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# Contamination of soil and sand with parasite elements as a risk factor for human health in public parks and playgrounds in Pula, Croatia

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**ABSTRACT**

The objectives of the present study were to screen soil and sand samples collected from public parks and children's playgrounds in the city of Pula for the presence of infective stages of parasites and to determine the prevalence of helminth eggs and protozoan cysts and oocysts in two seasons. 90 samples of soil and sand were collected from a total of nine parks and surveyed on two occasions, in June and December. Throughout the study 4 genera of nematode eggs and 2 genera of protozoan cysts and oocysts were detected. The contamination rate was different in the dry and rainy season. The most frequent finding was *Toxocara* sp. eggs and had the highest prevalence in both seasons. Of 90 samples 15.5% were positive for *Toxocara* spp. eggs in June and 23.3% in December. Our results show that *Ascaris lumbricoides* eggs from human faeces were detected in 4.4% samples in June and 15.5% in December. In June 45% of all parasite eggs were embryonated and in December the percentage rose to 75%. The increase in the number of positive findings in December was only found to be statistically significant ($P<0.05$) for *A. lumbricoides* eggs. There was no significant difference in the number of positive findings between soil and sand samples.

**Key words**: soil contamination, parasite eggs, parks, Croatia

## Introduction

The presence of animals, pets or animals without an owner, especially dogs and cats in urban public areas, is common in many cities of the world. There is a large population of pets, as well as stray dogs and cats, and it is still increasing. Studies performed in various cities show a variable prevalence of soil contamination with different parasite species

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and the variations could be explained by the characteristics of the human population, environmental (climate) conditions, the type of urbanization and the density of the canine and feline population (RUBEL and WISNIVESKY, 2005).

Intestinal canine and feline parasitic infections transmissible to humans, such as toxocarosis, giardiosis etc, have a cosmopolitan distribution and are among the ten most common infections in the world. Mainly in developing countries, so called soil-transmitted helminthes (STH) still remain a formidable public health problem of cosmopolitan importance (ANONYM., 1987, 1997).

Soil-transmitted parasites are a group of nematodes causing human infections through contact with parasite infective eggs or people can also become infected by skin penetration of parasitic larvae if their bare skin comes in contact with contaminated soil.

Visceral larva migrans (VLM) is a parasitic disease of humans caused by infection with *Toxocara* spp. from animals, dogs and cats. As these eggs must undergo a period of development in the environment before they become infective, direct contact with infected animals is not likely to give rise to infection to humans. Infected eggs can remain viable in the soil for a long time depending on several factors, such as climatic conditions, seasonal air temperatures, humidity or desiccation of soil, exposure to sunlight etc. (STOREY and PHILLIPS, 1985). Thus, contamination of soil with parasite eggs in public parks, particularly playgrounds with sandpits for children, may be an important source of infection and constitutes a great risk factor for human infections, especially for small children aged lower than 12 years with a history of pica (O'LORCAIN, 1994). VLM is considered one of the most important parasitic zoonosis (SCHANTZ, 1991).

This study was undertaken to determine the prevalence of helminth eggs and protozoan cysts and oocysts, especially those which represent a risk for human health, in public places and children's playgrounds.

## Materials and methods

The survey was carried out and samples were collected in urban areas of Pula, twice, in June and December 2007, with the agreement of Pula City Council.

*Soil sample collection sites.* At the beginning of the study nine locations were chosen and only parks with children's playground sites were selected. The chosen parks were in the urban part of Pula with a high human population density which is increased by many visiting tourists with pets during the summer as the city of Pula is located on the coast of the Adriatic Sea. The parks and children's playgrounds were unfenced with free dog and cat access. Each location was numbered as 1-9.

Ten samples of soil and sand from 10 different sites, taken at a distance of 20 m were collected from each location, making a total of 90 samples. At each location samples were taken from shady sites and sites exposed to direct sunlight. Samples were collected during the spring/summer, in June, representing the dry season and in winter, in December, representing the rainy season.

At each site approximately 500 g of top soil or sand was taken using a small shovel, in the area inside a square 25 × 25 cm and 10 cm in depth. The samples were taken from the sites without grass to avoid intensified drainage on grassy soil. The samples were closed in marked plastic bags and transported to laboratory.

