CONTAMINATION WITH TOXOCARA SPP. EGGS OF ENVIRONMENTAL SAMPLES OF PUBLIC PLACES OF VARNA CITY, BULGARIA

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ABSTRACT:
Toxocariasis in humans is an unknown and under-diagnosed disease with increased risk of distribution determined by the large canine and cat populations. Contamination of the environment with pets feces containing helminth eggs is the key route of transmission toxocariasis, threatening especially the youngest children.

The purpose of this study is preliminary research of the degree of pollution of the environment with Toxocara spp. eggs by examining soil and sand samples from different public places of Varna, Bulgaria.

Material and methods: In 2018, 40 soil and sand samples were collected from various locations of Varna city: public park areas, areas for dog-walking, playgrounds, sandboxes and yards with domestic animals. The samples were processed using modified Romanenko’s method.

Results: Soil analysis showed Toxocara spp. eggs in one sample from a public park area in the central region of Varna. In another soil sample of a private yard with domestic dog typical ova of the Taeniidae family was observed (possibly of E. granulosus). In two other samples, eggs from other canine parasites were discovered.

Conclusion: The first research of the environmental contamination with helminthic eggs in Northeastern Bulgaria showed Toxocara spp. eggs in one of the central and most populated area of Varna city. Furthermore, at least 10% of the tested materials were contaminated with canine excrements that testify for the lack of proper control of domestic and stray animals, which represents a real and imminent danger from zoonotic infection for the public health in the region.

Keywords: Toxocara, Toxocariasis, Visceral Larva Migrants, Environmental Exposure, Public Health

INTRODUCTION:
Toxocara canis and Toxocara cati are roundworms from genus Toxocara - one of the most common parasites of dogs and cats, respectively [1, 2]. Toxocariasis in humans is a result of the migration of the Toxocara larvae in different tissues and internal organs of the organism. The clinical forms include asymptomatic or subclinical course with mild and unspecific symptoms known as covert toxocariasis and the clinically significant involvement of the lungs, liver and local or systemic allergic reactions – visceral toxocariasis or visceral larva migrans. The involvement of the retina and other ocular structures (ocular toxocariasis) may lead to blindness and the migration of the larvae through the brain, and spinal cord (neurotoxocariasis) causes severe neurological complications - meningitis, encephalitis, cerebral vasculitis and epilepsy [1, 3-6].

In Bulgaria, the increased risk for the humans of this zoonotic infection is determined by the large numbers of dogs and cats, especially the stray ones [4, 7]. In more than 20 years the “stray dogs population” is a widespread and overwhelming problem of our country that remains unresolved and continues to exist, unabated with all of its consequences [7]. Furthermore, no official data exist regarding the population of the domestic or feral cats.

Toxocariasis in humans is caused by occasional ingestion of Toxocara ova from the environment [4, 6, 8]. In the European cities, public places, including parks, playgrounds and sandboxes are the major areas of exposure to the helminthic eggs, because those areas serve as common places for pets walking and usual habitat of stray dogs and cats [9, 10]. Children playing in these areas are at higher risk of exposure due to their improper personal hygiene [11]. Recent studies show widespread contamination of urban public places with eggs of Toxocara spp. in different parts of the world [8, 9, 12, 13]. In Europe the prevalence of such positive finding varies from 13.0% to 87.0%, in US – from 4.4 to 20.6%, in Asia – from 6.6% to 63.3% and in Latin America from 17.4 to 60.3% [10, 13–16]. Uncontrolled and untreated definitive hosts lead to a widespread contamination of the environment that, in warm and humid climates, facilitates the survival of Toxocara spp. eggs [3, 5, 17, 18].

The purpose of the current study is to perform a preliminary investigation of the environmental contamination with Toxocara spp. eggs of soil and sand samples of several public places of Varna city, Bulgaria.
MATERIALS AND METHODS:

In February-March 2018, 40 environmental samples (n = 34 soil and n = 6 sand) were taken from various locations of Varna city with an increased risk of infestation with helminthic eggs (Figure 1). The sampling sites were assigned and categorized in the following groups: public park green areas (n=11), specified public areas for dogs (n=4), public playgrounds (n=7), separately sandboxes of playgrounds, kindergartens and schools (n=6), as well as private house yards with dogs, cats and other domestic animals (n=12).

The soil and sand samples were gathered in an initial volume of 200g from the different spots of superficial ground layer (1-3 cm) of the assigned places. The samples were placed in polyethylene bags with individual labels containing information about the place, date and environmental conditions. The samples were stored in room temperature and analyzed within a week after collection. The samples were investigated using modified Romanenko’s centrifugation and flotation technique [4]. After thorough mixing 25 g of the initial soil/sand samples were measured and processed by adding an equal quantity of 3% NaOH. After continuous homogenization for 20-30’ min the samples were sieved, centrifuged and then washed several times depending on the debris. The final stage of flotation was performed with a concentrated solution of NaNO3 in 20 ml plastic vials for 20-30 min, and the superficial layer was examined with light microscopy.

