Research article

ENVIRONMENTAL CONTAMINATION BY PARASITES IN PUBLIC PARKS IN BELGRADE IN THE CONTEXT OF ONE HEALTH APPROACH

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This paper studies the presence of developmental forms of gastrointestinal parasites in soil and samples, as well as in dog feces collected from public green spaces in Belgrade. The paper incorporates the spread analysis of contamination over different segments of parks. Four public green spaces in Belgrade were chosen, all containing an open-spaced children's playground and a fenced dog park. Sample analysis of soil/sand was examined using qualitative methods without concentration (native slide) and with concentration (passive sedimentation and gravitational centrifugal flotation). In total, 106 samples have been collected out of which 60 samples of soil, 36 of dog faeces and ten samples of sand. Seven different agents have been detected, out of which five nematodes - Ancylostoma/Uncinaria spp., Trichuris spp., Capillaria spp., Toxocara spp., Toxascaris leonina and two protozoa - Isospora spp. and Giardia intestinalis. The overall prevalence of contamination of soil samples was 31.67% and a statistically significant difference in the prevalence of ancylostoma eggs was found between different locations. The most common agent detected in the dog's faeces was Isospora spp., with a prevalence of 5.56%, followed by Giardia intestinalis and ancylostomatids with a prevalence of 2.78% each. The sand samples had no parasitic elements found. The contamination by parasites and by dogs' faeces was equally dispersed in all segments of the examined locations.

There is a great need to raise public awareness on the issue, and by the joint action of veterinarians, medical doctors, pet owners and people using public parks for recreation - a precondition is created for the sustainability of the "One Health" concept which implies the preservation of the environment and human and animal health.

Key words: Belgrade; contamination; dogs; One Health; parasites; parks

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INTRODUCTION

After the onset of the Covid-19 pandemic outbreak and social isolation, an increasing number of people in urban areas decided to have pets, especially dogs [1]. Walking with pets affects both the physical and psychosocial health of their owners. Arranged city parks and urban green areas are the most important places for recreation and children's play, and for dog walking. Even though there is a beneficial recreative and social role of public parks, developmental forms of parasites (especially of canine origin) which may have zoonotic potential, can contaminate urban green areas. Due to a large number of dogs in densely populated urban areas - city streets, public parks and other green city areas, but also schoolyards and children playgrounds, are becoming a source of constant contamination by developmental forms of different parasites [2,3]. The main source of environmental contamination by parasites is the feces of pet dogs, but also stray dogs and cats [4,5]. Parasites in the environment pose a health risk for both dogs and humans, especially children. Children are at high risk of infection from the environment because of their characteristic forms of behaviour such as lack of hand hygiene, onychophagy and geophagy [6]. Ingestion of infective parasite eggs is the main cause of infection or by direct penetration of larvae through the skin. The soil as the source of contamination by parasite elements represents a constant hazard for reinfection of dogs that visit certain city zones with their owners. Parasite eggs can also be brought into homes on dog's paws and people's shoes [7].

There are few studies conducted in Serbia in the past years concerning the issue of environmental contamination by zoonotic parasites. Thereby, public areas and playgrounds in Belgrade [8], Požarevac and Kostolac [9], as well as Niš [3] are found to be contaminated by developmental forms of zoonotic parasites. Numerous stray dogs are identified as an important source of contamination in Niš [5].

This study aimed to examine the presence of developmental forms of gastrointestinal parasites in dogs in samples of soil, sand and dog feces. All samples were collected in the capital of Serbia – Belgrade from city parks, which have both children's playgrounds, as well as playgrounds for dogs. Additionally, the distribution of contamination in different segments of parks was also examined.

MATERIAL AND METHODS

Ethical statement

The conducted research is not related to the use of animals. No ethical approval was obtained because this study did not involve animals and only involved non-invasive procedures (collecting soil, sand and faecal samples from the environment).

Samples and sampling locations

The city of Belgrade is located in southeastern Europe on the Balkan Peninsula (44°49'14" northern latitude and 20°27'44" eastern longitude) at the point where the

river Sava merges into the Danube, and the river waters surround it from three sides. Belgrade has a humid subtropical climate with four seasons with uniformly spread precipitation with about 691 mm precipitation a year. The study was conducted from November 2020 to February 2021 at four city parks in Belgrade. The samples were collected in the morning, on days without precipitation at an average temperature of 10-15 °C. In total, 106 samples were collected out of which 60 samples of soil, 10 samples of sand and 36 samples of dog's feces. The obligatory criteria for the location selection were a fenced playground for dogs and a playground for children within the park. Four parks were chosen - two in the old city area (Park I - Karadordev Park, Park II - Čuburski Park) and two parks in the new city area (Park III - Bežanijska kosa, Park IV - Novi Beograd - district 30). All four parks are located in densely populated parts of the city that lacks large green areas for daily dog walking (Figure 1). The parks are unfenced so free access is available to stray dogs and cats. Within every examined park, there is a playground for dogs surrounded by a wired fence and an open-spaced playground for children. The samples of soil and feces were obtained from all four parks, and samples of sand from three parks. The samples of soil/ sand were collected from different parts of the park (soil) and sandpits (sand), using a metal spatula from a square surface, 10 cm x 10 cm in width and lenght, and 5 cm depth, weighing approximately 100 g. Fresh samples of feces were collected from all four parks. Sampling was performed only once to avoid possible repetition of samples from the same animal and obtain falsely a higher prevalence of positive samples.