*Survey method.* The samples were analyzed by the modified zinc sulphate ($ZnSO_{4)}$ flotation technique previously described (DADA and LINDQUIST, 1979; GIACOMETTI et al., 2000) with our modification. Briefly, 500 g of the soil or sand sample was stirred and chopped and 100 g was placed in a cup and stirred again with 100 mL water and sieved to remove larger particles. The homogenized solution was placed into sedimentation cups, filled with 500 mL water and left overnight. After the supernatant was decanted, 20 g of the sediment was re-suspended with 50 mL water, placed in 2 centrifuge tubes and centrifuged at 1500 rpm for 5 min. Finally, the sediment was re-suspended in 15 mL saturated $ZnSO_4$ solution and poured into centrifuge tubes which were filled to the brim and the coverslip was superimposed. The samples were centrifuged at 1500 rpm for 5 min, the coverslip was removed onto a microscopic slide and examined for the presence of parasite eggs.

All samples were also examined for the presence of *Giardia* sp. cysts and *Cryptosporidium* sp. oocyst by the direct immunofluorescence method described before (JOHNSTON et al., 2003) using the commercial Merifluor *Cryptosporidium/Giardia* test. A drop of the sediment specimens, prepared as described above, was transferred to a treated slide well and left to dry for 30 min. A detection reagent containing FITC labeled anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies and counterstain reagent was placed in each well. After incubation in a humidified chamber for 30 min at room temperature the slides were washed with buffer.

Each well was covered with coverslip and examined under fluorescent microscope using 100-200× magnification for *Giardia* sp. cysts and 400× for *Cryptosporidium* sp. oocysts.

*Statistical analysis.* Statistical analysis was performed using the computer package Statistica 7.1. (STATISTICA 7.1, Copyright Statgraf, 1984-2005).

The significance of the differences in the frequency of positive findings for particular parasite genera during the investigations in June and December was established by the Chi square test.

**Results**

Our survey concentrated on parasitic elements which represent a risk for human health. However, we also recorded parasite eggs which are not infective for humans, such as *Trichuris vulpis* eggs, but their presence shows the range and intensity of contamination with canine faeces on some areas. In most samples we found also strongylid, ascarid and capillarid eggs, as well as *Eimeria* sp. oocysts, without zoonotic potentional, probably originating from birds and small mammals in the parks.

A total of 90 samples were examined in June and 90 samples in December 2007 from nine locations, parks and playgrounds.

In June, 8 locations out of 9 were found to be positive for at least one parasitic species (Table 1).

Overall, 6 genera of parasite eggs, cysts and oocysts were recovered. They included 4 genera of nematode eggs and 2 genera of protozoan cysts and oocysts, originating from human and animal faeces.

Most samples were positive for *Toxocara* spp. eggs. *Toxocara* spp. eggs were found at 8 out of 9 locations and 15.5% samples (14 out of 90) were positive. In the positive locations, out of the 10 examined sites 1-3 samples were positive, with mean egg density of 2 eggs/sample and total of 19 eggs found. At 3 locations *A. lumbricoides* eggs were found in 4 samples (4.4%) with a total of 7 eggs found. *T. vulpis* eggs were found at 6 locations, with 9 positive samples (10%) and a total of 17 eggs, ranging from 2 to 5 eggs/sample and mean egg density was 1.8 eggs/sample. Eggs of *Ancylostoma* sp. were found in only 2 samples in 2 locations.

All samples were negative for *Strongyloides* sp., *Taenia* sp. eggs as well as hookworm and rhabditid larvae.

*Giardia* sp. cysts were found in only 1 sample and oocysts of *Crypto*s*pridium* sp. in 2 samples.

In the second sample collection, in December, all 9 locations were positive for at least one parasitic species (Table 2).

As in June, the most frequent finding were *Toxocara* spp. eggs, found in 8 locations. 23.3% samples (21 out of 90) were positive with a range of 3-7 eggs/sample and a total of 36 eggs found.

The number of positive locations for *A. lumbricoides* eggs rose from 3 in June to 5 positive locations, as did the number of positive samples, which rose from 4 (4.4%) in June to 14 (15.5%) in December. The total number of *A. lumbricoides* eggs found in December was 24 with a range of 3-7 eggs/sample.

