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Geohelminths eggs contamination of sandpits in Vladivostok, Russia

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ABSTRACT

Objective: To detect nematode eggs in sandpits in urban area in Vladivostok.**Methods:** Totally 60 samples were collected from five districts. Sand samples were analysed using combined flotation-sedimentation method. In result, 18 sand samples were positive for at one or two parasites species.**Results:** Overall, only 3 genera of helminths were recovered. Most samples were positive for *Toxocara* spp. eggs. *Toxocara* spp. eggs were found in each district. More samples with *Toxocara* spp. eggs were found in Pervomaiskii district. *Ascaris* spp. eggs were also appeared in each district, however *Ascaris* eggs rate of contamination was less than rate of *Toxocara* spp. contamination. *Toxascaris leonina* eggs were the less frequent species in sandpits, it was found only in Sovetskii and Pervomaiskii districts. *Ascaris* eggs rate of contamination was less than rate of *Toxocara* spp. contamination. *Toxascaris leonina* eggs were the less frequent species in sandpits, it was found only in two districts. No correlation between districts and helminths eggs present in soil samples was found ($P > 0.05$).**Conclusions:** Considering the high sand contamination with parasite infective elements of both human and animal origin, measures to improve environmental and sanitary conditions are indicated, as well as promoting the concept of responsible pet ownership.

1. Introduction

Contamination of soil with parasite eggs in public parks, particularly playgrounds with sandpits for children, is an important source of infection in urban environment and constitutes a great risk factor for human infections, especially for small children [1].

Soil-transmitted helminths infections are the most prevalent of human infections worldwide [2]. Children are most exposable to parasitic infestations. They often contact with pets, which can be infested large number of zoonotic parasites, they also contact with contaminated soil in sandboxes and playgrounds [3,4]. Dogs and cats feces are major source of environment contamination by helminths' eggs. *Toxocara* spp. and *Ancylostoma* spp. are the most widespread and economically important zoonotic parasites worldwide [2]. Human toxocariasis caused by canine

Toxocara canis (*T. canis*), rare feline *Toxocara mystax* (*T. mystax*) (syn. *T. cati*) manifest vary symptoms included ocular and cutaneous larva migrants, neurotoxocariasis and common toxocariasis [5,6]. Whereas, *T. canis* infestation is also causes morbidity in industrial nations, populations, especially in children and socio-economical disadvantage populations [7].

Another parasites as *Ascaris lumbricoides* and *Trichuris trichiura* are also the most prevalent and widespread helminths in the world [8]. Around a billion people are infested with *Ascaris* spp., and 500 million with *Trichuris* spp. [9,10]. Infected eggs can survive in the soil for a long time depending on several factors, such as climatic conditions, temperatures, humidity or desiccation of soil [11].

The aim of this study is to determine of nematodes contamination in sand samples collected in sandpits of registered pre-schools, hospitals and kinder gardens in Vladivostok.

2. Materials and methods

Vladivostok is the largest city in the Far Eastern Region (43°06'20"83 N, 131°52'24" E), and capital of Primorskii

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region. It is situated on the coast of Sea of Japan, an area characterized by a monsoonal climate, with cold and snowy winters and warm and rainy summers. The average maximum and minimum temperatures over 2009 to 2015 were -23.9°C and 30°C , respectively. Humidity levels for Vladivostok are 57–60% per year. Average amount of snowfall in the winter is 89×47 cm. The Vladivostok population is approximately 600000.

Sand samples were collected in urban areas of Vladivostok during July to December 2015. Sand samples were divided into 5 groups according their collection area accordance with 5 districts: Pervorechenskij, Sovetskij, Pervomajiskiji, Leninskij and Frunzenskij. At least 9 samples were collected from each district.

Five sand samples of approximately 25 g each per sandpit were taken (four samples from corners and one sample from the centre). Each sample removed from sandbox was taken from different depths: from the top 5–6 cm and from 10 to 15 cm below the surface. The five samples from one sandbox were placed in one plastic bag with label. Sand samples were stored at 4°C . Totally 60 samples from five district were collected and analysed.

Sand samples were analysed using Romanenko's method [12]. It's a combined method based on flotation and sedimentation techniques. Sand samples 25 g each per sandbox are mixed with water (1:2) in 50 mL centrifuge tubes, and centrifuged 1200 rpm \times 4 min. Then the sediment is centrifuged again with NaNO_3 (1 L water per 1 kg NaNO_3) added. Finally, the sediment was re-suspended in 50 mL saturated and poured into centrifuge tubes, which were filled to the brim, and the coverslip was superimposed. The samples were stored at the room temperature for 30 min, the coverslip was removed onto a microscopic slide and examined for the presence of parasite eggs. Correlation between number of positive samples and district made using the Pearson chi-squared test with Yates correction. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

In result, 18 sand samples were positive for at one or two parasites species. Overall, only 3 genera of helminths were recovered. Most samples were positive for *Toxocara* spp. eggs (Figure 1). *Toxocara* spp. eggs were found in 14 samples, maximum value of intensity was 23 eggs/25 g soil; *Toxascaris leonine* eggs were found in 2 samples, maximum value of intensity was 6 eggs/25 g soil. *Ascaris* spp. eggs were found in 7 samples, maximum value of intensity was 3 eggs/25 g soil. A higher rate of contamination of helminths eggs was detected in

sandpits in Frunzenskij district (14%) and Pervomajskij district (12.5%). The least contamination was found in sandpits in Sovetskij district (7.1%) (Table 1). No correlation between districts and helminths eggs present in soil samples was found ($P > 0.05$). *Toxocara* spp. eggs were found in each district. More samples with *Toxocara* spp. eggs were found in Pervomajskii district. *Ascaris* spp. eggs were also appeared in each district, however *Ascaris* eggs rate of contamination was less than rate of *Toxocara* spp. contamination. *T. leonina* eggs were the less frequent species in sandpits, it was found only in Sovetskij and Pervomajskij districts.

