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DOI 10.2478/helm-2020-0018

HELMINTHOLOGIA, 57, 2: 109 – 119, 2020

Soil and sand contamination with canine intestinal parasite eggs as a risk factor for human health in public parks in Niš (Serbia)

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Article info

Received November 15, 2019
Accepted March 6, 2020

Summary

Regarding geographical distribution and clinical relevance, the most common canine geohelminths are *Toxocara canis*, ancylostomatids, and *Trichuris vulpis*. Canine intestinal parasites from the soil and sand present an important potential serious human health hazard, especially for the children preschool and school – age. This paper aimed to establish the degree of contamination of soil and sand with zoonotic parasites from the canine feces and the degree of risk they could pose for human health in public places and playgrounds in the city of Niš. Our parasitological study involved 200 soil samples and 50 sand samples from the public parks in the city of Niš in southeastern Serbia (43°19'15"N, 21°53'45" E). From several locations, about 100 g of soil and sand was collected based on the bioclimatic indices. Parasitological diagnosis was performed using conventional qualitative and quantitative coprological methods, abiding by the recommendations about the diagnosis of parasitic diseases. In 38 – 46 % of soil samples and 40 % of sand samples seven species of endoparasites were diagnosed. In the samples of soil, a medium and high degree of contamination with the ascarid *T.canis* (14 – 22 %) was detected, as well as a low and medium degree of contamination with ancylostomatids (4 – 12 %), and in the samples of sand, a variable degree of contamination with the helminths *T. canis* (26 %) and *A. alata* (16 %) was found. A statistically significant difference was found in the contamination with *A. alata* eggs between the samples of sand and samples of soil. The studied public surfaces represent the reservoir of zoonotic parasites, which is a public health problem requiring a synergistic action of several factors to be successfully resolved, i.e. the implementation of prevention, surveillance, and control measures.
Keywords: intestinal parasites; dog; contamination; public spaces; public health

Introduction

More than 2 billion people worldwide are exposed to a risk for geohelminth infections. Regarding geographical distribution and clinical relevance, the most common canine helminths are *Toxocara canis*, *Ancylostomatidae*, and *Trichuris vulpis*, but the significance of these pathogens has been often underestimated by both veterinary and human medical professionals, as well as the general public (Traversa *et al*., 2014).

Human contact with the soil is one of the possible ways of spread-

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ing the canine intestinal parasites. These pathogens reach the soil via canine and human feces and can survive in this environment for a long period, making it a potential reservoir of infection (Tudor, 2015). The developing forms of geohelminths can survive the longest and enter the organism of susceptible individuals via skin or wounds in the visible mucosa (*Ancylostoma caninum*, *Uncinaria stenocephala*, *Strongyloides stercoralis*), causing a cutaneous larva migrans (Heukelbach & Feldmeier, 2008; Jaleta *et al*., 2017), or by ingestion (*T. canis*), when a visceral or ocular larva migrans is formed, which may persist in human tissues even for as long as several years (Aydenizöz-Özkayhana *et al*., 2008**;** Overgaauw & van Knapen, 2013). The infections such as these are mainly sporadic, but in extraordinary situations (natural disasters and wars), they can also occur in epidemic scales (Tudor, 2015).

According to the information from the available literature, different degrees of soil contamination with helminth eggs and larvae from canine feces have been established. In Poland, the percentage was between 7.9 % and 10.6 % (Blaszkovska *et al*., 2012) and 18.6 % (Bojar & Kłapeć, 2012); in Italy, 24 % (Habluetzel *et al*., 2003); in Portugal, 63.3 % (Otero *et al*., 2014); in Slovakia, 79.2 % (Rudohradska *et al*., 2011); in India, 12.84 % (Sudhakar *et al*., 2013); in Spain, 71.33 % (Martínez-Moreno *et al*., 2007); and in Romania, 22.22 % (Tudor, 2015).

The studies performed in public parks in several Serbian cities, such as Belgrade (Pavlović *et al*., 2015), Požarevac, Kostolac (Pavlović *et al*., 2015) and Kruševac (Raičević & Pavlović, 2019), have demonstrated a degree of contamination of urban green spaces, soil, and sand ponds in day-care facilities for pre-school children with zoonotic protozoa, nematodes, and cestodes.

