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Presence of *Toxocara* spp. eggs in children's recreation areas with varying degrees of access for animals

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Abstract

Introduction and objective. The contamination, seasonal and vertical distributions of *Toxocara* eggs in children's recreation areas were estimated with respect to their accessibility to domestic and stray animals.

Materials and methods. During autumn 2011 and spring 2012, a total 88 composite samples of soil/sand (300g each) were taken twice, from 2 depths, from 11 sandpits and 11 play areas situated in the city of Łódź, Poland. From the collected material, 528 samples (20g) were tested using the flotation method. Half the sample sites were secured from access to dogs and cats, while the other half were not.

Results. The difference in the numbers of positive samples from sandpits and playing areas was significant ($\chi^2 = 13.72$, $p = 0.0002$). The highest rate of contamination was observed in poorly-secured play areas (15.8% of positive samples and 1.2 eggs/100 g of soil/sand). The average density of *Toxocara* eggs in secured play areas was 6 times less than that found in unsecured areas, while secured sandpits were 3 times less contaminated than those unsecured. The contamination rate was similar in autumn 2011 and spring 2012 (6.4% and 6.8%, respectively). An inverse relationship between the sand/soil depth and number of recovered *Toxocara* eggs was observed. Additionally, other intestinal helminth eggs (Ancylostomidae, *Ascaris* spp., and *Trichuris* spp.) and oocysts of *Isospora* spp. were also detected from soil samples collected from playing fields.

Conclusions. The number of *Toxocara* eggs recovered decreased following fence construction around the examined children's play areas, but it did not sufficiently prevent the contamination by eggs. These data indicate the necessity for educational programmes which should be implemented for the protection of the local child population from zoonotic infection.

Key words

Toxocara eggs, zoonotic infections, geohelminths, soil contamination, sandpits

INTRODUCTION

It is widely recognized that sand and soil from areas accessible to dogs and cats are likely to be contaminated by intestinal parasites from these animals. The genus *Toxocara* contains parasitic nematodes of human and animal health significance, such as *Toxocara canis*, *Toxocara cati* and *Toxocara vitulorum*. Two species *T. canis* and *T. cati*, are among the most prevalent parasites of dogs and cats with a worldwide distribution [1]. Epidemiological surveys have indicated that the prevalence of *T. canis* in dogs ranges from 3.1% – 82.6%, depending on their environmental status, and the prevalence of *T. cati* in cats ranges from 8% – 62.5% [2].

Detection of *Toxocara* eggs in the outdoor environment is recognized internationally as an important indicator of zoonotic contamination. Humans become infected by ingestion of invasive eggs, either from soil, dirty hands, raw fruit and vegetables, or larvae from the undercooked meat of paratenic hosts. Some researchers have reported the occurrence of fully-embryonated *Toxocara* eggs on dog coats, implicating them as a possible additional route of transmission [3]. However, contact with soil or eating soil are still believed to be the most important routes for human infection. Young children are the main population at risk for *Toxocara* infection due to geophagia and onychophagia, poor hygiene, and frequent contact with dogs or cats. As reported by Geissler et al. [4], the median daily consumption of soil by geophagous Kenyan primary schoolchildren was 28g. Most commonly, children are infected after exposure to contaminated soil while playing in sandpits or playgrounds. For this reason, numerous studies have assessed the presence of *Toxocara* eggs in children's play areas, public parks, kindergardens and school areas. The results of studies showing the average density of the *Toxocara* eggs in children's recreational areas in various parts of the world are demonstrated in Table 1. Very different levels of soil contamination with *Toxocara* spp. can be seen and vary from 0.067 [5] – 31 eggs/100 g of sand [6]. Such a great variation in findings results from the fact that the recovery of *Toxocara* eggs from soil samples varies depending on environmental conditions, size of dog and cat population, choice of sampling sites, type of soil, and type of technique employed for egg recovery.