Table 1

Helminths eggs contamination in sandpits and playgrounds in different cities from Russia.

City	Total number of collected samples	Positive samples (%)	Parasites eggs
Vitebsk [13]	388	20.1	<i>T. canis</i> , <i>T. mystax</i>
Kurskii Region [14]	777	5.9	<i>Ascaris</i> sp., <i>Toxocara</i> spp., <i>Enterobius</i> sp.
Cheboksary [15]	–	39.1	<i>T. canis</i> , <i>T. leonina</i> , <i>Trichuris vulpis</i> , <i>Dipylidium caninum</i>
Voronezh [16]	40	27.5	<i>Toxocara</i> , <i>Trichuris</i> sp., <i>Ascaris</i> sp., <i>Enterobius</i> sp.
Present study (Vladivostok)	60	30.0	<i>T. mystax</i> , <i>T. leonina</i> , <i>Ascaris</i> sp.

4. Discussion

These results are comparable with those of studies conducted on children's playgrounds located in various Russian cities, such as Vitebsk, Kursk, Cheboksary and Voronezh [13–16] (Table 1). Rate of contamination in present survey don't significantly differ from other cities, except Kurskii region, with low rate of contamination.

Toxocara spp. and *Ascaris* sp. eggs are the most frequency parasites founded in sandpits in Russia. 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 [17].

Eggs of *Trichuris* spp. don't found in present study. The source of eggs of *Trichuris* spp. in urban areas are domestic animals (cats and dogs), rare humans. *Trichuris vulpis* infested dogs and *T. serrata* and *T. campanula* infested cats [18,19]. These results are due to the fact, that cats and dogs infested *Trichuris* spp. were not found in Vladivostok in recent years [20] and cases

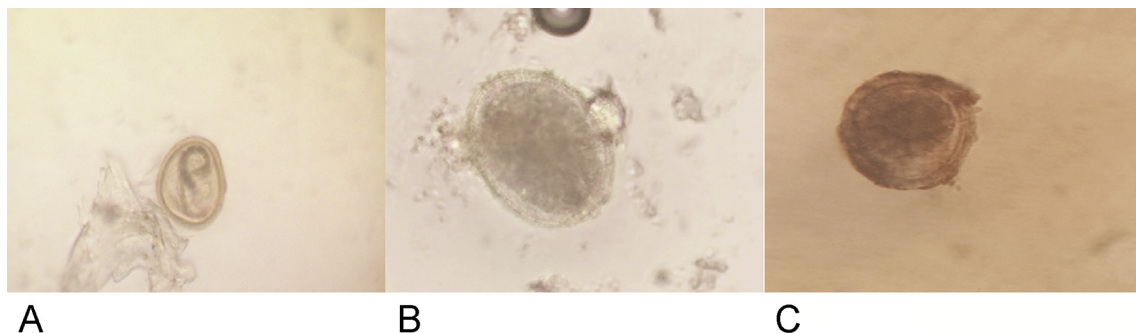


Figure 1. Embryonated *Toxocara* sp. egg (A), *Ascaris* sp. egg (B) and *Toxascaris leonina* egg (C).

of people infestation by *Trichuris* sp. were not registered in Pymorskii region in the last years [21].

Enterobius vermicularis is the most frequency helminth found in peoples in Primorskii Region in recent years, There are 75.1% cases of *E. vermicularis* infestation in people, especially in children under 14 years, among other helminthiasis [21].

So, lack of *E. vermicularis* in sandboxes is a good tend to decrease of *E. vermicularis* eggs in children.

Major measure to prevent *Toxocara* eggs contamination of sandpits is protected them from access to animals. Covering sandpits is a safe and inexpensive procedure for preventing contamination by animal faecal deposits, which also results in decrease of parasites eggs contamination. In present survey, only 4 sandboxes (6.66%) were protected from animals, helminthes eggs didn't found in these places. However, that method don't completely prevent eggs contamination.

Moreover, other methods to prevent contamination of sandpits with helminthes eggs have been studied: covering sandpits, sand pasteurisation, sand replacement and also installation of fences. But, these methods do not completely prevent egg contamination [22].

In conclusion, this study showed that environmental pollution with parasite eggs poses a significant threat to public health. Considering the high sand contamination with parasite infective elements of both human and animal origin, measures to improve environmental and sanitary conditions are indicated, as well as promoting the concept of responsible pet ownership.

Conflict of interest statement

We declare that we have no conflict of interest.

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