This paper aimed to establish the degree of soil contamination and sand with zoonotic parasites from the canine feces and the degree of risk they could pose for human health in public places and playgrounds in the city of Niš.

Material and Methods

Study design

In the parasitologic examination performed in the period February – May 2019, we collected and analyzed 200 samples of soil and 50 samples of sand. The soil was sampled in three public parks in Niš: Tvrđava (Park 1) – 50 samples; Čair (Park 2) – 50 samples; Sveti Sava (Park 3) – 50 samples; and „Pet park“ (Park 4) – 50 samples (representing a special, isolated group of sampled material), enclosed and situated within the Čair park. The sand was sampled only from the „free“ part of Čair park (50 samples), since in other parks of interest for the study there were no sandpits.

Study area

The city of Niš (43°19'15" N, 21°53'45" E) is the largest in southeastern Serbia and is the seat of the Nišava administrative district. It is situated at an altitude of 194 m and occupies an area of around 596.73 km^2. According to the European NUTS (Nomenclature of territorial units for statistics) classification, it belongs to the NUTS3 category. Two important European transport corridors (Corridors X and VII) directly or indirectly connect the city with the border European surroundings. The city has a moderate continental climate (warm summers and moderately cold winters), with an average yearly temperature of 12.08°C, average precipitation of 577.79 mm/m^2 (the highest in October; the lowest in February), and average air humidity of 70.4 % (the highest in January; the lowest in August) (source: Spatial plan of the Niš City Council).

The parks of interest for this study are situated in urban parts of the city, with a high population density and considerable fluctuation of pet and stray dogs. The Sveti Sava park is in one of the newer and largest communities in Niš, covering the area of 4.31 hectares. The Čair park is the largest in Niš situated in the city core, covering the area of 16.4 hectares. In 2016, an enclosed pet park was open within the Čair park, with the area of 5.000 m^2, in which pet owners may walk or train their pets without leashes. The Tvrđava park is a complex cultural monument and an urbanistic hallmark of the central part of Niš. It is situated on the right bank of the Nišava river and occupies the area of 22.1 hectares.

Samples

From each of the above four locations, about 100g of soil and 100g of sand, for each sample was collected with a small shovel from the square area of 25 x 25 cm and depth of 10 cm. The material was collected from the sites without any vegetation (grass) to avoid more intense draining of a grass-covered soil. The sampling was performed based on the bioclimatic conditions indexes, abiding by the method of bioclimatogram by Uvarov (1931) and taking into account the parameters of average temperatures and humidity values for the studied area.

The number of soil and sand samples was determined by the location size and degree of apparent contamination with canine feces. The samples were packed in PVC bags and appropriately labeled (with date of sampling, location, number, and type of samples). After that, the samples were stored in a handheld refrigerator at +4 °C and transported to the laboratory of the Department of Parasitology, Faculty of Veterinary Medicine, University of Belgrade. Parasitological diagnosis was performed in the first 24 – 48 hours after the sample receipt.

Parasitological examination

The diagnosis of parasitic sample contamination was performed using the qualitative method without any concentration of parasitic elements – native examination by Pataki (Soulsby, 1986) and qualitative methods with the concentration of parasitic elements – the method of gravity flotation with saturated NaCl solution, specific weight 1,200 and saturated $ZnSO_4$ solution, specific weight 1,400, at 20 °C (Urquhart *et al*., 1996), sedimentation method (Hansen and Perry, 1994), the method by Fülleborn (Euzeby, 1982), and sedimentation-flotation method for soil and sand (Pavlović, 2017).

In all examined samples of soil/sand, using the method by McMaster (Kochanowski *et al*., 2013), we were able to diagnose fewer than 50 parasitic elements per 1 g. That was the reason why the result of quantification was done using the qualitative FEC method (Pittman *et al*., 2010). Qualitative FEC defines the obtained results as „positive" or „negative". In the method of conventional gravity flotation the number of parasite eggs is valued as „minus" (-) for negative findings, or „plus" (+) for positive findings (with three-level grading as „+, ++, +++"). The number of plus marks denotes the examiner's subjective opinion about the number of parasitic elements present beneath the two coverings of a microscopic specimen.