Table 1. Results of soil and sand examination in June

| Location number | *Toxocara* sp. (*T. canis, T. cati*) | | *Ascaris* sp. (*A. lumbricoides*) | | *Trichuris* sp. (*T. vulpis*) | | *Ancylostoma* sp. | | *Strongyloides* sp. | | *Giardia* sp. | *Cryptosporidium* sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | No. of positive samples /10 |
| 1. | 2 | 2 | 1 | 1 | 2 | 3 | 1 | 2 | 0 | 0 | 0 | 0 |
| 2. | 2 | 3 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3. | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4. | 1 | 2 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 5. | 3 | 4 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6. | 2 | 3 | 0 | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 1 | 0 |
| 7. | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 |
| 8. | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 1 |
| 9. | 2 | 3 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 14 | 19 | 4 | 7 | 9 | 17 | 2 | 4 | 0 | 0 | 1 | 2 |

Table 2. Results of soil and sand examination in December

| Location number | *Toxocara* sp. (*T. canis*, *T. cati*) | | *Ascaris* sp. (*A. lumbricoides*) | | *Trichuris* sp. (*T. vulpis*) | | *Ancylostoma* sp. | | *Strongyloides* sp. | | *Giardia* sp. | *Cryptosporidium* sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | Total No. of eggs/ loc. | No. of positive samples /10 | No. of positive samples /10 |
| 1. | 4 | 5 | 2 | 4 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2. | 3 | 7 | 2 | 3 | 3 | 5 | 2 | 3 | 0 | 0 | 0 | 0 |
| 3. | 2 | 3 | 3 | 7 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4. | 2 | 5 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5. | 4 | 6 | 0 | 0 | 4 | 7 | 1 | 2 | 0 | 0 | 0 | 0 |
| 6. | 2 | 3 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7. | 1 | 3 | 4 | 6 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 0 |
| 8. | 0 | 0 | 0 | 0 | 3 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9. | 3 | 4 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 21 | 36 | 14 | 24 | 16 | 31 | 5 | 9 | 0 | 0 | 0 | 1 |

Table 3. Number and frequency of positive findings by months

| | Number and frequency of positive findings/months | | Chi² |
|---|---|---|---|
| Parasites | June | December | |
| *Toxocara* spp. | 14 (15.5%) | 21 (23,3%) | P>0.05 |
| *Ascaris* sp. | 4 (4.4%) | 14 (15.5%) | P<0.05 |
| *Trichuris* sp. | 9 (10.0%) | 16 (17.8%) | P>0.05 |
| *Ancylostoma* sp. | 2 (2.2%) | 5 (5.5%) | P>0,05 |

*T. vulpis* eggs were discovered at 7 locations, in 17.7% of samples and a total of 31 eggs were found with range of 2-7 eggs/sample.

The increase in the number of *Ancylostoma* sp. eggs from 4 to 9 eggs in December was not significant.

All samples were negative for *Strongyloides* sp. eggs and *Giardia* sp. cysts while *Crypto*spor*idium* sp. oocysts were detected in only one sample.

While in June 45% eggs of all parasitic species were embrionated, in December the percentage number of embrionated eggs rose to 75%.

A larger number of eggs as well as embryonated eggs were found in the samples taken from shady sites under the trees.

The increase in number of positive findings in December was only found to be statistically significant (P<0.05) for *A. lumbricoides* eggs (Table 3).

**Discussion**

In our study, we found that the parasite contamination of soil and sand in parks and playgrounds in Pula is characterized by a small number of parasite species, as a total of only 6 genera of endoparasites were found.

The survey was conducted in the dry and rainy seasons and the prevalence of intestinal helminth eggs and protozoan cysts and oocysts in the soil and sand from parks and children's playgrounds, as well as the number of parasite species recovered were compared. In both sample collections, in June and in December, the *Toxocara* sp. eggs were the most frequent finding and had the highest prevalence. This is in correlation with the findings of O'LORCAIN (1994), who found prevalence of *Toxocara* sp. eggs of 15% in Dublin and AYDENIZOZ (2006) who recovered *T. canis* eggs in 15.6% samples in playgrounds in Turkey. From the literature we found that, depending on climate and geographic differences, the level of contamination varies in different countries and within countries and is determined by local factors. So, in Japan the frequency rate of *Toxocara* sp. eggs from public parks ranged from 19.2 to 68.8% (SHIMIZU, 1993). GILLESPIE et al. (1991) reported contamination of soil in parks with *T. canis* was 6.3% in London. In Spain, more than 67% of parks and 1.24% of soil samples were contaminated (RUIZ et al., 2001) while in Italy, the contamination of soil in the urban area of Ancona with *Toxocara* spp. was 63.6% (GIACOMETTI et al., 2000) and HABLUETZEL et al. (2003) found 3 out of 6 parks examined were *Toxocara* spp. positive in the Marche region of Italy.

