Soil contamination by *Toxocara canis* and human seroprevalence in the Attica region, Greece

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Abstract

Background *Toxocara canis* is one of the most widespread public health and economically important zoonotic parasitic infections humans share with canids, mainly dogs. Human infection occurs by the accidental ingestion of embryonated eggs or larvae from a range of wild and domestic paratenic hosts. The aim of the present study was to examine the soil contamination of public places by the parasitic ova and to estimate serologically the prevalence of *T. canis* human infection in the Attica region, Greece.

Methods In this region, public areas are permanently inhabited by dogs, mostly stray dog population that is hardly kept down to a manageable level. A total of 1,510 soil samples were collected from 33 public places of six regional units of Attica from March 2014 to April 2014 and ova were detected using a microscopic assay. In addition, sera were collected from 250 residents, routinely active in the sampled areas, and tested for *T. canis* IgG antibodies using an enzyme immunoassay.

Results *T. canis* eggs were isolated from 31 (94%) of the examined public areas. Of the total samples, *T. canis* ova were recovered from 258 samples, suggesting an overall *T. canis* ova contamination of 17.2%. The areas of higher socioeconomic status presented lower percentages of soil contamination in a statistically significant level, compared to the areas of lower socioeconomic status. *T. canis* IgG seropositivity was detected in 40 (16%) serum samples. Similar rates were established among *T. canis* seropositivity and soil contamination within the same geographical areas. The proportion of seropositive samples in the group of children was significantly higher compared to the proportion of adults (48% versus 8%, $p < 0.001$).

Conclusion The level of environmental *T. canis* contamination as well as human infection found in the Attica region calls for a greater awareness towards this public issue. Preventing measures should be implemented to control the spread of this parasitic infection.

Keywords *Toxocara canis*, toxocariasis, soil contamination, *Toxocara* ova, seroprevalence, Greece

Introduction

Toxocariasis is a human infection caused by larvae (immature worms) of either *Toxocara canis* or *Toxocara cati*. Humans normally become

infected by ingestion of embryonated eggs (each containing a fully developed larva, L2) from contaminated sources (soil, fresh or unwashed vegetables, or improperly cooked paratenic hosts).¹ Three syndromes are commonly recognized: visceral larva migrants, ocular larva

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migrants and covert toxocariasis.² Covert toxocariasis is the most common form of the disease and is frequently asymptomatic or can cause mild symptoms such as headache, cough, fever, and wheezing. Consequently, many *Toxocara* infections remain underdiagnosed and underappreciated.

According to several studies worldwide, *T. canis* human infection rates are linked mostly with soil contamination with infectious stages of the parasite.³ Due to the growth in the number of animals kept as domestic pets, public areas of urban recreation, such as parks, playgrounds and squares have become serious risk factors for the transmission of such zoonotic parasites to humans.^{4,5}

In Greece, apart from the increased number of dog owners, a significant raise in the number of stray dogs that freely circulate in public places has become an important issue in recent years, constituting a significant threat to the spread of parasitic infections to humans, mainly in urban areas. However, only limited number of Greek studies report data on either human or animal *Toxocara* spp. infection, mostly referring to rural Greek areas.⁶ Furthermore, no studies exist reporting data with reference to soil contamination by *Toxocara* spp. in any part of Greece.

The aim of the present study was to investigate the presence of *T. canis* ova in soils of public areas of a broader region of Athens, the capital and most populated city of Greece, as well as to estimate the seroprevalence of human toxocariasis in healthy residents.

Methods

Collection sites and sampling design

From March 2014 to April 2014, soil samples were collected from 33 most popular public places of six large regions throughout the total area of the Attica region: (A) Central Athens area, (B) West Attica area (C) North suburbs area (D) Southern suburbs area (E) Piraeus area (F) Elefsina area (Figure 1). Those areas differ in terms of: 1) social status of the inhabitants and 2) 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 to soil such as gardeners, land workers, etc.) or hobbies related to plants, flowers or herb collection.

Figure 1. Demonstration of the examined areas throughout Attica region

The number of soil samples taken from each place was calculated on the basis of the selected areas' dimensions (Table 1). Sample takers carried out a rough calculation of the place dimensions by measuring its diagonal with steps (one step = approximately one meter). Places where the ground was covered by gravel, cement or asphalt-based materials were excluded from the sampling procedure.