All the parasitological methods used in the study conform to the recommendations by the ISID (*International Society for Infectious Diseases*), OIE (*World Organisation for Animal Health*), WHO (*World Health Organization*) and WAAVP (World Association for the Advancement of Veterinary Parasitology) related to the diagnosis of parasitic diseases.

Statistics

The data was processed with the GraphPad Prism statistics software. The study results were presented in tables. The statistical analysis of the data involved the application of descriptive tests and analytical non-parametric tests (the Chi-square test). The descriptive statistics were performed to report the data analysis that was presented as mean and standard deviations. The categorical variables were shown as frequencies and percentages. The statistical significance cut-off was set at $p < 0.05$.

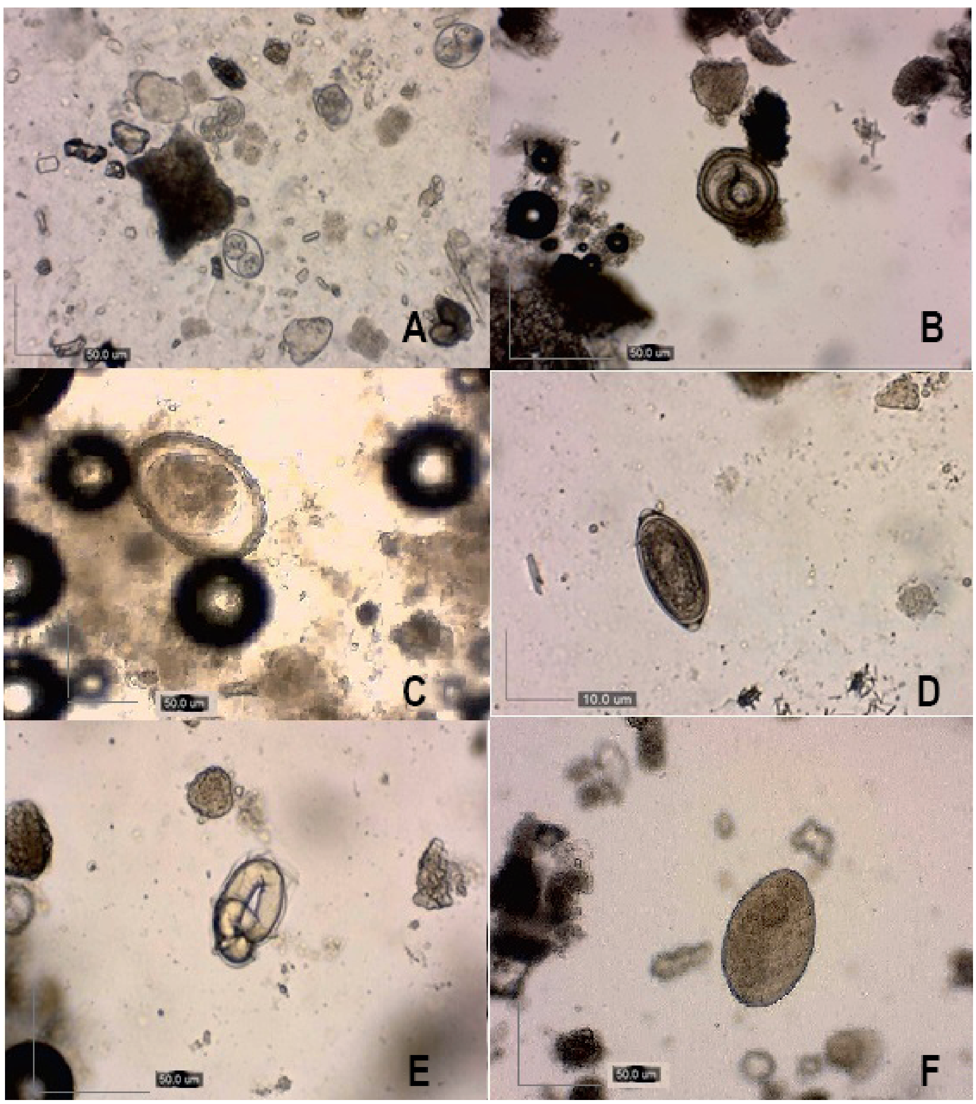

Figure 1. Parasitology diagnosis of soil samples. A) sporulated oocysts of *Cystoisospora* spp.; B) embryonated egg of *T. canis* (stage 1 larva); C) egg of *T. leonina*; D) egg of *T. vulpis*; E) embryonated egg of ancylostomatidae (stage 1 larva) (gravitation flotation method, $ZnSO_4$); F) egg of *A. alata*, sedimentation method), 100x

Table 1. Qualitative assessment of soil samples from the public parks in Niš – diagnosed endoparasites.