Figure 1. Sampling locations in Belgrade city (adapted from https://maps.google.com with original pictures of the locations) (1 - Karađorđev park; 2 – Čuburski park; 3 – Bežanijska kosa; 4 – Novi Beograd, district 30)

The samples were categorized based on the park segments: the playground for dogs (inside the fenced area and its direct surrounding), playground for children (inside

the playground and its direct surrounding) and the rest of the park (the area of the park that is not included in the dog's area neither the children's area); and sampling locations within these areas as subsegments: under the trees/bushes (shaded places, protected from sunlight), beside the benches for rest and from the remaining areas of the parks (in direct sunlight). All samples were packed in plastic bags, properly marked (according to the sampling site), transported on the same day to the laboratory of the Department for Parasitology at the Faculty of Veterinary Medicine University of Belgrade and examined in the following 48 hours.

Methods

Analysis of soil/sand samples was done using the concentration method – passive gravitational sedimentation and centrifugal flotation with saturated salt solution $(ZnSO_4, specifical density 1.18 at 20 °C)$. Faecal samples were examined using the native slide preparation with potassium iodide and centrifugal flotation with saturated salt solution $(ZnSO_4, specifical density 1.18 at 20 °C)$ [10]. All samples were examined in duplicate, by two experienced specialists, under light microscope (Olympus CX 23) using 100x i 400x magnifications.

Passive sedimentation. Approximately 50 g of soil/sand was homogenized in distilled water and filtered through a sieve to remove large particles. The homogenized solution was poured into a sedimentation cup and left to stand overnight. The supernatant was decanted and distilled water was poured again and this step was repeated until the supernatant was completely clear. After the last casting of the supernatant, the sediment was poured on a microscopic slide using a micropipette and covered with a coverglass and examined under the microscope.

Native slide with the addition of potassium iodide. A drop of physiological saline was placed on the microscopic slide and a part of the faecal sample (1:1) was homogenized in it. A drop of potassium iodide was added to the homogenate, covered with the coverglass and examined under the microscope.

Centrifugal flotation with saturated zinc sulphate solution. A sample weighing 3-5 g was homogenised in 30 ml of aqueous saturated zinc sulphate solution. The homogenized mixture was then filtered through a sieve to remove large particles and the filtered solution was poured into 15 ml glass tubes. The tubes were centrifuged at $500 \times g$ for 5 min. After centrifugation, the tubes were filled with flotation solution until a positive meniscus appeared, covered with a coverglass and left to sit for another 10 min at room temperature. During this time, parasitic elements, which are lighter than the specific gravity of the flotation solution, adhere to the underside of the coverglass. The coverglass was then removed from the test tube, placed on the microscope glass slide and observed under the microscope.

Statistical analysis

All the results were processed in *Graph Pad Prism* software. *Chi*-squared test was used to determine the statistical significance of differences in the prevalence of parasitic elements. Differences were determined at the level of statistical significance of p < 0.05 and p < 0.001. Results are shown in tables.

RESULTS

Seven different parasites were detected in the total of 106 samples: five nematodes -*Ancylostoma/Uncinaria* spp., *Trichuris* spp., *Capillaria* spp., *Toxocara* spp., *Toxascaris leonina* and two protozoa - *Isospora* spp. and *Giardia intestinalis* (Table 1). The overall prevalence of contamination in all examined samples was 21.7% (23/106). A statistically significant difference (p<0.05) in the prevalence of ancylostomatid eggs was detected between soil samples collected in different parks with the highest prevalence of 24.14% (7/29) in park IV. In the soil samples, an overall prevalence of 31.67% (19/60) was detected. A very statistically significant difference (p<0.05) in the prevalence of ancylostomatid eggs was detected in the soil samples between examined parks with the highest prevalence of 43.75% (7/16) in park IV. The most prevalent agents in the soil samples were Ancylostomatidae - 20% (12/60), followed by *Trichuris* spp. and *Capillaria* spp. with the same prevalence of 3.33% (2/60), while other agents were present with a prevalence of 1.67% (1/60) (Table 2, Figure 2).