RESULTS AND DISCUSSION:

Soil analysis showed helminth ova or larvae in 45% (n=18) of the tested sampling sites, and 55% of the materials remained negative (Figure 1). In one (2.5%) of the positive samples from a public park area in the central region of Varna (Fig.1, sampling site #3) Toxocara spp. eggs were discovered. Their specific morphology includes spherical shape, thick dark shell and serrated surface. In a soil sample (2.5%) of a private yard with domestic dog (Fig.1, sampling site #9) typical Cestode eggs were observed – small, oval with radially striated thick shell and central oncosphere. Although the ova of all Taeniidae family are with the same morphology, we suspect that this is probably Echinococcus granulosus egg – another canine parasite with great clinical significance in Bulgaria. A soil sample (2.5%) of an enclosed public area for dog walking (Fig.1, sampling site #15) showed eggs with the characteristic lemon shape of Trichocephalus spp. Since human trichuriasis in Varna city was not diagnosed as endemic in the last 10 years [19], we assume that these are eggs of some of the canine whipworms - probably Trichuris vulpis. In one of the sand samples (2.5%) from a playground of a central city, area elongated eggs with thin shell, and several blastomeres were discovered. Due to their morphological identity, we couldn’t differentiate if this is Ancylostoma or Strongyloides spp. egg but either genus may represent a treat for human health. In 11 soil (27.5%) and two sand (5.0%) samples, a wide variety of nematode larvae were observed. They have not been defined by species because they have a limited clinical significance for humans and are not relevant to this study.

Fig. 1. Varna city map with sampling sites and positive findings.
★ - sampling sites with canine parasites, ● - sampling sites with Nematoda larvae.
This is the first study researching the environmental contamination with helminthic eggs in Northeastern Bulgaria, and the as a pilot enquiry was focused on several high-risk places of Varna city. Even in these small numbers of samples in our preliminary research, we were able to detect *Toxocara* spp. eggs in a public park area of one of the most populated central part of the city. Furthermore, in the other 3 sampling sites ova of canine parasites were discovered which shows that at least 10% of the tested materials are contaminated with canine excrements. These results testify for the lack of proper control of domestic and stray animals in the city area and represent a real and imminent danger from zoonotic infection for the public health of the human population of the region.

Additional sampling of soil and other environmental materials from small villages and countryside areas of the Varna District would be necessary to determine the real threat of toxocariosis and other helminths because most of the studies in the European countries display the even higher presence of *Toxocara* spp. eggs in the rural areas [6, 8, 13, 16, 18]. At the same time, the specific regional bioclimatic factors of the Black Sea coast – warm winter and humid summer, are favorable for the maturation, survival and longevity of *Toxocara* eggs in the soil [9, 11]. In a similar enquiry from Stara Zagora region (South Bulgaria), significantly higher levels of contamination are reported - *Toxocara* eggs in 56.76% of the studied soil and sand samples, probably associated with difference in sampling sites, advantageous climate or socio-economic conditions [20]. Additional and consecutive research in Northeastern Bulgaria would be essential to reveal the actual levels of contamination with *Toxocara* eggs of the environment and would expose the major determinants responsible for the detected discrepancies in the different parts of our country.

**CONCLUSIONS:**

The ova of all soil transmitted Nematoda (including *Toxocara* spp.) and other helminths (incl. *E. granulosus*) are resistant to the exterior conditions and remain infective in the soil for years. There are no methods for reducing the contamination from the environment, so the only practical approach requires prevention of the initial pollution [2, 8]. This can include measures for and even mandatory removing of the excrements of the companion animals by the owners; regular anthelmintic treatment of domestic cats and dogs, especially puppies; active improvement of the health education of pet owners by the veterinarians; preventing access of cats and dogs to public areas (children’s playgrounds, schools etc.). Several measures in Varna city in this direction are already applied by the authorities like the establishment of specified enclosed areas for dog-walking, fencing of playgrounds and reduction of sand-boxes. In addition, one of the major control measures of the municipalities should include limiting the population of the stray dogs and feral cats.

Such multidisciplinary and integrated approach would require collaboration between physicians, veterinarians, pet owners, health and public authorities to limit the distribution of toxocariasis, echinococcosis and other zoonotic helminthiases that present a significant and in the case of toxocariasis unknown and underdiagnosed threat for the public health.

**Conflict of Interest:**

No conflict of interest to declare.

**REFERENCES:**


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