ENDOPARASITE	PARKS IN NIŠ (soil)								χ2	P
	Park 1 N=50		Park 2 N=50		Park 3 N=50		Park 4 N=50			
	n	%	n	%	n	%	n	%		
Cystoisospora spp.	2	4	1	2	1	2	0	0	2.04	0.56
Toxocara canis	9	18	8	16	11	22	7	14	1.21	0.75
Toxascaris leonina	1	2	2	4	1	2	0	0	2.04	0.56
Ancylostoma caninum/ Uncinaria stenocephala	4	8	5	10	6	12	2	4	2.25	0.52
Trichuris vulpis	3	6	2	4	3	6	0	0	3.13	0.37
Alaria alata	0	0	1	2	1	2	1	2	1.02	0.80
Total positive	**19**	**38**	**19**	**38**	**23**	**46**	**10**	**20**		

n – number of positive samples; N – total number of samples

Ethical Approval and/or Informed Consent

Not applicable.

Results

In the examined soil and sand samples, seven endoparasites were identified: protozoa from the *Cystoisospora* genus (Fig. 1A), nematodes (*Toxocara canis* – Fig. 1B, *Toxascaris leonina* – Fig. 1C, *Trichuris vulpis* – Fig. 1D, ancylostomatidae – Fig. 1E) and a trematode *Alaria alata* (Fig. 1F).

Each of the 4 methods of parasitological diagnostics applied was used according to the degree of sensitivity of the selected method for the appropriate type of parasite elements, as expected in the samples of the test material.

Results of qualitative examination of soil samples

Developing forms of endoparasites were found in 38 % (19/50) soil samples from Park 1, 38 % (19/50) samples from Park 2, 46 % (23/50) samples from Park 3, and 20 % (10/50) samples from Park 4 (Table 1).

Table 2. Qualitative assessment of soil samples from the public parks in Niš – diagnosed coinfections.

MIXED ENDOPARASITIC CONTAMINATION	PARKS IN NIŠ (soil)								χ2	P
	Park 1 N=50		Park 2 N=50		Park 3 N=50		Park 4 N=50			
	n	%	n	%	n	%	n	%		
Cystoisospora spp. *Toxocara canis*	1	2	0	0	1	2	0	0	2.02	0.57
Toxocara canis *Toxascaris leonina*	1	2	0	0	1	2	0	0	2.02	0.57
Toxocara canis *Ancylostomatidae*	1	2	3	6	2	4	1	2	1.63	0.65
Toxocara canis *Trichuris vulpis*	0	0	0	0	1	2	0	0	3.05	0.39
Toxascaris leonina *Trichuris vulpis*	0	0	1	2	0	0	0	0	3.05	0.39
Toxocara canis *Alaria alata*	0	0	0	0	0	0	1	2	3.05	0.39
Ancylostomatidae *Trichuris vulpis*	1	2	0	0	0	0	0	0	3.05	0.39
Cystoisospora spp. *Toxocara canis* *Trichuris vulpis*	0	0	1	2	0	0	0	0	3.05	0.39
Toxocara canis *Ancylostomatidae* *Trichuris vulpis*	1	2	0	0	0	0	0	0	3.05	0.39
Total positive	5	10	5	10	5	10	2	4		

n – number of positive samples; N – total number of samples

Table 3. Quantitative assessment of soil samples from the public parks in Niš.