| | Park | | | | | | | | | | | | |
|--|-------------|-------|--------------|-------|---------------|-------|--------------|-------|------------------|-------|------|-------|--|
| Parasitic elements | I (n=29) | | II (n=22) | | III (n=26) | | IV (n=29) | | Total (n=106) | | χ2 | р | |
| | Ν | % | Ν | % | Ν | % | Ν | % | Ν | % | | | |
| Ancylostomatidae | 5 | 17.24 | 1 | 4.55 | 0 | 0 | 7 | 24.14 | 13 | 12.26 | 9.32 | 0.02* | |
| Trichuris spp. | 0 | 0 | 1 | 4.55 | 1 | 3.85 | 0 | 0 | 2 | 1.89 | 2.50 | 0.47 | |
| <i>Capillaria</i> spp. | 0 | 0 | 1 | 4.55 | 1 | 3.85 | 0 | 0 | 2 | 1.89 | 2.50 | 0.47 | |
| Toxocara spp. | 0 | 0 | 1 | 4.55 | 0 | 0 | 0 | 0 | 1 | 0.94 | 3.86 | 0.28 | |
| Toxascaris leonina | 1 | 3.45 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.94 | 2.68 | 0.44 | |
| Isospora spp. | 0 | 0 | 0 | 0 | 2 | 7.69 | 1 | 3.45 | 3 | 2.83 | 3.48 | 0.32 | |
| Giardia intestinalis | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3.45 | 1 | 0.94 | 2.75 | 0.43 | |
| Total | 6 | 20.69 | 4 | 18.18 | 4 | 15.38 | 9 | 31.03 | 23 | 21.70 | | | |
| Mixed contamination | | | | | | | | | | | | | |
| <i>Trichuris</i> spp./ Ancylostomatidae | 0 | 0 | 1 | 4.55 | 0 | 0 | 0 | 0 | 1 | 4.55 | 3.86 | 0.28 | |
| Total | 0 | 0 | 1 | 4.55 | 0 | 0 | 0 | 0 | 1 | 4.55 | | | |

Table 1. Prevalence of parasitic contamination in the examined parks

*p<0.05, n – total number of samples, N – positive samples

| | Soil samples in the examined parks | | | | | | | | | | | | |
|--|------------------------------------|----|--------------|-------|---------------|-------|--------------|-------|-----------------|-------|------|------------|--|
| Parasitic elements | I (n=20) | | II (n=11) | | III (n=13) | | IV (n=16) | | Total (n=60) | | χ2 | р | |
| | Ν | % | Ν | % | Ν | % | Ν | % | Ν | % | | | |
| Ancylostomatidae | 4 | 20 | 1 | 9.09 | 0 | 0 | 7 | 43.75 | 12 | 20 | 9.71 | 0.02^{*} | |
| Trichuris spp. | 0 | 0 | 1 | 9.09 | 1 | 7.69 | 0 | 0 | 2 | 3.33 | 3.14 | 0.37 | |
| <i>Capillaria</i> spp. | 0 | 0 | 1 | 9.09 | 1 | 7.69 | 0 | 0 | 2 | 3.33 | 3.14 | 0.37 | |
| Toxocara spp. | 0 | 0 | 1 | 9.09 | 0 | 0 | 0 | 0 | 1 | 1.67 | 4.53 | 0.21 | |
| Toxascaris leonina | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1.67 | 2.03 | 0.56 | |
| Isospora spp. | 0 | 0 | 0 | 0 | 1 | 7.69 | 0 | 0 | 1 | 1.67 | 3.68 | 0.30 | |
| Total | 5 | 25 | 4 | 36.36 | 3 | 23.08 | 7 | 43.75 | 19 | 31.67 | | | |
| Mixed contamination | | | | | | | | | | | | | |
| <i>Trichuris</i> spp./ Ancylostomatidae | 0 | 0 | 1 | 9.09 | 0 | 0 | 0 | 0 | 1 | 1.67 | 4.53 | 0.21 | |
| Total | 5 | 25 | 3 | 27.27 | 3 | 23.07 | 7 | 43.75 | 1 | 0 | | | |

Table 2. Prevalence of parasitic elements in soil samples in the examined parks

*p<0.05, n – total number of samples, N – positive samples

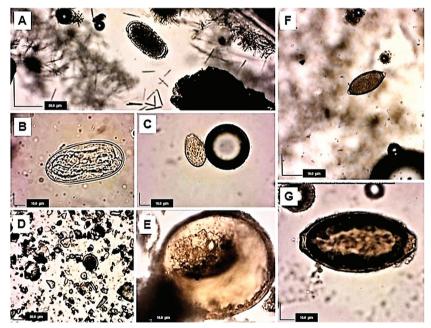


Figure 2. Parasitic elements detected in the soil samples (**A** – unembrionated ancylostomatid egg, 100x; **B** – embryonated ancylostomatid egg, 400x; **C** – *Isospora* spp. oocyst, 400x; **D** – *Toxocara* spp. egg 100x; **E** – *Toxascaris leonina* egg 400x; **F** – trichurid egg 100x; **G** – capillarid egg 400x) (original).

Out of a total of 36 samples of feces, the most prevalent agent was *Isospora* spp. with a prevalence of 5.56% (2/36), while *G. intestinalis* and ancylostomatid eggs were present with the same prevalence of 2.78% (1/36) (Table 3, Figure 3).

| | | Feces samples in the examined parks | | | | | | | | | | | |
|-----------------------|------------|-------------------------------------|-------------|---|---------------|----|--------------|----|--------------|-------|------|------|--|
| Parasitic elements | I (n=9) | | II (n=7) | | III (n=10) | | IV (n=10) | | Total (n=36) | | χ2 | р | |
| | Ν | % | Ν | % | Ν | | | % | Ν | % | | - | |
| Ancylostomatidae | 1 | 11.11 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2.78 | 3.09 | 0.38 | |
| Isospora spp. | 0 | 0 | 0 | 0 | 1 | 10 | 1 | 10 | 2 | 5.56 | 1.69 | 0.64 | |
| Giardia intestinalis | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 10 | 1 | 2.78 | 2.67 | 0.44 | |
| Total | 1 | 11.11 | 0 | 0 | 1 | 10 | 2 | 20 | 4 | 11.11 | | | |

| Table 3. | Prevalence of | parasitic elements i | n the feces san | nples in the | examined parks |
|----------|---------------|----------------------|-----------------|--------------|----------------|
|----------|---------------|----------------------|-----------------|--------------|----------------|

