Contamination of selected recreational areas in Lublin Province, Eastern Poland, by eggs of Toxocara spp., Ancylostoma spp. and Trichuris spp.

Hubert Bojar¹,², Teresa Kłapeć³,², Bá-C

¹Department of Toxicology and Food Safety, Institute of Rural Health, Lublin, Poland
²Department of Biological Threats of Health and Parasitology, Institute of Rural Health, Lublin, Poland

Abstract

Introduction. While using recreational areas, people take with them accompanying animals (dogs, cats). These animals are the main source and reservoir of dangerous zoonoses, including parasitoses caused by genera of nematodes, also called roundworms, Toxocara spp., Trichuris spp. and Ancylostoma spp. Attention should also be paid to the possibility of contamination of recreational areas with the eggs of intestinal parasites by wild animals (e.g. red fox).

Materials and Methods. Sand was collected on beaches and leisure terrains located in recreational areas in Zwierzyniec, Rudka reservoir on the river Wieprz, ‘Echo’ reservoir, accessible to tourists to the Roztocze National Park, and the ‘Florianiecki’ reservoir, which is inaccessible to tourists due to its location in the depths of the National Park. Material for the research was also collected from the ‘Zemborzycki reservoir in Lublin, A total of 300 sand samples were collected. The samples, approximately 500 g each, were collected into plastic bags from the superficial layer of sand, at a depth up to 3 cm. For egg detection, flotation with the Wasylikowa method and the modified Quinn method were used. In the Quinn method, the flotation solution is NaCl saturated solution, in the Wasylikowa method, 5% solution of NaOH is used.

Results. The most contaminated grounds were areas by the water ‘Zemborzycki’ reservoir in Lublin. The eggs of intestinal parasites were isolated from 13 out of 24 samples examined. In sand collected from the beach by the ‘Echo’, ‘Florianiecki’ and ‘Rudka’ water reservoirs in Zwierzyniec, no eggs of intestinal parasites were detected.

Conclusions. Such a dissemination of results may indicate a connection between the degree of occurrence of geohelmint eggs and the intensity of human movement, together with accompanying animals, in places that can be used for recreation.

Key words

contamination, recreational areas, geohelminths eggs, Trichuris, Ancylostomatidae, Toxocara

INTRODUCTION

Commonly, recreational areas consist of open spaces which are spontaneously used for leisure purposes. The system of recreational areas covers all types of parks, urban squares, bicycle paths, sports complexes, holiday camp areas, playgrounds for children, beaches, and even lawns spontaneously used for recreational purposes.

Human modus vivendi, and associated recreational behaviors changed together with the political and economic transformations observed in Poland in the 1990s.

The majority of people spend their free time in the open air only in the vicinity of their place of residence, while small groups use home gardens. This results not only from the reluctance to travel, but also from the necessity of walking their dogs, usually in the nearest surroundings. The greatest intensification of traffic in recreational areas is noted on weekends, holidays and during the vacation season. Guarded swimming places are usually very populous; therefore, an increasing number of places with the access to water have become places for summer recreation.

While using recreational areas, people take with them accompanying animals (dogs, cats, and others). These animals are the main source and reservoir of many dangerous zoonoses, including parasitoses caused by genera of nematodes also called roundworms Toxocara spp., Trichuris spp. and Ancylostoma spp. Attention should also be paid to the possibility of contamination of recreational areas with the eggs of intestinal parasites by wild animals (e.g. red fox Vulpes vulpes) [1, 2, 3]. Recently in Poland, as well as in many other European countries, there has occurred the phenomenon of synanthropization of wild living animals into anthro-po-ecosystem.

Often, urbanized areas are visited increasingly by species of large and medium-sized mammals, such as wild boars (Sus scrofa) and red foxes (Vulpes vulpes). The presence of wild animals in areas habited by humans, apart from problems of a psychological nature (agrizoophobia), creates a serious health risk for humans and their domesticated animals [4].

The eggs of roundworms Toxocara spp are the main cause of contamination of soil and sand in recreational areas in Poland. Worldwide, infections in dogs vary from 3–80%, and nearly 100% of puppies are born with the parasite. Toxocariasis is a serious clinical problem, in humans associated with the occurrence of symptoms defined as visceral larva migrans (VLM) or ocular larva migrans (OLM). This disease is most frequently observed in children [5].
The subsequent cause of contamination of recreational areas is the whipworm *Trichuris vulpis*. The parasite occurs in dogs, foxes and other canids, and is typical for older animals. In Poland, dog whipworm infections are found in 3–10% of the population, especially among children living in rural areas [6]. In addition, cases of infection with other dog and cat intestinal parasites non-specific for humans, such as *Dipylidium caninum* and *Ancylostoma* spp, are also relatively often noted [7].