According to literature data, the worldwide seroprevalence of anti-*Toxocara* IgG antibodies has been found to vary from 2.4% – 92.8% [1]. No data exists regarding the overall seropositivity of the child population in Poland. However, in recent years, about 30 cases of paediatric clinical toxocarosis have been diagnosed per year in central Poland. In the years

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Table 1. *Toxocara* egg contamination of children's recreational areas in various parts of the world

Reference	Country/City	Public place/kind of samples	Mean No. of eggs/100 g of sample
Aydenizöz-Özkayhan [15]	Turkey/Kirikkale	Playgrounds/soil	0.3
Avcioglu & Burgu [25]	Turkey/Ankara	Parks/sand	5.1
Blaszkowska, et al. [10]	Poland/Lodz	School sports fields/soil Playgrounds/sand	1.1 0.4
Dubna, et al. [20]	Czech Republic/ Prague	Parks/soil	6.2
Oge & Oge [18]	Turkey/Ankara	Parks/soil	5.6
O'Lorcain [14]	Ireland/Dublin	Playgrounds/sand	1.4
Rokicki, et al. [12]	Poland/Gdansk	Sandpits/sand	2.0
Ruiz de Ybanez, et al. [19]	Spain/Murcia	Parks/soil	16.7
Stojcevic, et al. [26]	Croatia/Pula	Parks/soil; playgrounds/sand	2.0
Toparlak, et al. [5]	Turkey/Istanbul	Sandpits/sand	0.067
Uga [6]	Japan/ Hyogo Prefecture	Sandpits/sand	31.0

2004–2007, toxocarosis was confirmed in 178 children at the Polish Memorial Hospital in Łódź [7]. Examination of soil samples collected from 53 courtyards in the vicinity of the patients' places of residence revealed a high prevalence of ground contamination with *Toxocara* eggs in both rural (30.4%) and urban (23.3%) areas of the Łódź province [8]. Another study revealed high *Toxocara* spp. egg contamination in soil collected from yards situated in 5 villages near Łódź [9]. Recently, preliminary studies conducted in randomly-selected localities in the city of Łódź have shown varying degrees of geohelminth egg contamination in children's play areas, depending on the nature of the examined site [10].

OBJECTIVE

The aim of the study was to evaluate the variation in the degree of *Toxocara* spp. egg contamination of soil from children's recreation areas situated in locations in the city of Łódź which had not been previously examined. The time of sampling, type of site, and especially the accessibility of the site to domestic and stray animals, were all taken into account. The study also evaluated the association between the implementation of precautionary measures and contamination of these sites.

MATERIALS AND METHOD

Study area. Łódź is the third-largest city in Poland, with a population of 737,100 residents. The city covers 293 square kilometres with 34 public parks. According to estimates available at the public health service, the pet dog population of the city exceeds 70,000.

The investigations were carried out in selected locations in the city of Łódź, on 2 occasions during autumn (October-November) 2011 and spring (April-May) 2012. The composite samples of soil and sand were taken from 22 localities: 11 sandpits and 11 children's play areas (playgrounds or playing fields). Six examined localities were situated in the 3 public parks located in the city centre: Staszica Park (small) constituting an area of 3.9 ha, and 2 larger, partially wooded parks – Źródliska Park and 3rd May Park, covering areas of 17.2 and 23.5 ha, respectively. Recreation facilities are present: numerous playgrounds, sports fields and tennis courts. These parks are often visited by adults with children and favoured by dog walkers, are unfenced with free dog and cat access. Ten of the tested childen's recreation sites were situated in large housing estates belonging to 2 districts of Łódź: Bałuty and Górna. Many families living in blocks of flats own pets, mainly dogs, with a pet dog to family ratio of 1: 3–4. The 6 remaining locations were situated in school or kindergarten areas with no access for domestic animals. Thus, half the examined sandpits, playgrounds and playing fields were properly protected from entry by domestic and stray animals. They were enclosed by tall fences exceeding 1.5 m in height, with a 1 m wide gate closed at all times and conspicuous 'No Dogs Allowed' signs posted around the fence. Additionally, the sand in the sandpits is replaced at least twice every year. The other half of the examined localities were unfenced, or fenced by either a low hedge or fences not exceeding 0.5 m in height. The sand in the sandpits is replaced perhaps once every few years. Based on the quality of site maintenance, sand, fences and accessibility to dogs and cats, the examined sites were classified as secured (S) or as poorly secured (PS).

Collection of soil samples. A total 88 of composite samples were collected during autumn 2011 and spring 2012 at the same 22 localities. The samples of sand and soil were taken both from the surface (0–3 cm superficial layer) of each examined site, and from a depth of about 15 cm. The material from these 2 depths were packed into separate collecting bags. From each sandpit, 300 g of sand was taken (material mixed from 6 points: one from each corner of the sandpits, and 2 from the centre – 50 g each). From play areas, soil of 280–300 g within a 10 m^2 area was collected (material mixed from 9 points – about 33 g each).