Endoparasites	DEGREE OF CONTAMINATION (QUALITATIVE FEC method)																	
	Park 1						Park 2						Park 3					
	Low		Medium		High	Total positive	Low		Medium		High	Total positive	Low		Medium		High	Total positive
	n (%)	Mean ± SD	n (%)	Mean ± SD	n (%)		n (%)	Mean ± SD	n (%)	Mean ± SD	n (%)		n (%)	Mean ± SD	n (%)	Mean ± SD	n (%)	
Cystoisospora spp.	2 (100)	5.5±3.54	0	0	0	**2**	1 (100)	7	0	0	0	**1**	1 (100)	5	0	0	0	**1**
Toxocara canis	6 (66.66)	5.67±1.97	3 (33.33)	35±8.89	0	**9**	6 (75)	4.67±2.94	2 (25)	27±11.31	0	**8**	7 (63.63)	6.86±2.91	4 (36.36)	35.50±14.53	0	**11**
Toxascaris leonina	1 (100)	10	0	0	0	**1**	2 (100)	4±2.83	0	0	0	**2**	1 (100)	6	0	0	0	**1**
Ancylostomatidae	3 (75)	5.33±3.51	1 (25)	44	0	**4**	3 (60)	7.67±3.21	2 (40)	34±9.90	0	**5**	4 (66.66)	3.75±2.22	2 (33.33)	28.50±14.85	0	**6**
Trichuris vulpis	3 (100)	6.67±3.06	0	0	0	**3**	1 (50)	10	1 (50)	37	0	**2**	2 (66.66)	5.50±3.54	1 (33.33)	25	0	**3**
Alaria alata	0	0	0	0	0	**0**	0	0	1 (100)	43	0	**1**	1 (100)	9	0	0	0	**1**

Low: 1 – 10 eggs, oocysts; Medium: 11 – 49 eggs, oocysts; High: ≥ 50 eggs, oocysts (number of oocysts/eggs calculated per one covering – opc/epc)

Table 4. Qualitative assessment of samples of sand in Park 2 – free space.

ENDOPARASITES	**Sand samples N=50**	
	n	**%**
Cystoisospora spp.	3	6
Toxocara canis	13	26
Toxascaris leonina	1	2
Ancylostoma caninum/ Uncinaria stenocephala	4	8
Trichuris vulpis	2	4
Alaria alata	8	16
Total positive samples	**20**	**40**
MIXED ENDOPARASITIC CONTAMINATION	**n**	**%**
Cystoisospora spp. *Toxocara canis*	2	4
Toxocara canis *Ancylostomatidae*	2	4
Toxocara canis *Alaria alata*	3	6
Ancylostomatidae *Alaria alata*	2	4

n – number of positive samples; N – total number of samples

The most prevalent intestinal parasites were *T. canis* (18 % – 9/50) and ancylostomatids (8 % – 4/50) in Park 1, *T. canis* (16 % – 8/50) and ancylostomatids (10 % – 5/50) in Park 2, and *T. canis* (22 % – 11/50) and ancylostomatids (12 % – 6/50) in Park 3. The species from *Cystoisospora* genus were most prevalent in Park 1, with a prevalence rate of 4 % (2/50), *T. canis* nematode, with a prevalence rate of 22 % (11/50) was predominant in Park 3, *T. leonina,* with a prevalence rate of 4 % (2/50) in Park 2, *A. caninum*/*U. stenocephala* with a prevalence rate of 12 % (6/50) in Park 3, and *T. vulpis* with a prevalence rate of 6 % each (3/50) in Parks 1 and 3. The *A. alata* trematode was diagnosed in two soil samples, one from Park 2 (2 % – 1/50) and one from Park 3 (2 % – 1/50). In the examined soil samples we established the presence of eight mixed endoparasitic pathogens, and the most common was the double infection toxocariasis-ancylostomatidosis, with a prevalence of 6 % (3/50) in Park 2, and 4 % (2/50) in Park 3 (Tables 1 and 2). By analyzing

Table 5. Quantitative assessment of samples of sand in Park 2 – free space.

Endoparasites	**DEGREE OF CONTAMINATION (QUALITATIVE FEC method)**						**Total positive**
	Park 2						
	Low		**Medium**		**High**		
	n (%)	Mean ± SD	n (%)	Mean ± SD	n (%)	Mean ± SD	
Cystoisospora spp.	3 (100)	6±2	0	0	0	0	**3**
Toxocara canis	8 (61.54)	4.75±2.31	2 (15.38)	34±8.49	3 (23.07)	77±24.56	**13**
Toxascaris leonina	0	0	1 (100)	36	0	0	**1**
Ancylostomatidae	1 (25)	4	3 (75)	35.33±8.50	0	0	**4**
Trichuris vulpis	1 (50)	9	1 (50)	16	0	0	**2**
Alaria alata	5 (62.5)	4.60±3.05	3 (37.5)	20.67±10.97	0	0	**8**

Low: 1 – 10 eggs, oocysts; Medium: 11 – 49 eggs, oocysts; High: ≥ 50 eggs, oocysts (number of oocysts/eggs calculated per a covering – opc/epc)

Table 6. Comparison of the results obtained for soil and sand samples in the free space of Park 2.

