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Testing sand from selected playgrounds in Romania, Hungary, Slovakia and Poland for eggs of nematodes at the time of massive migration of people from Ukraine to these countries – a pilot study

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ABSTRACT

Nematodes are invertebrates that inhabit the water and soil environment. Numerous nematodes are parasites of plants, animals and humans, especially children. Among the parasitic diseases of the gastrointestinal tract, diseases caused by nematodes are very common: eneterobiasis, ascariasis, and toxocarosis. From February 2022, we observe an intensive migration of people from Ukraine to the European Union countries: mainly to Poland (4.5 million) and to a lesser extent to Slovakia, Hungary and Romania. They are mostly women with children. In places where they find shelter, they try to provide children with the best possible conditions for their development. Hence the authors' interest in hygienic conditions in the playgrounds of the European Union countries bordering Ukraine.

The aim of the study was to examine samples from selected playgrounds in Romania, Hungary, Slovakia and Poland for eggs of nematodes *Ascaris lumbricoides*, *Toxocara* sp., and *Trichuris trichiura*.

Twenty samples of sand and soil obtained from 4 playgrounds in Craiova (Romania), Miskolc (Hungary), Košice (Slovakia) and Lublin (Poland). In order to detect the presence of *Toxocara canis/cati*, *Ascaris lumbricoides* and *Trichiuris trichiura* eggs, a modified flotation method was used. No eggs of parasites were found in the examined sand samples. The pilot study suggests that refugees can safely play in the playgrounds in Romania, Hungary, Slovakia and Poland.

INTRODUCTION

Nematodes are invertebrates that inhabit the water and soil environment. Numerous nematodes are parasites of plants [1], animals [2] and people [3], especially children. Parasitic diseases are suspected in the case of abdominal pain, abnormal bowel movement, lack of weight gain in children, sometimes coughing, skin rashes, and hyperactivity and dark circles under the eyes. Gastrointestinal parasitic infections are common all over the world [4]. It is estimated that in Poland they concern 10% of the population [3].

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Prevention of parasitic diseases of the digestive tract consists in maintaining hygiene, ensuring good sanitary conditions, thorough washing of hands after playing in sandboxes and playgrounds, after contact with cats and dogs, washing fruit and vegetables before eating, avoiding raw and semi-raw meat, long freezing/cooking meat, prohibiting the fertilization of the soil with human excrement and emptying septic tanks onto farmland [3].

Among the parasitic diseases of the gastrointestinal tract, diseases caused by nematodes are very common, and include: enterobiasis, ascariasis, toxocarosis [3].

Dogs and cats play an important role in the spread of parasitic diseases caused by nematodes. They shed *Toxocara*

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canis/*cati* eggs in their feces (Fig. 1), which become invasive after staying in the soil [4]. In turn, the eggs of the *Trichiuris trichiura* and Ascaris *lumbricoides* are excreted by humans with feces. The eggs also need to be incubated in moist soil (hence the term 'geohelminths'). They develop invasiveness in soil after 17 days at 30 °C to 21 days at 25 °C [4]. A person becomes infected with *Trichiuris trichiura* or *Ascaris lumbricoides* by eating unwashed fruit and vegetables contaminated with human feces or through self-contamination through contact with fouled soil. Young children are most at risk of being infected with these parasites due to games in playgrounds in sandboxes, in parks and gardens, as well as the tendency to put everything in their mouths. Parks and playgrounds are democratic – they are designed for everyone. According to landscape architects, there should be a green area/park/playground for recreation for the urban population for every 400 flats.

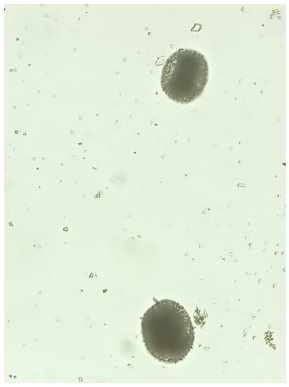


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Microscope Eclipse E200, Nikon, magnification 100×

Figure 1. Immature *Toxocara canis* eggs obtained from a female parasite in the laboratory

From February 2022, we observe an intensive migration of people from Ukraine to the European Union countries: mainly to Poland (4.5 million) and to a lesser extent to Slovakia, Hungary and Romania. The refugees are mostly women with children. In places where they find shelter, they try to provide children with the best possible conditions for their development. Hence the authors' interest in hygienic conditions in the playgrounds of the European Union countries bordering Ukraine.