Table 1. The estimated number of the soil samples taken, according to the size of the examined areas

Size of area (m ²)	Place size	No. of samples
50-150	Small	4
200-100	Medium	8
250-350	Large	12

With a small garden shovel marked down with color 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.^{7,8} Where sample-takers noted that the soil was less dense, they dug below 3 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).

Soil processing for isolation and identification of *Toxocara* ova

The ova detection in soil samples was based on the technique proposed by Kazacos.⁹ In this method a saturated solution of NaNO₃ was used as flotation medium. About 30 g of carefully mixed soil aliquot was placed into a 50 mL conical centrifuge tube and 30 mL of 0.5 percent Tween 40 solution was added. The whole mixture was shaken thoroughly for 4 min and sieved through a nylon sieve of 1 mm² pore size. The filtrate was later centrifuged at 1500 rpm (327×g) for 3 min and the supernatant was discarded. Afterwards the sediment was washed twice with distilled water. After centrifugation, the supernatant was discarded and the sediment was suspended in 30 mL of saturated NaNO₃ solution (sp.gr-1.35) and centrifuged for 3 min at 1500 rpm (327×g).

All samples were examined microscopically using ×10 and ×40 magnification lenses for parasitic ova. Two laboratory clinicians examined in parallel and independently the samples. When recognition of *T. canis* or *T. cati* eggs was impossible they were classified to *Toxocara* genus.

The criteria used for recognition of the ova were the following: 1) oval or round shape; 2) cortex (thickness); 3) color (light-colored shell compared with the egg interior); 4) existence of pronymph; 5) size between 65-90 µm (measured by the microscope graded scale). A sample with at least a single egg detected was considered positive. Discrimination between *T. canis* and *T. cati* ova by microscopic examination was based on the ova sizes (85 by 75 µm and 75 by 65 µm, respectively) verified by an ocular micrometer, the shape, color and the wall thickness. *T. canis* eggs are thick-walled and have oval or spherical shapes with pits on the surface bigger than those observed on the eggs of *T. cati*.

Residents' healthcare records

Blood samples were collected from residents routinely visiting the examined areas for at least a decade, selected while they were undergoing specific activities such as playing soccer, collection of plants, herbs, etc. from the sampled places. The selection of residents and the collection of blood samples were carried out in parallel with soil investigation for *Toxocara* ova. All residents that were present at the time of soil sampling and were appropriate for the study, were asked to participate. Twenty-three of them refused to participate. Sera were collected from 200 adults (104 males, 96 females, from 16 to 56 years) and 50 children <14 years (32 males, 18 females) present at all examined areas throughout Attica during the soil sampling. Each participant signed an informed consent form and filled an anonymous questionnaire including the following data: A) date of birth, sex, age, place of residence, profession, and education level; B) evidence of fever, cough, wheezing, hepatomegaly, and splenomegaly; C) personal hygiene habits that may raise the likeliness of infection by *Toxocara* parasite such as: 1) not washing hands before meals; 2) indirect geophagia e.g., when cleaning herbs, vegetables, etc.; 3) presence of dogs in the house or in the backyard/front yard. Blood samples for serology were collected after appointment and the serum was stored at -20°C until the day of the test.

Serological testing procedure

Serum samples were treated following the instructions of RIDASCREEN® *Toxocara* IgG test (r-Biopharm, Darmstadt, Germany), which is an enzyme immunoassay for the qualitative determination of IgG antibodies against *T. canis* in human serum. The test is performed through the binding of serum antibodies to purified antigens coated to a micro-well plate. The result is obtained by dividing the absorption value of the sample by the cut-off value. According to the procedure, ratios are accepted as negative (<0.9), equivocal (0.9-1.1) and positive (>1.1). The specificity of the test is considered 98.4% and the sensitivity 100%.