ENDOPARASITES	PARK 2				
	Soil N=50		Sand N=50		P
	n	%	n	%	
Cystoisospora spp.	1	2	3	6	0.62
Toxocara canis	8	16	13	26	0.33
Toxascaris leonina	2	4	1	2	1
Ancylostoma caninum/ Uncinaria stenocephala	5	10	4	8	1
Trichuris vulpis	2	4	2	4	1
Alaria alata	1	2	8	16	0.04*
Total positive	**19**	**38**	**20**	**40**	

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

the soil sampled from the "Pet Park", contamination with endoparasites was detected in 20 % (10/50) of samples. Three types of endoparasites were diagnosed: *T. canis* nematode 14 % (7/50), which was the most prevalent, ancylostomatids 4 % (2/50), and *A. alata* trematode 2 % (1/50) (Table 1).

Results of quantitative examination of soil samples

In the majority of soil samples, low levels of *T. canis* ascaridide contamination (66.66 %; 5.67±1.97 epc in P1, 75 %; 4.67±2.94 epc in P2 and 63.63 %; 6.86±2.91 epc in P3) and ancylostomatids (75 %; 5.33±3.51 epc in P1, 60 %; 7.67±3.21 epc in P2 and 66.66 %; 3.75±2.22 epc) were found in P3. Soil samples positive for the presence of *T. leonina* and *Cystoisospora* spp. showed low levels of contamination with this nematode in all three tested parks. A low degree of contamination of *T. vulpis* nematode (100 %; 6.67±3.06 epc in P1, 50 %; 10 epc in P2, 66.66 %; 5.50±3.54 epc in P3) was diagnosed in all the parks tested. In one sample from Park 2 (50 %; 37 epc) and Park 3 (33.33 %; 25 epc), a moderate degree of contamination with *T. vulpis* was determined. An average degree of contamination by trematode *A. alata* in the sample originating in Park 2 and a low degree of contamination in the sample from Park 3 were determined (Table 3).

Results of qualitative examination of sand samples

In the examined sand samples collected from the free space in Park 2, we diagnosed the protozoa from the *Cystoisospora* genus (6 % – 3/50), nematodes *T. canis* – 26 % (13/50), *T. leonina* – 2 % (1/50), ancylostomatids – 8 % (4/50), *T. vulpis* – 4 % (2/50) and a trematode *A. alata* – 16 % (8/50). The most common coinfection was toxocariasis-alariosis (6 % – 3/50), while other mixed infections (*Cystoisospora* spp. – *T. canis*; *T. canis* – ancylostomatids and ancylostomatids – *A. alata*) were present in 4 % (2/50) (Table 4).

Results of quantitative examination of sand samples

In most sand samples we detected a low degree of contamination with *T. canis* (61,54 %; 4.75±2.31 epc), while in 23,07 % (77±24.56 epc) of positive samples a high degree of contamination with this ascarid was seen. We also established a medium degree of contamination with the species *T. leonina* (100 %; 36 epc), *T. vulpis* (50 %; 16 epc), *A. alata* (37,5 %; 20.67±10.97 epc) and ancylostomatids (75 %; 35.33±8.50 epc) (Table 5).

The analysis of soil samples from public city parks in Niš and Park 4 did not reveal any statistically significant difference (p>0.05) (Tables 1 and 2). The analysis of soil and sand from the free space in Park 2, we found a significant difference (p<0.05) in the prevalence of individual endoparasitic infection with the trematode *A. alata*, with a higher prevalence in sand samples – 16 % (8/50) (Table 6).

