of *Toxocara canis* in the area of Athens

Area	Soil samples	Positive soil samples	Rate	Total number of women	Positive samples from women	Rate
Central Attika	270	44	16.3%	25	4	16%
West Attika	390	89	22.8%	40	10	25%
Northern Suburbs	510	89	17.4%	20	5	10%
Southern Suburbs	90	11	12.2%	10	1	10%
Piraeus	150	15	10%	30	3	10%
Elefsis	100	10	10%	9	1	11.5%
Total	1510	258	17.08%	134	23	17.16%

with a rate of 10%. The blood samples collected from this area were nine and from them one was positive for specific *Toxocara* IgG antibodies, giving a rate of 11.5%.

Discussion

Bibliographical research reveals evidence about variation in contamination by the *Toxocaracanis* throughout the world and thus Perth (Australia) has contamination rates close to zero [5] and Prague (Czech Republic) has high contamination rates in the ground [6].

In the area of Thessaloniki the rate was 35.5 % in winter of 1992 [7]. The importance of this result is high considering the fact that each study on the sample data differs according to the use of each investigated place (i.e. public parks against children's playgrounds).

The fact that the proportion between contaminated places and non-contaminated places by *Toxocara* varies across the various parts of the country is due to striking differences between urban, semi-urban and rural areas (differences in the build and natural environment, i.e. streets, number of straw dogs etc). Despite that and according to the work of Habluetzel et al., lack of statistical significance in the results does not produce reliable statistical conclusions [8].

In the present study, particularly within the Athens & Attica wider district, the frequency rate of positive soil samples does not vary significantly across the various places of the same area.

In our own study the overall frequency rate of soil contamination throughout Attica was found to be 16.43%. Statistical differences between the various areas of Attica are not significant. It is worth noting that microenvironmental conditions are more or less equal across the various areas examined.

With regard to the seropositive blood samples collected, it was considered that those people came in contact with the roundworm only in the places where soil samples had been collected because

- all surveyees who might have potentially come in contact with the *Toxocara canis* roundworm anywhere else other than the aforementioned Attica areas (Table 1) were excluded from the data elaboration and
- the elaborated data belonged to people who were not dog owners.

The seropositivity rates did not vary significantly across the different places of the same area. Bibliographical research reveals that according to the study of Pavlinova et al., 5.5% in pregnant women blood samples collected were *Toxocara* seropositive (*Toxocara*-IgG ELISA) [9]. In Brazil, antibodies against *Toxocara canis* antigens were determined in 51.6% of the children's blood samples, while there were no significant differences observed between positive serology and the presence or absence of intestinal worms [10]. However, the ELISA test carried out in Romania by Cojocariu et al. was 100 % positive in blood samples of people who showed a variety of symptoms associated with toxocariasis (diagnosis was facilitated through *Toxocara* larvae detection in biopsy) [11].

Preventive measures

As a result of the survey carried out some preventive measures are suggested:

- Care should be taken to deworm household pets and treat their illnesses
- Pet owners should regularly visit veterinarians to get informed on how to administer pet medications
- Local government welfare programs for preventing illnesses or treating ill straw dogs should be implemented. Municipal authorities and voluntary organizations should ensure that stray animal's feces are picked up from public spaces whereas dog owners should collect themselves domestic animal waste.

Particular care should be taken to prevent dogs' access to public spaces where regular human activities take place (children's playgrounds, outdoor kindergardens, etc). This can be obtained using fences or railings, or, where possible, guards to prevent animals from moving among people.

Visitors (adults, children) and staff working in those places should be regularly informed on maintaining proper hygiene rules (i.e. not eating food before washing hands, not placing bread on places likely to be contaminated such as benches, ground etc).

It goes without saying that children's playgrounds, public parks and school yards should be properly fenced with railings or chain link fencing so that animals are prevented from accessing them.

At beaches animals should not circulate among people.

Physicians should be trained to show a higher degree of sensitivity to the *Toxocara canis* roundworm and consequent diseases (particularly the Ocular larva Migrans) in order to establish an appropriate differential diagnosis. Similar surveys on compliance with the standards set by the National School of Public Health laboratory and using its experience are already being carried out in Greece with successful results.

In conclusion in a Greek population of pregnant women the prevalence of *Toxocara canis* (infection) was found (23 positive blood samples) in a total number of 134 samples, rate 17.16%. This due to the soil contamination as in 1510 soil samples 258 positive samples were found giving a rate: 17.08%

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