Detections of eggs. Dried composite samples were sieved through a 4 mm mesh to remove larger contaminants. From composite samples of each location, 12 samples of 20 grams each were examined: 6 samples from the surface and 6 from the deeper layer. Thus, a total of 528 samples were tested in total. The soil samples were investigated for the present of *Toxocara* eggs using a flotation method, as presented previously [10]. The eggs were recovered from 20 g amounts of soil by flotation in saturated sodium nitrate solution (SG 1.30). After the flotation process, 24 × 24mm cover slips were placed on the tubes, and after a 10 min waiting period were examined for the presence of eggs at 100× and 400× magnifications. No attempt was made to differentiate eggs to species level. The viability of the detected eggs was evaluated as described previously [9]. Eggs with a moving larva were recognized to be potentially invasive.

Statistical methods. The data was analyzed using STATISTICA 10 software. Differences between groups were compared by the Chi-square test. If any of the frequencies was less than 5, Fisher's exact test was applied. Values of $p < 0.05$ were taken as significant.

RESULTS

The contamination of soil and sand with *Toxocara* eggs in children's play areas located in Łódź are shown in Tables 2 and 3. *Toxocara* eggs were found in 40.1 % of sandpits and 50% of play areas, and the overall egg contamination rate of examined localities was 6.6%. The frequencies of positive samples from sandpits and playing areas were found to be significantly different ($\chi^2 = 13.72$, $p = 0.0002$). A higher rate of contamination was found in play areas (9.1% of positive samples), which was twice the frequency of positive samples from sandpits (4.2%). Of the 528 examined samples, 35 were positive with a mean egg density of 0.4/100g of soil/sand for the overall examined sites (Tab. 4). The number of eggs for positive samples varied from 1–3 eggs per sample (20 g), and the average concentration of *Toxocara* eggs was 6.6 eggs/100 g of sand/soil. A high proportion of eggs (56.5%) were embryonated and 42.9% of isolated eggs contained moving larvae.

Table 2. Contamination of examined sites with *Toxocara* eggs

Examined sites	Date of sampling	No. of sites sampled	No. of sites with eggs (%)
Sandpits			
S	autumn 2011	5	1 (20.0)
PS	autumn 2011	6	4 (80.0)
	Total	11	5 (45.5)
S	spring 2012	5	1 (20.0)
PS	spring 2012	6	3 (50.0)
	Total	11	4 (36.4)
Play areas*			
S	autumn 2011	6	2 (33.3)
PS	autumn 2011	5	4 (80.0)
	Total	11	6 (54.5)
S	spring 2012	6	1 (16.7)
PS	spring 2012	5	4 (80.0)
	Total	11	5 (45.5)

S – secured from access to animals; PS – poorly secured;
* Playgrounds or playing fields

More *Toxocara* eggs were found in the samples from localities with poor protection against animals than places with limited access to dogs and cats (Tab. 3). The average number of *Toxocara* eggs per 100 g of sand was 0.1, and 0.3 for samples from secured and poorly-secured sandpits, respectively. However, the differences between the frequencies of positive samples of these sandpits were not statistically significant ($\chi^2 = 1.53$, $p = 0.216$). The highest rate of contamination was observed in the poorly-secured play areas (15.8%). The frequencies of positive samples from secured and poorly-secured play areas were significantly different ($\chi^2 = 12.10$, $p = 0.0005$) and average egg density in samples collected them were 0.2 and 1.2 eggs/100 g, respectively.

Regardless of the time of sampling, 3 times the number of eggs were recovered from the surface of the sites (34 eggs) than from the deeper layer (12 eggs). A significant difference was observed between the frequency of positive samples taken from the surface and those taken from a depth of 15 cm ($\chi^2 = 6.88$, $p = 0.0087$).

Table 3. *Toxocara* eggs recovered from samples collected from outdoor children's recreation areas in the city of Łódż in autumn 2011 and spring 2012

Examined sites year, type (No. of sites sampled)	No. of samples*	No. of samples with eggs (%)	No. of *Toxocara* eggs		
Sandpits			T	D	T+D
2011 S (5)	60	1 (1.7)	1	0	1
PS (6)	72	5 (6.9)	4	1	5
Total (11)	132	6 (4.5)	5	1	6 2
2012 S (5)	60	2 (3.3)	2	0	2
PS (6)	72	3 (4.2)	2	1	3
Total (11)	132	5 (3.8)	4	1	5
Play areas					
2011 S (6)	72	4 (5.6)	5	0	5
PS (5)	60	7 (11.7)	7	1	8
Total (11)	132	11 (8.3)	12	1	13
2012 S (6)	72	1 (1.4)	0	1	1
PS (5)	60	12 (20)	13	8	21
Total (11)	132	13 (9.8)	13	9	22
SUM TOTAL	528	35 (6.6)	34	12	46

S – secured from access to animals; PS- poorly secured;
T – top; D – deep layer
* sample = 20g of sand/soil from composite sample

More helminth eggs (27) were detected from samples collected during spring 2012 than autumn 2011 (19 eggs) (Tab. 4). The frequencies of positive samples taken during spring and autumn were similar, 6.4% and 6.8%, respectively. No significant differences were found between seasonal distributions ($\chi^2 = 0.031$, $p = 0.860$).