Discussion

The study was performed in dry and humid periods in the spring season of 2019, investigating the prevalence of intestinal helminths eggs and protozoan oocysts in the samples of soil and sand from public parks and playgrounds for children in the city of Niš. The eggs of *T. canis* were diagnosed with the highest prevalence (16 – 22 % in the soil; 26 % in the sand), which agreed with other authors' findings, demonstrating the prevalence of this ascarid at 15.6 % in the playgrounds in Turkey (Aydenizoz, 2006) and 15.5 % – 23.3 % in the playgrounds in Croatia (Stojčević *et al*., 2010). The literature data suggest that depending on the climate and geographical location, the level of contamination varies among different countries and within individual countries, where it is determined by the action of local geoclimatic factors. Accordingly, the contamination of public parks in Italy with eggs of *T. canis* was 63.6 % (Giacometti *et al*., 2000), in Slovakia 61.3 % (Rudohradská *et al*., 2011), in Serbia (Kruševac) 50.1 % (Raičević & Pavlović, 2019), while in Spain over 67 % of parks and 1.24 % of soil samples were contaminated (Ruiz *et al*., 2001).

The results of this study agree with the data about soil contamination with *T. canis* eggs obtained by the authors from the Czech Republic 5.0 to 20.4 % (Dubná *et al*., 2007), Croatia – 15.5 do 23.3 % (Stojčević *et al*., 2010), Spain – 16.4 % (Dado *et al*., 2012), Poland – 16.6 % (Bojar & Káapeü, 2012), Romania – 17.17 % (Tudor, 2015) and Greece – 17.2 % (Papavasilopoulos *et al*., 2018).

The degree of contamination of sand samples with the ascarid *T. canis*, as was found in the study, is following the authors' findings from Slovakia – 6.8 to 27.0 % (Ondriska *et al*., 2013), markedly higher than that from the Czech Republic – 11.9 % (Dubná *et al*., 2007), Slovakia – 11.8 % (Papajová *et al*., 2014) and slightly higher than the degree of contamination established in India – 17.64 % (Sudhakar *et al*., 2013).

The contamination with ancylostomatids eggs was diagnosed in 8 – 12 % of soil samples and 8 % of sand samples, while the presence of *T. vulpis* eggs was established in 4 – 6 % of soil samples and 4 % of sand samples. The studies from Croatia report a higher degree of contamination of soil and sand samples from the playgrounds in Pula with *T. vulpis* eggs – 10 – 17.7 % (Stojčević *et al*., 2010).

A statistically significant difference in the degree of contamination with *A. alata* eggs was found between sand (16 %) and soil samples (2 %). In the samples of soil from Park 1, the eggs of this trematode were not found. The reason might have been a dry period of sampling (May) since a higher degree of contamination and a greater number of identified species are always reported in humid periods of the year (Nurdian, 2004). An increased number of soil samples positive for the presence of ascarids and ancylostomatids, as well as sand samples for the presence of *A. alata*, can be explained by favorable climatic conditions, with moderate temperatures, appropriate soil humidity and adequate environmental conditions in general. During the spring months, the eggs accumulated and concentrated in the soil, and due to low precipitation, they were not washed down to deeper layers of soil. The analysis of soil sections revealed that most of the eggs were situated at the depth of 0 – 4 cm (Storey & Phillips, 1985). Since they are not situated on the surface, the eggs are probably protected from the action of direct sunlight and decay.

Such a long survival of ascarid eggs can be explained by the fact that these are most resilient helminth eggs, able to preserve their vitality for up to several years. On the other hand, protozoan cysts and oocysts have a reduced ability to survive in the natural environment (the cysts of *Giardia* spp. are less resilient than *Cryptosporidium* spp. oocysts). This fact may partly explain the absence of these protozoa in our study, in contrast to other authors' findings (Martínez-Moreno *et al*., 2007; Dado *et al*., 2012; Pavlović *et al*., 2015).

A difference was established in the number of positive soil (13) and sand samples (20). The sand was sampled only from the „free" part of Čair park (50 samples), since in other parks of interest for the study there were no sandpits.