Our findings of a statistically significant (P<0.05) increase in the number of positive samples in December, after the summer months, as well as an increase in the number of *A. lumbricoides* eggs originating from human faeces, probably reflecting the lack of public latrines as these parks are situated near the railway station and harbour in Pula.

Our results showing a higher contamination rate in December than in June correlate with the findings of UGA et al. (1995), RAI et al. (2000) and NURDIAN (2004) who established a significant difference in the dry and rainy seasons, finding a higher prevalence and number of detected species in the rainy season.

In our survey the increase in the number of positive samples, as well as the increase in the number of helminth eggs found in December can be attributed to the very suitable environmental and climate conditions, with moderate temperature and sufficient soil humidity. During the summer and autumn months the eggs accumulated and because of the low quantity of rainfall that year (2007), the eggs were not washed into the deeper parts of the soil. In the soil profile, the most eggs are found at a depth of 0-4 cm (STOREY and PHILLIPS, 1985) and since they are not on the surface, eggs are probably protected from destruction by sunlight. In addition, the long survival and the increase in the number of infective embryonated eggs in December, can be explained by fact that ascarid ova are amongst the most resistant eggs, well suited to prolonged survival and can remain viable for several years. On the other hand protozoan cysts and oocysts survive less well, and *Giardia* sp. cysts are less resistant than *Cryptosporidium* sp. oocysts (SMITH, 1998). Our findings of *T. vulpis* ova in 6 locations with 10% positive samples in June and 7 locations with 17.7% positive samples of soil as well as sand from children's sandpits in December, besides other canine parasite eggs, show that due to the absence of any fence around playgrounds, pet dogs and stray dogs have free access to places assigned to children.

There was no significant difference in the number of positive findings in the samples of soil or sand. As the texture and humidity of the samples make a difference to the long survival of parasite eggs in environment, it is important to point out that in our investigation the sand and soil samples were almost equally moist, probably because of the proximity of the sea or the fact that the sand originated from the sea coast and was somewhat salty and had the ability to retain moisture. Our results are not in agreement with the findings of O'LORCAIN (1994) who found a higher contamination rate in soil than in sand.

The larger number of eggs as well as embryonated eggs found in samples taken from shady sites under trees can be attributed to the soil's ability to retain moisture and protection from sunlight, as well as the fact of the presence of dogs' behavioural patterns involving the selection of defecation sites (RUBEL and WISNIVESKY, 2005).

In conclusion, this study showed that environmental pollution with parasite transmissive stages poses a significant threat to public health. Considering the high soil and 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.

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**STOJČEVIĆ, D., V. SUŠIĆ, S. LUČINGER: Kontaminiranost zemlje i pijeska parazitskim elementima opasnim za ljudsko zdravlje u javnim parkovima i dječjim igralištima u Puli, Hrvatska. Vet. arhiv 80, 733-742, 2010.**

**SAŽETAK**

Svrha ovog istraživanja bila je utvrditi prisutnost parazitskih elemenata u uzorcima zemlje i pijeska iz parkova i dječjih igrališta u Puli kao i stupanj kontaminiranosti uzoraka s helmintskim jajima i protozojskim cistama i oocistama. Izabrano je devet parkova s dječjim igralištima i sakupljeno 90 uzoraka zemlje i pijeska, dvokratno, u mjesecu lipnju i prosincu. Nađena su jaja 4 nematodska roda i ciste i oociste iz 2 protozojska roda. Stupanj kontaminacije u prosincu se povećao u odnosu na lipanj. Od 90 uzoraka 15,5% bilo je pozitivno na jaja roda *Toxocara* u lipnju, a postotak se u prosincu povećao na 23,3%. Jaja vrste *A. lumbricoides*, podrijetlom iz ljudskog fecesa nađena su u 4,4% uzoraka u lipnju odnosno u 15,5% uzoraka u prosincu. U lipnju je 45% svih nađenih parazitskih jaja bilo embrionirano dok je u prosincu postotak embrioniranih jaja bio 75%. Statistički značajan porast ($P<0,05$) broja pozitivnih nalaza u prosincu u odnosu na lipanj utvrđen je samo za jaja vrste *A. lumbricoides.* Nije utvrđena značajna razlika u nalazima između uzoraka zemlje i pijeska.

**Ključne riječi**: kontaminacija zemlje, parazitska jaja, parkovi