Statistical analysis

Categorical variables were compared between groups using the Chi-square test. Continuous parametric variables were compared between groups using the T-test. A p value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Soil contamination

Based on the dimensions of the 33 public areas, a total of 1,510 soil samples were collected. According to our results, all 6 regional areas of Attica were contaminated with *T. canis* ova. More specifically, *T. canis* eggs were isolated from 31 out of the 33 examined public areas (94%). Of the total 1,510 examined samples, *T. canis* ova were recovered from 258 samples, suggesting an overall *T. canis* ova contamination percentage of 17.2%. Sixteen samples contained *Toxocara* eggs that none of the laboratory clinicians could confidently classify in species, therefore they were excluded from the positive *T. canis* samples.

Statistically significant differences in soil contamination rates were established between the six regional areas of Attica. The highest contamination rate was observed in West Attica area (22.8%) and the lowest in Piraeus area (10%) (Table 2). In particular, a difference in contamination rates was established among Piraeus area and North Athens area ($p=0.055$), West Attica and Piraeus area ($p=0.004$), and among West Attica area and Elefsina area ($p=0.005$). No differences in contamination rates were established among West Attica, Central Athens and North Athens areas. Similarly, no differences in contamination rates were established among South Athens, Piraeus and Elefsina areas.

No correlation could be made between dogs' population visiting the examined areas and the *Toxocara* ova soil contamination since the presence of dogs at the sampling sites was infrequent during the study conduct.

Toxocara seroprevalence

The epidemiological characteristics of the participants are presented in Table 3. The average age was 19.9 ± 5.8 years in the *T. canis* IgG seropositive group and 47.8 ± 5.4 years in the *T. canis* IgG seronegative group ($t\text{-value}=22.549$, $df=248$, $p<0.001$).

Positive *T. canis* antibody titers were found in 40/250 (16%) of serum samples in total. The proportion of seropositive samples was 48% (24 out of 50) in the group of children and 8% (16 out of 200) among adults ($p<0.001$). Differences in rates of *T. canis* antibody elevated IgG titers were observed between the various areas of Attica, although not in a statistically significant level. The highest *T. canis* seropositivity rate was observed in West Attica area (25%) and the lowest in Piraeus area (9.4%) (Table 2). Within the same geographical areas, no statistically significant differences were established between percentages of *T. canis* seropositivity and soil contamination rates (Table 2).

Discussion

Soil transmitted and zoonotic helminths, such as *Toxocara* spp., are still part of the most important health issues in the world, mainly in developing countries.¹⁰ In several cities, there are countless dogs walking freely in public places and it is very common that, stimulated by their owners, they defecate in these places contaminating the soil with several types of parasites with zoonotic importance. A consequence of this phenomenon is the widespread contamination of the public areas with the eggs of *T. canis* originating from defecation of dogs. Several studies regarding soil sampling from different parts of the world have a wide variation in contamination rates by *Toxocara* ova in public areas.¹¹ One of the main reasons *T. canis* prevalence reports vary significantly across the various parts of the world is the striking difference between urban, semi-urban and rural areas. Moreover, the lack of standardization of methods by means of the number and volume of samples, depth of sampling, season of examination, method of egg recovery, preservation of samples and laboratory skills,¹²

Table 2. Distribution of *T. canis* contaminated soil samples and residents' *T. canis* IgG seropositivity among the tested geographical Attica regions

Region	<i>T. canis</i> (+) soil samples	<i>T. canis</i> IgG seropositivity	Chi-square value (df=1)	P-value
Central Athens	16.3% (44/270)	16% (8/50)	0.003	0.998
West Attica	22.8% (89/390)	25% (20/80)	0.177	0.674
North Athens	17.4% (89/510)	10% (5/50)	1.824	0.176
South Athens	12.2% (11/90)	10% (2/20)	0.078	0.780
Piraeus Area	10% (15/150)	9.4% (3/32)	0.012	0.922
Elefsina Area	12% (12/100)	11.1% (2/18)	0.012	0.922
Total	17.2% (260/1510)	16% (40/250)	0.120	0.728

Table 3. Epidemiological characteristics among *T. canis* IgG seropositive and seronegative residents

Residents' characteristics	<i>T. canis</i> IgG seropositive (n=40)	<i>T. canis</i> IgG seronegative (n=210)	Chi-square value (df=1)	P-value
Sex (male/female)	23/17	113/97	0.184	0.667
Fever	1	2	0.693	0.405
Cough	2	4	1.374	0.241
Wheezing	3	4	3.865	0.049
Hepatomegaly	0	1	1.734	0.187
Splenomegaly	0	2	0.679	0.410
Washing hands before meals	14	65	0.255	0.613
Indirect geophagia	7	43	0.186	0.666
Dog ownership	23	78	5.783	0.016

hamper the comparison of findings of different studies,¹³ and the evaluation of their impact to public health.