*p<0.05, n - total number of samples, N - positive samples



Figure 3. Parasitic elements detected in the samples of feces of dogs (A-) *Isospora* spp. oocyst, 400x; (B-) ancylostomatid egg, 400x; (C-) *Giardia intestinalis* cyst, 400x) (original)

In three different park sand samples there were no parasitic elements.

| Table 4. | Prevalence of | parasitic elements in | different segments of | parks |
|----------|---------------|-----------------------|-----------------------|-------|
|----------|---------------|-----------------------|-----------------------|-------|

| | | Examined segments of parks | | | | | | | | | | |
|--|--------------------------------------|----------------------------|--------------|----------------------------------|--------|-------------------------|-------|----------------|------|------|--|--|
| Parasitic elements | Playground for children (n=38) | | Playg for | Playground for dogs (n=40) | | rest of park =28) | ר | lotal =106) | χ2 | р | | |
| | Ν | % | Ν | % | Ν | % | Ν | % | | | | |
| Ancylostomatidae | 5 | 13.16 | 5 | 12.5 | 3 | 10.71 | 13 | 12.26 | 0.09 | 0.95 | | |
| Trichuris spp. | 1 | 2.63 | 0 | 0 | 1 | 3.57 | 2 | 1.89 | 1.31 | 0.52 | | |
| <i>Capillaria</i> spp. | 1 | 2.63 | 1 | 2.5 | 0 | 0 | 2 | 1.89 | 0.73 | 0.69 | | |
| Toxocara spp. | 0 | 0 | 0 | 0 | 1 | 3.57 | 1 | 0.94 | 2.81 | 0.25 | | |
| Toxascaris leonina | 1 | 2.63 | 0 | 0 | 0 | 0 | 1 | 0.94 | 1.81 | 0.41 | | |
| Isospora spp. | 3 | 7.89 | 0 | 0 | 0 | 0 | 3 | 2.83 | 5.25 | 0.06 | | |
| Giardia intestinalis | 0 | 0 | 0 | 0 | 1 | 3.57 | 1 | 0.94 | 2.81 | 0.25 | | |
| Total | 11 | 28.95 | 6 | 15 | 6 | 21.43 | 23 | 21.70 | | | | |
| | | | Mixed | contami | inatio | n | | | | | | |
| <i>Trichuris</i> spp./ Ancylostomatidae | 1 | 2.63 | 0 | 0 | 0 | 0 | 1 | 0.94 | 1.81 | 0.41 | | |
| Total | 1 | 2.63 | 0 | 0 | 0 | 0 | 1 | 0.94 | | | | |

*p<0.05, n – total number of samples, N – positive samples

Comparing different examinated segments on the described locations (Table 4) no statistical significance in the prevalence of parasitic elements in playgrounds for

dogs, playgrounds for children and the rest of the park was detected. In addition, no statistical significance in the prevalence of parasitic elements was detected according to the different sampling sites (Table 5) on the examined locations (under the trees/ bushes, beside benches and the rest of the parks).

| | Sampling sites within the parks | | | | | | | | | | | |
|--|---------------------------------|-------|-------------------|-------|-----------------------------------|-------|--------------------|---|------------------|-------|------|------|
| Parasitic elements | Trees/ bushes (n=32) | | Benches (n=12) | | The rest of the park (n=51) | | Sandpits (n=11) | | Total (n=106) | | χ2 | р |
| - | Ν | % | Ν | % | Ν | % | Ν | % | Ν | % | | |
| Ancylostomatidae | 2 | 6.25 | 1 | 8.33 | 10 | 19.61 | 0 | 0 | 13 | 12.26 | 5.34 | 0.15 |
| Trichuris spp. | 0 | 0 | 1 | 8.33 | 1 | 1.96 | 0 | 0 | 2 | 1.89 | 3.52 | 0.32 |
| <i>Capillaria</i> spp. | 1 | 3.13 | 0 | 0 | 1 | 1.96 | 0 | 0 | 2 | 1.89 | 0.71 | 0.87 |
| Toxocara spp. | 1 | 3.13 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.94 | 2.37 | 0.50 |
| Toxascaris leonina | 1 | 3.13 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.94 | 2.37 | 0.50 |
| Isospora spp. | 0 | 0 | 0 | 0 | 3 | 5.88 | 0 | 0 | 3 | 2.83 | 3.27 | 0.35 |
| Giardia intestinalis | 0 | 0 | 0 | 0 | 1 | 1.96 | 0 | 0 | 1 | 0.94 | 1.10 | 0.77 |
| Total | 5 | 15.63 | 2 | 16.67 | 16 | 31.37 | 0 | 0 | 23 | 21.70 | | |
| Mixed contamination | | | | | | | | | | | | |
| <i>Trichuris</i> spp./ Ancylostomatidae | 0 | 0 | 1 | 8.33 | 0 | 0 | 0 | 0 | 1 | 0.94 | | |
| Total | 0 | 0 | 1 | 8.33 | 0 | 0 | 0 | 0 | 1 | 0.94 | | |