Table 4. Occurrence of *Toxocara* eggs in soil and sand samples in different seasons

Examined sites	No. of samples (No. of positive samples)	Total no. of detected eggs	Viable eggs (%)	Eggs with moving larva (%)	Mean egg density per 100 g
Sandpits					
autumn 2011	132 (6)	6	66.7	33.3	0.2
spring 2012	132 (5)	5	40	40	0.2
Total	264 (11)	11	54.5	36.4	0.2
Play areas					
autumn 2011	132 (11)	13	53.8	38.5	0.5
spring 2012	132 (13)	22	59.1	36.4	0.8
Total	264 (25)	35	57.1	37.1	0.7
SUM TOTAL	528 (35)	46	56.5	36.9	0.4

Additionally, other intestinal helminth eggs were discovered during microscopic examination of soil samples collected from playing fields situated in the larger parks (Park Źródliska and 3rd May Park). Three Ancylostomidae eggs, 9 eggs of *Ascaris* spp. and 2 eggs of *Trichuris* spp. were found. Moreover, 2 oocysts of *Cystoisospora* spp. were found in 2 sand samples from the children's playground located in the 3rd May Park.

DISCUSSION

The presented study shows differential *Toxocara* contamination rates for the examined outdoor child recreation areas in the city of Łódź. The contamination of soil with *Toxocara* eggs in the samples from play areas was found to be more than twice (9.8% positive samples) than that of sandpits (4.2%). The present and earlier studies by the authors of the current study indicate that among the examined sites, sandpits were the least contaminated with *Toxocara* eggs [10]. These results are comparable with those of studies conducted on children's playgrounds located in various Polish cities, such as Gdańsk, Elbląg and Warsaw [8, 11, 12]. An examination of the distribution of probable sources of *Toxocara* infection in urban environments located in 5 regions of Poland shows that the highest egg concentrations are found on pavements and city green spaces (19.3% positive samples), followed by public parks (10.5%), playgrounds and playing fields (9.4%) [13]. In the presented study, the average density of *Toxocara* eggs in children's recreation areas varied from 0.1–1.2 eggs per 100 g of samples, depending on the type of locality, and are comparable to those from sandpits and playgrounds situated in other European cities: Kirikkale and Istanbul in Turkey and in Dublin, Republic of Ireland [5, 14, 15]. Higher *Toxocara* egg concentrations can be found in many other cities in the world (Tab. 1).

It was confirmed that the prevalence of toxocarosis in children depends on the degree of soil contamination with *Toxocara* eggs [8]. To prevent faecal contamination, it is important to identify the main animal responsible for defecating in play areas for young children. Mizgajska-Wiktor and Jarosz [16] showed that *T. cati* eggs were much more prevalent in urban areas (97% of all eggs recovered), while *T. canis* were more often found in rural areas (84% of all recovered eggs). It was recognized that *T. canis* eggs are most common in public parks, and most of the sandpits are polluted with *T. cati* eggs [17]. It should be noted that it is very difficult to differentiate between eggs belonging to the *Toxocara* genus by means of morphological similarity under the light microscope; correct identification of *Toxocara* eggs is possible by using molecular biology methods.

The numbers of *Toxocara* eggs found in the the presented study were generally low, but the presence of even a few geohelminth eggs in play areas is associated with a risk of infection. It should be noted that a review of sand or soil collection, processing and laboratory examination procedures indicate an extreme lack of standardization, and the consequent possibility that low densities of *Toxocara* eggs may be missed during analysis. It should be emphasized that environmental and technical factors are thought to influence the recovery of helminth eggs [18, 19]. Generally, the recovery rates of geohelminth eggs from soil samples using standard centrifuge-flotation techniques is low. The percentage of eggs recovered by 6n different tested techniques ranged from 6% – 15% for 50 g sand samples containing 100 eggs, and after artificial contamination of a sand sample by 500 *Toxocara* eggs, only 24–73 were recovered [18]. The results of these studies can suggest that the use of such flotation techniques can detect only a small number of the eggs present in samples.