In Greece, the environmental contamination by *Toxocara* spp. is practically unknown. This is the first Greek study providing data regarding soil contamination by *T. canis*. The analysis of our sampled areas confirmed a considerably high *T. canis* contamination level, revealing *T. canis* positive soil samples in almost all the examined public regions in Attica except for two (94%). However, the percentage of positive soil samples was 17.21%, lower than the rates reported by most other European countries. More specifically, *Toxocara* spp. prevalence in sandpit samples from Toulouse, France was estimated at 38%, 50% in the Marche region of Italy,¹⁴ 5.45% in Prague, Czech Republic,¹⁵ and research also suggests that the prevalence of the disease in North America is

significant.¹⁶ Our results are similar to those from other European cities and countries such as Lisbon, Portugal, where the rate of *T. canis* contaminated fecal samples collected from public sandpits and public parks was 16%,¹⁷ Poland where reports demonstrate soil contamination percentages of 16%,¹⁸ and Madrid, Spain reporting *Toxocara* spp. as the most frequent parasite species found in the examined soil samples (16.4%).¹⁹

The microenvironmental conditions were relatively similar throughout the various areas examined, whereas the type of Attica soils is predominantly clay. Moreover, all soil samples were collected during a short time-period (two-month) in order to avoid considerable weather changes.

The method implemented was that of flotation in a saturated and hypertonic solution

of NaNO₃ (sodium nitrate) with specific gravity 1.35. This technique is considered the most suitable for the detection of ova of various roundworms. Furthermore, the use of saturated NaNO₃ is now efficient for detecting *Toxocara* ova in the soil with a success rate of 65% to 82.5%.¹² We have chosen to apply this method, even though a number of previous studies have used molecular techniques to diagnose *Toxocara* infections, mainly to analyze genetic variations. However, the gold standard to diagnose infections caused by protozoa or helminths remains the microscopic or macroscopic detection of the parasite, particularly in epidemiological studies, similar to ours.

Statistically significant differences in soil contamination rates were established between the six areas of Attica. These areas differ in terms of population density and socioeconomic status of the inhabitants. The areas of higher socioeconomic status presented lower percentages of soil contamination in a statistically significant level, compared to the areas of lower socioeconomic status. Previous reports have also shown that toxocariasis mainly affects socioeconomically disadvantaged populations.²⁰

Finally, our serological findings indicate that human *T. canis* infection actually occurs in the Attica Region. A proportion of 16% of examined sera samples had elevated *T. canis* IgG antibodies, suggesting a contact with the larva migrans of the nematode. We also demonstrated a major *Toxocara* IgG seroprevalence in children. Indeed, children are widely considered to be particularly vulnerable for acquiring infection, since they are likely to be more exposed than adults in contaminated environments²¹ by playing and putting their fingers in their mouths, either deliberately or by accident. Prevalence of toxocariasis in children ranges from very low to over 50%²²⁻²⁴ in studies from different parts of the world and is generally considered to be underestimated.

An interesting finding is that similar percentages were established among *T. canis* seropositivity and soil contamination within the same geographical areas. High human seroprevalence has been indicated in areas with

documented soil contamination, while the risk for transmission may be increased in proportion analogous to the degree of environmental contamination.

Conclusion

The level of environmental *T. canis* contamination found in the Attica region calls for a greater awareness towards this public issue and the implementation of specific preventive measures, such as maintaining dogs free of worms by regular administration of anthelmintics, removal of dog feces from the environment before the eggs are able to embryonate and good personal hygiene. Also, a program to control the stray dog population should be implemented and may include the improvement of health and welfare of the stray dog population, the reduction of stray dogs' numbers to an acceptable level and the minimization of zoonotic diseases risk.

Authors' contributions statement: RHR performed the laboratory experiments, collected the data and performed the statistical analysis. NAL collected the data, interpreted the findings and drafted the manuscript. AAE designed the study, supervised the laboratory experiments and drafted the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to disclose.

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