Table 5. Prevalence of parasitic elements in different sampling sites within the examined parks

*p<0.05, n - total number of samples, N - positive samples

DISCUSSION

The intensity of environmental contamination by parasites depends on numerous factors i.e. climatic, sociodemographic, type of samples, etc. so the prevalences and parasite species may vary in different studies. The most prevalent gastrointestinal parasites of dogs and cats are nematodes Ancylostoma/Uncinaria spp., Toxocara spp. and T. vulpis [11,12] and protozoa G. intestinalis and their developmental forms can survive in the environment for a long period. Only the zoonotic potential of T. vulpis is still controversial, while all the forementioned agents can cause diseases in humans. One of the most important nematodoses with zoonotic potential is toxocarosis [13]. Ascaridid nematodes from Toxocara genera enter the organism of true hosts by ingestion of embryonated eggs, and transplacental (T. canis) and galactogenic (T. canis, Toxocara cati) routes are important in cubs [14]. Infection of humans with *Toxocara* spp. can cause two sindromes - viscelar larva migrans, which can be found in the liver, lungs, brain and other visceral organs and ocular larva migrans, which have an affinity for the eyes and the optical nerve [14]. In the seroepidemiological study of toxocarosis in children and adults in Belgrade [15] it was concluded that contact with dogs is not the main route of infection but contaminated soil, because most of the positive patients denied contact with dogs. In addition, no significant difference in the prevalence between positive children and adults was detected. In the current study, the eggs of Toxocara spp. were found in the free segment of park II. The samples of feces from all four parks were negative for the presence of T. canis eggs. This can be explained by the fact that the eggs of T. canis are mostly found in the feces of puppies from three to six weeks of age (transplacentar and galactogenic infection) [16] when dogs are quarantined because of the period required to acquire immunity after compulsory vaccination against infectious diseases. Results in the study in Japan show that cats have a leading role in the contamination of sandpits in the playgrounds for children and that the contamination is mainly caused by T. cati eggs [17,18]. In the current study in Belgrade, no parasite elements were found in the sand samples (0/10) taken from one playground for children and two playgrounds for dogs. These results are in concordance with a similar study conducted in Japan [18], where only nine samples were positive out of 107 examined samples of sand from sandpits. Also, a low prevalence (18.6%) of helminth eggs was detected in the samples of sand in the parks in Poland [19]. The cause of the negative results in the recent study in Belgrade can be the period of sampling (autumn, rainy season) and faster drainage and leaching of sand compared to the soil. In addition, cats usually avoid spaces where a high number of dogs is constantly present, such as the examined locations in densely populated city areas. Sand samples from parks III and IV were collected from fenced playgrounds for dogs, and dogs less often than cats express a form of behaviour related to defecation in the sand substrate, which results from the need of cats to bury their faeces.

The global prevalence of *T. leonina* infection is estimated to be about 2.9% in dogs and 3.4% in cats, with it occurring more frequently in the eastern Mediterranean [20]. Since the infection in dogs and cats is strictly caused by ingestion of embryonated eggs/ transport hosts [16], the contaminated environment represents a significant route of infection. The presence of *T. leonina* eggs was detected in Park I (5.0%) in the sample collected under a tree, where the risk of transmission is higher due to the behavior of dogs related to sniffing and territory marking.

A very high prevalence (43.75%) of ancylostomatid eggs was detected in soil samples in Park IV, as well as a statistically significant difference (p<0.05) in the prevalence in the examined parks, and also these were the only nematodes found in the feces samples. The possible reason for this may be the position of this park – it is located in a very densely populated part of the city with numerous high buildings lacking large green areas. Similar results were obtained in Lisboa [21], where ancylostomatid eggs were the only contaminants of the soil (27.8%) and the most prevalent parasites in the faeces of dogs (16.5%). The most prevalent protozoans in the research were *Cryptosporidium* spp. (11.9%) and *Giardia* spp. (11.4%), while *Cystoisospora* spp. were detected only in 1.1% samples, and nematodes *T. leonina* and *Toxocara* spp. only in 1.1% and 0.5%, respectively, which is in concordance with the current study in Belgrade. In the study in Niš, [5] the authors concluded that the main cause of contamination of public parks is numerous stray dogs that inhabit these locations. Stray dogs are the population of free-living animals; they are not protected with ectoantiparasitics or other hemoterapeutics, which makes them an important source of contamination of public areas with canine parasites present in the feces [22]. In the current study in Belgrade, no stray dogs or cats were spotted either in the examined parks or the area, so it is justified to assume that the collected samples origin from pet dogs. This assumption is supported by the fact that the organized sterilization/castration of stray dogs (followed by obligatory vaccination and dehelmintisation) is being implemented on the territory of Belgrade since 2011, as a part of the strategy for solving the problem of stray dogs on the territory of Belgrade (Official Gazette of the City of Belgrade, No. 37/2011). Pavlović et al. concluded back in 2013 [23] that the number of stray dogs in Belgrade is significantly lower since the implementation of the strategy and, consequently, the contamination of public green areas is lower compared to previous years, which is also in accordance with the current study.