Since the texture and humidity of samples have an impact on the length of survival of parasite eggs in the external environment, it should be emphasized that the samples of soil and sand in our study were almost identical as to their humidity. The reason is the vicinity of the Nišava river and its tributary Gabrovačka river, i.e. the fact that sand has an increased capacity to hold moisture. Nišava flows past the Parks 1 and 3 (from which exclusively soil was sampled), while the Park 2 is located further in the city core (the only park with free sand for sampling). In the Park 2, a higher degree of sand contamination (40 %) was found compared to soil contamination (38 %), which agreed with the results reported by Bojar and Kłapeć (2012), who detected the highest degree of contamination of sand (40 %) on the lake beaches in southeastern Poland. The authors stressed that in these places there was an increased chance for contamination of recreational areas with eggs of intestinal parasites from wild animals. The results are supported by the fact that in Poland and other European countries the phenomenon of synanthropization of wild animals, especially red foxes and feral pigs, has been observed, which opens new, additional opportunities for soil and sand pollution in public places in urban areas.

Most parasite eggs (especially embryonated ones) were found in the samples from shadowy places (under the trees or small shrubs). The reason is the ability of soil to hold humidity in such places and protection from direct sunlight, which significantly prolongs egg viability. The finding is also directly correlated with the behavior of dogs and their choice of spots for defecation (Rubel & Wisniveskyy, 2005).

In our study, 5 – 20g of soil and 5 – 20g of sand were examined for each of these methods. The amount of sample tested was dependent on the method used. According to literature data from Mandarino-Pereira *et al*. (2010) tested soil samples (25g) using a modified centrifugal-flotation technique with NaNO3 (Dunsmore *et al*. 1984) and an adaptation of Rugai's *et al*. method (Carvalho *et al*., 2005) is a spontaneous sedimentation method. This method checks 100g of soil and can be done the granulometric analyses of the samples were done and the soil classified into sand, silt, and clay according to their composition (Embrapa, 1997).

According to Kazacos (1983), the ability to diagnose parasitic elements in soil samples increases when a larger amount of soil (30g) is used for analysis. These authors claim that 30g of soil is the maximum amount of soil that can be effectively cultivated. In our study, the maximum amount of soil/sand sampled was 20g for technical reasons. Our experience shows that a larger amount of the sample leads to the formation of a dense suspension with the formation of air bubbles, which make it difficult to microscopically examine the preparations and to diagnose parasitic elements.

The experience of other authors shows that sedimentation techniques are more practical and economical than flotation for soil and sand examination, but leave more impurity particles in the supernatant, which may interfere with the detection of the parasite by microscopy (Carvalho *et al*., 2005). Therefore, the flotation method with a saturated ZnSO4 solution is always recommended as an alternative. The flotation technique requires the use of a solution of appropriate specific gravity, which makes this technique more expensive than sedimentation (Dunsmore *et al*., 1984).

The diagnosed canine intestinal parasites from the soil and sand present an important potential serious human health hazard, especially for the children aged 3 – 5 years. Supporting this notion are the diagnosed cases of parasitic zoonoses in Serbia (Gvozdenović *et al*., 2012; Mijatović *et al*., 2015; Miladinović Tasić *et al*., 2017; Perić *et al*., 2017), and that is the reason why a synchronized cooperation of professionals in the fields of both veterinary and human medicine is mandatory.

In conclusion, in the areas of public parks in the city of Niš, we established contamination with endoparasites of 38 – 46 % of soil samples and 40 % of sand samples. In the samples of soil, the contamination with *T. canis* ascarid (a low and medium degree) and ancylostomatids (a low and medium degree) was predominant, while in the samples of sand different degrees of contamination were present with *T. canis* and *A. alata*. Such a finding suggests a serious risk for human health since the above geohelminths are the etiological agents of visceral, ocular and cutaneous *larva migrans* syndrome, as well as of human larval alariosis. The finding of *A. alata* trematode in the samples of soil (2 %) and sand (16 %) indicates a significant circulation of stray dogs in the areas of public parks in the city of Niš. For owned dogs and people, they represent a reservoir of zoonotic parasites found in the soil and sand in the studied public spaces.

The resolution of this significant public health problem is therefore necessary through the Suggestion of measures, involving: a) control of parasite transmission in the environment, b) guidelines/information for dog owners for the prevention, maintenance of health, and spread of zoonotic diseases, and c) education of medical and veterinary professionals, pet owners and the general public.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgements

This paper was realized in keeping with the projects No. TR31084, No. 173001 and No. TR30188 financed by the Ministry of Education and Science of the Republic of Serbia.

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