The presented study demonstrates that sandpits and play areas protected from access to animals tend to have lower levels of contamination. The average density of *Toxocara* eggs in secured play areas was 6 times less than in unsecured play areas, while the density in secured sandpits was 3 times less than in unsecured sites. Hence, the current results confirm that prevention of animal access to public recreation areas is an effective method of limiting the presence of parasite eggs in the environment. However, a small number of *Toxocara* eggs were still found in samples collected from sandpits and play areas enclosed by tall fences (3.4% positive samples). This data corresponds with the results of studies conducted in Prague, Czech Republic, where the prevalence of *Toxocara* spp. in samples from unfenced sandpits was higher than from fenced sandpits [20]. These results suggest that the effectiveness of a fence around a play area mostly depended on the behaviour of the users and their observance of playground and park rules and regulations. According to the observations by authors of the current staudy, children often leave the gates to fenced areas open, and dog owners walk around playgrounds with unleashed pets. However, the presence of *Toxocara* eggs in secured sandpits could be explained by the territorial behaviour and defecation habits of cats. It has been experimentally confirmed that cats prefer a quiet place with sandy material to defecate, and they are able to climb over fences [17]. Many researchers have shown that the most common animals contaminating sandpits are cats [16]. These reports are in accordance with those of a 144-day camcorder observation of 3 sandpits in urban public parks [21].

Various methods to prevent contamination of sandpits with *Toxocara* eggs have been studied: covering sandpits, sand pasteurisation, sand replacement and also installation of fences. However, these methods do not completely prevent egg contamination [20, 22]. Abe and Yasukawa [23] describe that the number of eggs recovered from sandpits following fence construction decreases, but it is not sufficient to prevent contamination; *Toxocara* spp. eggs were detected 2 months after the installation of sandpit fences and sand replacement. Replacement of contaminated sand has been found to be ineffective; Uga and Kataoka [22] note the reappearance of eggs in the sand 6–9 weeks after replacement. However, covering sandpits is a safe and inexpensive procedure for preventing contamination by animal faecal deposits which also results in the destruction of existing eggs.

Seasonal conditions can be important factors affecting the degree of environmental contamination with *Toxocara* eggs. Some researchers have noted the presence of *Toxocara* eggs in sandpits located in public parks in Japan, showing a higher frequency of eggs recovering during the autumn, spring and early summer [24]. In other countries, the highest egg concentrations have also occurred in these seasons [22, 25], which is probably connected with the reproductive period of the definitive hosts which, in turn, leads to a greater presence of puppies and kittens in these seasons. In the presented study, more *Toxocara* eggs were detected from samples collected during April–May than October–November, and no significant differences were found between seasonal distributions. Other studies conducted in Poland, which is located in the temperate climatic zone, showed that in both rural and urban areas the degree of soil contamination with *Toxocara* eggs in spring and autumn was similar [13, 16]. On the other hand, in countries with dry and rainy seasons, the *Toxocara* contamination rate was higher in December than in June [26]. This fact can be attributed to the particular environmental and climatic conditions which are very suitable for the survival of *Toxocara* eggs deposited in colder months. During the dry seasons, the embryonated eggs are exposed to destruction by sunlight or poor humidity.

In natural conditions, geohelminth eggs, like soil particles, will be moved vertically and horizontally by the actions of rain, wind, and animals living in the soil. It is commonly known that the defecation behaviour of animals varies; most cats cover their excrement while dog faeces tend to be deposited on the ground. Some studies have shown that animal defecations are distributed evenly as a result of ground animals [21]. It has been confirmed that animals such as earthworms, soil nematodes, snails and rodents take part in the spreading of parasite eggs, transporting them from deeper layers to the surface [13]. Environmental observations reveal that after 17 months, *Toxocara* eggs can be found in the superficial layer [27].

CONCLUSIONS

The results of presented study show that over 45% of examined child recreation areas located in the city of Łódź are contaminated with *Toxocara* eggs, indicating that control measures, as well as greater education, particularly of dog and cat owners, is still needed for the protection of the local child population from zoonotic infection. Since no practical methods exist for reducing environmental egg burdens, prevention of initial contamination of the environment is the most important tool. This can be achieved by taking measures such as effectively eliminating infections in dogs and cats, preventing defecation by pets in public areas, improving hygiene, and better educating the public.

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