After ancylostomatids, the most prevalent contaminants in this research are Trichuris spp. and Capillaria spp. Trichuris trichiura is a human pathogen, while T. vulpis causes infection in dogs and other canids. The eggs of these two species are morphologically similar and their dimensions overlap so it is not possible to determine the parasite at the species level using light microscopy [24]. The zoonotic potential of T. vulpis and its possibility to cause visceral larva migrans and diarrhea in people is still controversial [25]. The eggs embryonate in the environment and in the soil it takes about 3-8 weeks for the eggs to become infective. The eggs are very resistant and can survive for a long period in shaded, moist places in the soil. This is the reason why the risk of reinfection is higher for the dogs which are in constant contact with the contaminated soil, i.e. dogs visiting the same parks daily [26]. The incidence is higher in adult dogs than in the puppies, because of the long prepatent period (2-3 months) and no transplacentar/ galactogenic route of transmission for T. vulpis. Savadelis et al. [27] examined 200 samples of dog's faeces of which 27% was positive for the presence of helminth eggs - 8.5% trichurid eggs, 17% ancylostoma eggs and 1.5% T. canis eggs. Most of the positive samples with acylosotma eggs were found within one park, while samples positive for T. vulpis and T. canis were from few different parks. Blaszkowska et al [28] detected helminth eggs in 15.7% of samples collected around children sports playgrounds, 7.7% from the playground for children within the parks and 1.4% from fenced sandpits. More samples that are positive were found in the spring than in the autumn. The authors state that the reason for the low contamination of sandpits in the playgrounds for children is that the sandpits are fenced and access for dogs is almost impossible. In the examined locations in Belgrade, there was no statistical significance in the contamination of different segments within the parks. However, the highest prevalence of the ancylostomatid positive samples (13.16%) and mixed contamination with ancylostomatid eggs/Trichuris spp. were found nearby the playgrounds for children. The reason for that may be the behaviour of pet owners who let the dogs play in the playgrounds for children, but also the fact that all the examined locations were not fenced. Larvae of A. caninum can penetrate the skin of humans and cause

cutaneous larva migrans syndrome, followed by the presence of folliculitis and papules/ pustules on the skin [29]. The infection is mainly caused by direct penetration of the infective larvae from the soil or sand through barefoot. The contamination from the faeces of infected dogs can be high because a large number of eggs is being excreted in the environment through feces in a few weeks [10]. The results obtained in the retrospective analysis of the prevalence of intestinal helminthoses of dogs and cats in Serbia [12] show that T. canis is the most prevalent in dogs younger than one year, because of the galactogenic and transplacentar route of transmission of this parasite. On the other hand, infections caused by ancylostomatids are not detected at all in dogs younger than one year, while T. vulpis is mainly detected in older dogs. In the current research in Belgrade, the most prevalent parasites in the faeces of dogs were protozoa - Isospora spp. and G. intestinalis. Unlike other agents they need a period for sporulation/embrionation in the environment, Giardia cysts are infective immediately after excretion and they can survive for up to 65 days at 4°C [30]. There are misconceptions among pet owners that only one dose of antiparasitic is enough for the treatment of parasitic infections. It is common for pet owners to self-administer anthelmintics only once every few months without prior coprological testing, which creates the possibility of improper treatment of parasite species and creates a constant risk for the development of resistance to antiparasitics [31,32]. Regular coprological testing (at least two times a year) is important because commercially available and most commonly used antiparasitics usually do not treat protozoa, which are the most prevalent species detected in dog's faeces in the current research. In addition, one dose of anthelmintic is not efficient for the treatment of infections caused by T. vulpis and Ancylostoma/Uncinaria spp. [33,16].

People's awareness of the risk of transmission of zoonotic parasites is very low [33]. Therefore, the role of veterinarians in educating pet owners about the existence of infections that can be transmitted from animals to people is very important. According to the World Health Organization, "One Health" is an approach for designing and implementing programmes, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes. By the joint action of veterinarians, medical doctors, pet owners and people using public parks for recreation - a precondition is created for the sustainability of the "One Health" concept which implies the preservation of the environment and human and animal health. It is necessary to educate pet owners about the importance of removing dog's faeces from public areas, as well as the importance of regular coprological testing in order to prevent parasitic infections and consequently - the environmental contamination. The structure of the park is of great significance - playgrounds for dogs should be fenced, there should be environmentally friendly disposal bags for feces, trash cans should be closed to prevent birds and other animals from spreading garbage; playgrounds for children should also be fenced, and people shouldn't let dogs in the areas of park intended for children, but children also shouldn't play in the areas intended for dogs.

CONCLUSION

In the public parks in Belgrade, which contain playgrounds for dogs and playgrounds for children, moderate contamination by parasitic elements has been determined. The contamination by parasites but also by dog's feces is equally represented in all different segments of public parks. The parasitic contamination of the environment, especially in urban areas, is impossible to eliminate, but it can be reduced by implementing proper preventive measures. Education of pet owners about the importance of removing dog's faeces from public areas. Besides, the importance of regular coprological testing in order to prevent parasitic infections and consequently the environmental contamination by parasites.