AIM

The aim of the pilot study was to examine samples from selected playgrounds in Romania, Hungary, Slovakia and Poland for eggs of nematodes *Ascaris, Toxocara sp*., and *Trichuris*.

METHOD

Twenty samples of sand and soil obtained from 4 playgrounds in Craiova (Romania), Miskolc (Hungary), *Košice* (Slovakia) and Lublin (Poland) were obtained (Tab.1).

Table 1. The playgrounds selected for testing

No	Address	Link to google maps	Fence	Neighborhood	Samples	Date
1	George Enescu, Craiova, Romania	https://goo.gl/maps/N54G4pLVZXBXLooN7	Yes	Playground among blocks of flats	1-5	9 June 2022
2	Bajcsy-Zsilinszky utca; Selyemret, Miskolc, Hungary	https://goo.gl/maps/KLSQsFNAcmda3fsG8	Yes	Playground among blocks of flats	6-10	10 June 2022
3	Osloboditel'ov 666/15, Košice, Slovakia	https://goo.gl/maps/SexbGgF4GzfkBr6d8	No	In the park	11-15	10 June 2022
4	Piłsudkiego 9-27, 20-011 Lublin, Poland	https://goo.gl/maps/YTrJvjkZvVr2Yysx7	No	Park Ludowy - revitalized in 2020; playgraoud for children under 7 years of age; by the river Bystrzyca; 300 m from the dormitory for refugees	16-20	04 May 2022

In order to detect the presence of *Toxocara sp*., *Ascaris* and *Trichiuris* eggs, a modified flotation method was used. These tests are based on the Polish Standard and PN-Z-19000-4/2001. Sand samples were obtained from the top layer of the sand/soil, to a depth of 2-3 cm of the sandbox using a clean spoon. The envelope method was used – sampling from five places (one from each corner and one from the central area). The mass of a single sample was at least 100 g. The samples collected in this way were poured into clean plastic containers. The samples were collected in May and June 2022. The tests were performed at the Independent Parasitology Laboratory of the Military Institute of Hygiene and Epidemiology, Kozielska 4 Street, 01-163 Warsaw, Poland in June 2022.

Characteristics of the cities where the samples were collected:

1. Craiova – a city in Romania – 81 km^2, 298000 inhabitants,
2. Miskolc – a city in Hungary – 236 km^2, 159000 inhabitants,
3. Košice – a city in Slovakia – 243 km^2, 238000 inhabitants,
4. Lublin – a city in Poland – 147 km^2, 337000 inhabitants [5].

RESULTS AND DISCUSSION

In the samples from Romania, free-living nematodes were detected (Fig. 2), but no eggs of *Toxocara* sp., *Ascaris* and *Trichiuris*. In the samples from Hungary, Slovakia and Poland, no eggs of *Ascaris*, *Toxocara* sp., and *Trichuris* were identified. The authors plan to extend the study to the cities where the largest number of refugees stay.

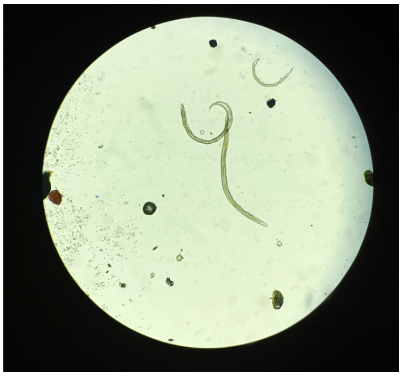


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Microscope Eclipse E200, Nikon

Figure 2. Free-living nematodes in sample 1 from Romania

The confirmation of infection with *Ascaris lumbricoides* is finding its eggs in the human stool. The study is difficult as there is a possibility of confusing them with pollen from plants. Serological tests are of little importance. The diagnosis of ascariasis is confirmed by finding the expelled adult form of the nematode in the feces. It is helpful to find slight leukocytosis and eosinophilia in the blood samples [3]. As part of toxocarosis diagnostics, an IgG ELISA test and/or a Western blot with the *Toxocara sp*. antigen are performed [3]. The diagnosis of the infection with *Trichiuris trichiura* is based on the detection of the eggs of this parasite in microscopic examination of the feces of a person suspected of being infected [4].