Hookworms (*Ancylostoma caninum*), apart from ascaris, are the most frequent dogs parasites. These parasites may cause the diseases in humans, such as cutaneous larva migrans (CLM) syndrome, induced by the penetration of invasive larvae through the skin of the host. A study in Berlin showed that an increase in temperature by 6 °C resulted in a growing incidence of (CLM), a skin disease in humans caused by *Ancylostoma caninum*, a nematode parasite that infects dogs. Therefore, this study investigated increasing local temperatures and global warming which may give rise to the emergence of CLM due to the ubiquitous presence of these zoonotic hookworms [9].

Studies of contamination of recreational areas with the eggs of geohelminths (i.e. soil-transmitted helminths) have been conducted in various regions of Poland, including: Wrocław, Lębork, Elbląg, Gdańsk, Poznań, Kraków and Warsaw. The objects of the study were primarily sand pits, playgrounds for children, and parks [8, 10, 11, 12, 13, 14, 15, 16, 17]. In the Lublin Region of Eastern Poland, investigations of the contamination of recreational areas (sand pits, playgrounds for children) with the eggs of parasites were conducted by Gundlach [18], and Rzymowska [19].

**MATERIALS AND METHOD**

**Sample collection.** Material for the study (sand) was collected on beaches and leisure terrains located in recreational areas in Zwierzyniec, ‘Rudka’ reservoir on the river Wieprz, ‘Echo’ reservoir accessible to tourists to the Roztocze National Park, the ‘Florianiecki’ reservoir which is inaccessible to tourists due to its location in the depths of the Park. The samples were collected under the appropriate permit (No. 15/2011) obtained from headquarter of the Roztocze National Park. Material for the research was been also collected in the city of Lublin, from the ‘Zemborzycki’ reservoir, from the areas of the former holiday centres, ‘Marina’ and ‘Dąbrowa’, as well as from new recreational area ‘Słoneczny Wrotków’. The studies in Zwierzyniec were carried out in 2011 and in Lublin in 2012, during which a total of 300 sand samples were collected.

Collected samples were used for parasitological studies prepared from 5 component samples; 60 averaged sand samples were examined: 8 from the beaches and places for recreation on the Rudka reservoir in Zwierzyniec (GPS coordinates 50°60'89.54"N 22°99'01.00"E), 16 from beaches by the ‘Echo’ water reservoir in Zwierzyniec (GPS coordinates 50°35'56.7"N 22°58'32.5"E), 12 from terrain surrounding the ‘Florianiecki’ reservoir (GPS coordinates 50°56'18.19"N 22°99'98.16"E), 12 from places for recreation in ‘Marina’, 8 from places for recreation in ‘Dąbrowa’ and 4 from places for recreation in ‘Słoneczny Wrotków’, by the water reservoir ‘Zemborzycki’ in Lublin (GPS coordinates 51°19'07.00"N 22°54'26.66"E). The samples, approximately 500 g each, were collected into plastic bags from the superficial layer of sand, at a depth up to 3 cm.

**Eggs detection.** The standardized flotation method according to the Polish Standard, and the flotation method by Quinn led to isolation of Geohelminth eggs from 100 g sand samples. [20, 21]. The samples were dried at room temperature for a period of 2–3 days. Larger contaminants were removed by sieving through a 2 mm mesh. Eggs of *Ascaris* spp. And *Trichuris* spp. were detected (Polish Standard PN-Z-19000–4/2001). Soil samples (100 g each) were transferred to volumetric flasks containing 100ml of 5% NaOH, then mixed and left for one hour. After the time, the samples were centrifuged for 10 min. The suspensions were transferred to centrifuge tubes, centrifuged at 1,600g for 2 min, after which the supernatant (not containing eggs) was decanted. Saturated flotation solution NaNO₃ was added, the tubes were vortexed again, then centrifuged 3 times at 1,600g for 2 min. After each centrifugation, 1 ml of the supernatant was transferred to a flask with 4–5 ml of water. The next step was a filtration, using a membrane vacuum pump. After that process, the filter paper was transferred to a glass slide and examined under x 10 magnification, and the number of *Ascaris* spp. and *Trichuris* spp. counted.

Eggs of *Toxocara* spp. were detected with the use of the flotation method by Quinn [21]. Dried soil samples were sieved through a mesh. 100 g soil samples were transferred to volumetric flasks containing 100 ml of 0.0025% Tween 80 detergent, and vortexed for 60 sec. The suspensions were transferred to centrifuge tubes, centrifugated at 2,600 g for 10 min., followed by the supernatant free of eggs been decanted. Saturated flotation solution NaCl was added and the tubes vortexed again, centrifugated at 2,600 g for 10 min., after which the solution was added to form a meniscus and a top of supernatant was overlayed. After 30 min., the top of supernatant was transferred to a glass slide, examined under x 10 magnification and the number of *Toxocara* spp. and *Trichuris* spp. eggs counted. The number of eggs and their genus were determined by microscope in each sample. Eggs of *Ancylostoma* spp. were sought for by the flotation method according to Quinn [21].