Authors' contributions

DB conceived and designed the study, and wrote the manuscript together with ND. DB, ND and NJ participated in collecting and analyzing the samples. TI, NS and ZK critically revised the manuscript and made substantial contribution to interpretation of data. KN performed the statistical analysis. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

- 1. Morgan L, Protopopova, Dupont Birkler RI, Itin-Shwartz B, Abbels Sutton G, Gamliel A, Yakobson B, Raz T. Human-dog relationships during the COVID-19 pandemic: booming dog adoption during social isolation. Humanities and Social Sciences Communications 2020, 7:155.
- 2. Phasuk N, Kache R, Thongtup K, Boonmuang S, Punsawad C: Soil Contamination with *Taxocara* eggs in public schools in rural areas of Southern Thailand. J Trop Med 2020, 9659640:1-6.
- 3. Ristić M, Miladinović-Tasić N, Dimitrijević S, Nenadović K, Bogunović D, Stepanović P, Ilić T: Soil and sand contamination with canine intestinal parasite eggs as a risk factor for human health in public parks in Niš (Serbia). Helminthologia 2020(a), 57:109-119.
- 4. Simonato G, di Regalbono AF, Cassini R, Traversa D, Tessarin C, Di Cesare A, Pietrobelli M: Molecular detection of *Giardia duodenalis* and *Cryptosporidium* spp. in canine faecal samples contaminating public areas in Northern Italy. Parasitol Res 2017, 116:3411-3418.
- Ristić M, Dimitrijević S, Višnjić A, Bogunović D, Gajić B, Stojanović M, Ilić T: Dogs from public city parks as a potential source of pollution of the environment and risk factor for human health. Indian J Anim Sci 2020(b), 90:535–542.

- 6. Cassenote A, de Abreu Lima A, Pinto Neto J, Rubinsky-Elefant G: Seroprevalence and modifiable risk factors for *Toxocara* spp. in Brazilian schoolchildren. Plos Negl Trop Dis 2014, 8:e2830.
- 7. Panova O, Khrustalev A: Dog walking brings *Toxocara* eggs to people's homes. Vet Parasitol 2018, 262:16-19.
- 8. Pavlović I, Terzin V, Terzin D, Stanković B, Ilin M: Parasitic contamination of the parks of the central municipalities of Belgrade during 2008 (in Serbian). Proceedings of the XII symposium epizootiological days with international participation 2008, Oplenac Topola, Serbia, pp. 177-180.
- Pavlović I, Teodor B, Stojanović D: Results of parasitological inspection of parks and sandpits in Požarevac and Kostolac (in Serbian). Proceedings of the expert conference "Pest control in the urban environment" VI Belgrade conference with international participation 2003, Belgrade, Serbia, pp. 159-163.
- 10. Dryden MW, Payne PA, Ridley R, Smith V: Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts, Veterinary therapeutics: research in applied veterinary medicine 2005, 6:15-28.
- 11. Anderson R: Nematode parasites of vertebrates. Their development and transmission, 2nd edition. CABI international; 2000, 672 pp.
- 12. Ilić T, Kulišić Z, Antić N, Radisavljević K, Dimitrijević S: Prevalence of zoonotic intestinal helminths in pet dogs and cats in the Belgrade area. J Appl Anim Res 2017, 45:204-208.
- 13. Ma G, Rostami A, Wang T, Hofmann A, Hotez PJ, Gasser RB: Global and regional seroprevalence estimates for human toxocariasis: A call for action. Adv Parasitol 2020, 109:273-288.
- Despommier D: Toxocariasis: clinical aspects, epidemiology, medical ecology and molecular aspects. Clin Microbiol Rev 2003, 16:265-272.
- Čolović Čalovski I, Jekić A, Stevanović O, Dubljanin E, Kulišić Z, Džamić AM: Anti-*Toxocara* antibodies in patients with suspected visceral larva migrans and evaluation of environmental risk of human infection in Belgrade, Serbia. Archives of Biological Sciences Belgrade 2014, 66:545-551.
- 16. Traversa D: Pet roundworms and hookworms: A continuing need to global worming. Parasit Vectors 2012, 5:91.
- 17. Uga S, Minami T, Nagata K: Defecation habits of cats and dogs and contamination by *Toxocara* eggs in public park and sandpits. Am J Trop Med Hyg 1996, 54:122-126.
- Matsuo J, Nakashio S: Prevalence of fecal contamination in sandpits in public parks in Sapporo City, Japan. Vet Parasitol 2004, 128:115-119.
- 19. Bojar H, Kłapeć T: Contamination of soil with eggs of geohelminths in recreational areas in the Lublin region in Poland. Ann Agric Environ Med 2012, 19:267-270.
- Rostami A, Riahi SM, Omrani VF, Wang T, Hofmann A, Mirzapour A, Foroutan M, Fakhri Y, Macpherson CNL, Gasser RB: Global prevalence estimates of *Toxascaris leonina* infection in dogs and cats. Pathogens 2020, 9:503.
- Ferreira A, Alho AM, Otero D, Gomes L, Nijsee R, Overgaauw PAM, de Carvalho LM: Urban dog parks as sources of canine parasites: contamination rates and pet owner behaviours in Lisbon, Portugal. J Environ Public Health 2017, 5984086.
- 22. Tudor P: Soil contamination with canine intestinal parasites eggs in the parks and shelter dogs from Bucharest area. Agric Agric Sci Proc 2015, 6:387-391.