Testing feces of dogs and cats for *Toxocara sp*. eggs, and testing of the sand from playgrounds for *Ascaris*, *Toxocara sp*., and *Trichuris* eggs allows estimating the risk of contamination of children using these recreational devices. According to Borecka, in 2005, 22% of all lawns and 31.6% of all sandboxes in Lublin were contaminated with the eggs of *Toxocara sp*.[6]. More and more frequently, the results of research on fruit and vegetables for egg contamination of *Ascaris lumbricoides* and *Trichuris trichiura* [7] have been published. Wherever human manure is used to fertilize crops, positive results are reported.

Before the conflict from February 2022, Ukraine had much in common with Russia, at least in the healthcare aspect. According to Lukashev *et al*., toxocarosis incidence in Russia is low (1.8 cases per 100,000 population). The parasitic diseases are not a major healthcare concern. However, in tests of primary hosts, 3-100% of dogs were infected with *Toxocara canis* in different regions of the country (on average 33%), and between 6% and 52% cats were positive for *Toxocara cati*. Levels of contamination of soil, children's playgrounds and sandboxes was 40 to 100% contamination rates. Authors underline seasonality in the prevalence of *Toxocara sp*. in primary hosts and the soil, with peaks in the summer and autumn. Interestingly, *Toxocara sp*. eggs were found in 4-10% of all vegetables and greenery samples tested in Russia, suggesting that they can contribute to the transmission of *Toxocara sp*. as well [7].

Simultaneously with the collection of data on the incidence of parasitic diseases in the gastrointestinal tract of humans and animals caused by nematodes, the amount of information on the potentially beneficial effects of infection with them is growing. There is evidence that parasitic helminth infections can protect against allergic airway inflammation a reduced risk of atopy and a reduced course of asthma. Moreover, the helminth *Trichuris suis* has demonstrated efficacy in treatment of inflammatory bowel disease [8]. However, Bakhshani *et al*. investigated the effect of *Toxocara cati* infection on experimental allergic airway inflammation using the murine model and came to opposite conclusions. In the work, BALB/c mice were infected with *Toxocara cati* eggs and then sensitized with followed ovalbumin (OVA) sensitization in order to induce allergic airway inflammation. Infection with *Toxocara cati* in combination with OVA treatment led to exacerbation of pulmonary inflammation, eosinophilia, airway hyper responsiveness [9]. Interestingly, in Australia, helminths are used to fight millipedes in seasons of mass occurrence of these arthropods disturbing the local people [10].

The National Health Program for 2021-2025 in Poland includes initiatives to prevent infectious diseases under the operational objective „Environmental health and infectious diseases" [11]. Ascariasis and trichurosis are treated as typical diseases of dirty hands in the program. The source of infection is soil contaminated with the eggs of these nematodes, sand, fruit and vegetables, and farmers are the most vulnerable groups. Stool tests for eggs of *Ascaris lumbricoides* and *Trichiuris trichiura* are very rarely performed in adults and children. The situation is similar with regard to detection of toxocarosis. It is being found incidentally, most often in children. The program particularly emphasizes hygiene recommendations aimed at reducing contagions, among others:

- strict adherence to personal hygiene,
- thorough washing of vegetables and fruits,
- neutralizing the source of infection (treating the sick people),
- protection of soil (including sandboxes) against contamination with feces of dogs and cats,
- systematic deworming of dogs and cats [11].

Playgrounds, including sandboxes, are considered to be small architecture objects in Poland, which means that they are subject to construction law, must be regularly inspected and care must be taken to keep them clean and safe [12-21]. The innovations observed at many of them in recent years are portable toilets and fences. Both are of great importance: the toilets prevent spread of eggs of *Ascaris lumbricoides* and *Trichiuris trichiura* and the fences prevent sand contamination with cat's and dog's feces.

CONCLUSION

The pilot study shows that refugees can safely use the playgrounds in Romania, Hungary, Slovakia and Poland.

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