**RESULTS**

The level of contamination with geohelminth eggs in selected recreational areas in the Lublin Region was demonstrated by the studies conducted in 2011–2012. Total contamination level – 21.6% (Tab. 1). The most contaminated grounds were areas by the ‘Zemborzycki’ water reservoir in Lublin. The eggs of intestinal parasites were isolated from 13 out of 24 samples examined (54.2%). The eggs of *Toxocara* spp. were found in 9 (37.5 %) of sand samples from among 24 samples collected from beaches of the water reservoir in Lublin. The eggs of *Trichuris* spp. were found in 11 (45.8%) of sand samples from among 24 samples collected from the grounds by the ‘Zemborzycki’ water reservoir in Lublin. In sand collected from the beach by the ‘Echo’ water reservoir in Zwierzyniec, no eggs of intestinal parasites detected. The same situation was observed in samples from grounds by the ‘Florianiecki’ water reservoir in Zwierzyniec, and the Rudka water reservoir in Zwierzyniec. Despite the search, the eggs of *Ancylostoma* spp. were not found in the examined material.
DISCUSSION

In Poland, the constantly increasing population of cats and dogs infested by parasites results in raised contamination of the human environment with invasive forms of parasites. The level of contamination of the natural environment may be evidenced by the fact that a single female roundworm can produce about 200,000 eggs a day. Thick egg capsules of Toxocara species allow the ovum to survive in soil for up to 10 years without losing invasive abilities, despite unfavourable environmental conditions.

The parasite soil contamination may determine a potential source of parasitic diseases in humans. Nowadays in Poland, the greatest attention is paid to studies of parasitological situation in urban areas. The examinations covered sand pits, playgrounds, urban recreational areas, lawns, squares and yards in various cities: Warsaw, Kraków, Poznań, Lublin, Puławy, Wrocław and Elbląg. Roundworms of Toxocara species were the parasites most frequently found in these areas. The observed contamination level of yards with the eggs of Toxocara spp. was as follows: Poznań – 27% [14], Kraków 61.9% [15] and Warsaw – 11.8% [17]. In Wroclaw, geohelmint eggs were found in 20% of tested soil samples. The presence of Toxocara spp. was detected in soil samples taken from squares near pavements [8]. In Katowice, 50.3% of samples were found to contain Toxocara spp eggs. The eggs of these nematodes were most commonly found on lawns and lawns located in large settlements and recreational areas and playgrounds, especially if they were equipped with a sandbox [11]. A comparable contamination with the eggs of roundworms of Toxocara species was noted for squares and lawns located in Poznań 8% [14], Kraków 28% [15], Warsaw 26.1% [17], Lublin and Puławy 22% [18].

Playgrounds located between blocks in housing estates are used in a variety of ways. First, by children to play in the sand, and secondly, for active recreation. Sand pits were also considerably contaminated with the eggs of Toxocara, and were clearly dominant in sand samples from the areas of Łębork 28% [10], Gdańsk 47.6% [13], Kraków 11.5% [15], Warsaw 5.4% [17], as well as Lublin and Puławy 31.6% [18]. Recent studies of recreational areas and sand pits in Lublin carried out in 2010, confirmed a high contamination of these places (23.3 %) [19].

The eggs of Toxocara spp. occurred considerably more rarely in rural than urban areas. In the sub-Krakowian villages of Grodkowice and Łązkowice, the level of soil contamination was 16% [15], in the Poznań Region – 2% [14], and in villages in the Lublin Region – 35.5% [18].

In Poznań, samples from 2 selected parks were collected in spring and autumn. In urban recreational areas, 2.7 % positive tests were found to be contaminated with Toxocara spp. In the 3 selected villages in Wielkopolska Region, samples were taken once, mostly in the spring. From the rural recreational areas together, 4.4% samples were positive [16]. The phenomenon may be associated with a lower population density of cats and dogs in the rural areas, and the tradition to chain the dogs at backyards. The latest studies conducted in the Łódź area in 2011, confirmed a high prevalence of the eggs of Toxocara spp. in examined yards – from 20–100% [22].

There are few reports concerning the helminthological contamination of beaches in Poland. The studies of the largest beaches of the Olsztyn lakes conducted in 2009 (Ukiel Lake, Kortowskie Lake) indicated an infection risk for people who spend their free time there. The eggs of Toxocara spp. were present in 7.5% of all samples examined [23].

Studies of the recreational areas by Lake Białe in Lublin Region conducted in 2010, revealed the presence of eggs of intestinal parasites in 40% of examined samples [28].

Studies concerning the parasitological contamination are conducted worldwide. The monitoring covers parks, playgrounds, school areas, municipal beaches, centres and suburbs of large cities, as well as farms, recreational centres, private properties, and areas around apartment building complexes. It is estimated that the contamination of soil with Toxocara spp. eggs worldwide varies from 1%-92% [24]. In Prague, Czech Republic, the eggs were found in 20.4% of parks, in 11.9% of sand pits and 45% of household gardens [25]. In the city of Shiraz in Iran, the level of contamination in municipal squares and playgrounds was 21.4%, including 12.4% of contamination with the eggs of Toxocara spp. [26]. In Khorram Abad, also in Iran, 63.3% of municipal squares were contaminated with the eggs of Toxocara spp. [27].

REFERENCES