- 23. Pavlović I, Samokovlija A, Elezović M, Marić J: Significance of parasitological control of green areas contamination in urban environments (in Serbian). Veterinarski žurnal Republike Srpske (Veterinary Journal of Republic of Srpska (Banja Luka)) 2013, 13:193-202.
- 24. Cutillas C, de Rojas M, Ariza C, Ubeda JM, Guevara D: Molecular identification of *Trichuris vulpis* and *Trichuris suis* isolated from different hosts. Parasitol Res 2007, 100:383-389.
- Dunn JJ, Columbus ST, Aldeen WE, Davis M, Carroll KC: *Trichuris vulpis* recovered from a patient with chronic diarrhea and five dogs. J Clin Microbiol 2002, 40:2703-2704.
- Taylor MA, Coop RL, Wall RL: Veterinary Parasitology, 3nd edition. Blackwell Publishing, New Jersey; 2007, 1032 pp.
- 27. Savadelis MD, Evans CC, Mabry KH, LeFavi LN, Klink BD, von Simson C, Moorhead AR: Canine gastrointestinal nematode transmission potential in muncipal dog parks in the southeast United States. Vet Parasitol Regional Studies and Reports 2019, 18:100324.
- 28. Blaszkowska J, Wojcik A, Kurnatowski P, Szwabe K: Geohelminth egg contamination of children's play areas in the city of Lodz (Poland). Vet Parasitol 2013, 192:228-233.
- 29. Caumes E, Ly F, Bricaire F: Cutaneous larva migrans with folliculitis: report of seven cases and review of the literature. Br J Dermatol 2002, 146:314-316.
- 30. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev 2011, 24:110-140.
- Kopp SR, Kotze AC, McCarthy JS, Coleman GT: High-level pyrantel resistance in the hookworm *Ancylostoma caninum*. Vet Parasitol 2007, 143:299–304.
- 32. Abongwa M, Martin RJ, Robertson AP: A brief review on the mode of action of antinematodal drugs. Acta Veterinaria-Beograd 2017, 67:137-152.
- 33. Traversa D: Are we paying too much attention to cardio-pulmonary nematodes neglection old-fashioned worms like *Trichuris vulpis*. Parasit Vectors 2011, 4.
- 34. Smith A, Semeniuk C, Rock M, Massolo A. Reported off-leash frequency and perception of risk for gastrointestinal parasitism are not associated in owners of urban park-attending dogs: A multifactorial investigation. Prev Vet Med 2015, 120:336-348.

KONTAMINACIJA ŽIVOTNE SREDINE PARAZITIMA U JAVNIM PARKOVIMA U KONTEKSTU KONCEPTA JEDNOG ZDRAVLJA

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U ovom radu je istraženo prisustvo razvojnih oblika gastrointestinalnih parazita u uzorcima zemlje i peska, kao i fecesu pasa, prikupljenim sa javnih zelenih površina u Beogradu. U okviru istraživanja izvršena je i analiza prisustva kontaminacije u okviru različitih segmenata parkova. Izabrane su četiri javne zelene površine u Beogradu, koje obuhvataju otvoreno dečje igralište i ograđeno igralište za pse. Analiza uzoraka zemlje/peska vršena je kvalitativnom metodom bez koncentrisanja (nativni preparat) i sa koncentrisanjem (pasivna sedimentacija i gravitaciona flotacija sa centrifugiranjem). Ukupno je sakupljeno 106 uzoraka, od čega 60 uzoraka zemlje, 36 uzoraka fecesa pasa

i 10 uzoraka peska. Ustanovljeno je sedam različitih uzročnika, od toga pet nematoda -*Ancylostoma/Uncinaria* spp., *Trichuris* spp., *Capillaria* spp., *Toxocara* spp., *Toxascaris leonina* i dve protozoe - *Isospora* spp. i *Giardia intestinalis*. Ukupna prevalencija kontaminacije uzoraka zemlje iznosila je 31,67% i utvrđena je statistički značajna razlika u prevalenciji ankilostomatida između različitih lokacija. Najčešći uzročnik u fecesu pasa je *Isospora* spp., sa prevalencijom 5,56%, a slede *Giardia intestinalis* i ankilostomatide sa prevalencijom od 2,78%. U uzorcima peska nije ustanovljeno prisustvno parazitskih elemenata. Kontaminacija parazitima i fecesom pasa bila je podjednako prisutna u svim segmentima ispitivanih lokacija.

Postoji velika potreba za podizanjem svesti ljudi o ovoj temi, a zajedničkim delovanjem veterinara, lekara, vlasnika kućnih ljubimaca i ljudi koji posećuju javne parkove za rekreaciju, stvara se preduslov za održivost koncepta "Jednog zdravlja", koji podrazumeva očuvanje životne sredine i zdravlja ljudi i životinja.