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Original Article

Canine intestinal parasites as a potential source of soil contamination in the public areas of Kruševac, Serbia

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Abstract

Introduction: Environmental contamination by infected canine faeces presents an indirect source of contamination in people. In this research the presence of parasitic elements in canine faeces found in the public areas of Kruševac was examined.

Methodology: During May and October 2018, 282 samples were collected from different public areas (ten kindergartens, six public squares and four parks). The examination of faeces was performed by means of direct smear and flotation. For statistical testing of the difference between the frequencies of attributes χ^2 test was applied.

Results: Out of 282 samples, 221 (78.4%) were positive, of which 17/20 (85.0%) from kindergartens, 125/160 (78.1%) from parks and 79/102 (77.5%) from public squares. The presence of *Ancylostomatidae* spp. was discovered in 113 (40.1%) samples, *Toxocara canis* in 82 (29.1%), *Dipylidium caninum* in 76 (27.0%), *Giardia intestinalis* in 45 (16.0%), *Taenia* spp. in 44 (15.6%), *Amoeba* spp. in 32 (11.3%), *Trichuris vulpis* in 19 (6.7%), *Toxascaris leonina* in 18 (6.4%), *Strongyloides stercoralis* in 17 (6.0%), *Isospora* spp. in 7 (2.5%) and *Cryptosporidium* spp. in 3 (1.1%). In May there were 115/141 (81.6%) positive samples, whereas in October there were 106/141 (72.5%) positive ones. No positive sample with three or four parasites was found in October.

Conclusions: This study shows that canine faeces from public areas in Kruševac can be the cause of a significant environmental contamination by eggs of canine intestinal parasites as well as of human infections.

Key words: Canine faeces; intestinal parasites; public areas; zoonoses.

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Introduction

A constant increase in the number of dogs is a serious hygienic, epidemiological and ecological problem of the urban areas worldwide. In urban areas, these animals defecate on public and green areas which they closely share with people. Apart from its unpleasant appearance and smell, canine faeces is a potential epidemiological hazard as dogs are carriers and true hosts of a large number of parasites. If most of the dogs are infected by zoonotic parasites which are transmitted through faeces, they pose a danger to human health. Eggs, cysts and oocysts are scattered in the surface soil layer by means of infected canine faeces, which causes the contamination of these areas [1-3]. The biological cycle of helminth eggs and developmental forms of protozoa in the surface soil layer is closely connected with the microclimate. Under favourable climatic conditions, infectious forms of parasites (especially geohelminths) remain vital for a long time on these surfaces, even after the disappearance of the faeces, acting as an indirect source of infection in humans [4]. People can get infected by accidental ingestion of the infectious forms of the parasites present in the environment [1,5]. The risk of infection in children is in correlation with spending time in contaminated public areas (green areas and sand) [6]. The connection between human toxocariasis and the contamination of public parks with the eggs of *Toxocara canis* from infected canine faeces is of special epidemiological significance and is present worldwide [7-10]. People can also get infected with nematode larvae from the Ancylostomatidae family (cutaneous larva migrans syndrome) [11-13] and *Strongyloides stercoralis* [14,15], with the eggs of tapeworms from Taeniidae family, *Echinococcus granulosus* being the most dangerous type [16,17], and with protozoa such as *Giardia intestinalis, Cryptosporidium* spp. and *Amoeba* spp. [16,18]. Being infected with the tapeworm *Dipylidium caninum* is also possible, but exclusively by means of transitional hosts – fleas [19,20].

The presence of a large number of infected dogs in a limited urban space meets the requirements for the appearance, spread and survival of zoonoses by the immediate contamination of public areas with parasite eggs, which has been proven worldwide. In 2017, there was a preliminary research of such contamination conducted in Kruševac where 70 soil samples from two parks and one public square were examined [21]. According to the performed analyses and obtained results, we concluded that it was necessary to conduct a much more extensive research that would include all public areas in town. Therefore, the aim of this research was to determine the presence of developmental forms of intestinal parasites in canine faeces in the public areas of Kruševac in order to detect the most contaminated sites and take remedial action which would contribute to reducing pollution in public green areas, kindergartens and public squares and, consequently, to reducing the risk of human infections. The results of this research are valuable as they can be used for conducting a study on reducing the number of stray dogs by opening new shelters, adopting dogs, etc., as a basis for educating people about responsible ownership (picking up after their dogs, regular deworming, etc.) and for environmental protection.

Methodology

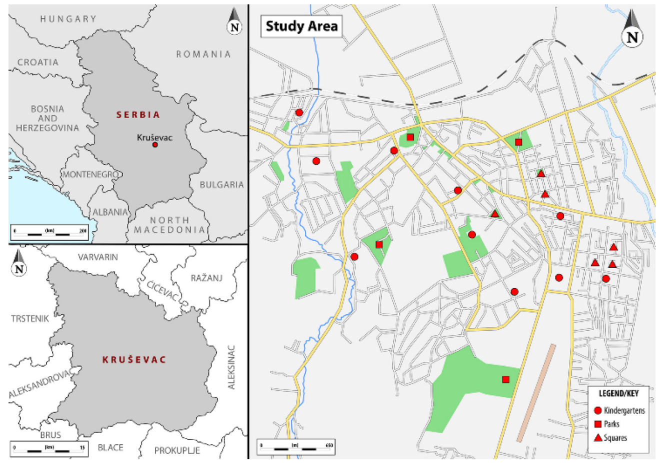
Study area

Kruševac is situated in the central part of the Republic of Serbia at 43˚22’21" and 43˚42’17" North and 21˚9’ and 21˚34’8" East geographical coordinates. The area of Kruševac is mostly situated in a basin and is characterised by the temperate continental climate. The territory of Kruševac (both its urban and its rural part) covers an area of 853.97 km^2 and it has the population of 131,368 inhabitants. 43.7% of the inhabitants live in the urban area of Kruševac where sampling points are located [22]. There is no analysis available concerning the number of dogs in town. No foxes have been noticed in the urban area so far. There are both stray dogs and owned dogs in Kruševac and they share the same public areas and defecate, while dog owners carrying poop bags are rarely seen.

Sampling

In 2018, canine faecal samples were collected twice, in spring and autumn. The spring sampling occurred in May (the average temperature was 18.9°C, the precipitation was 74.4 mm). The autumn sampling occurred in October (the average temperature was 13.6°C, the precipitation was 8.5 mm) [23]. The sampling was performed in 20 different public areas with the greatest concentration of people, especially children. Groups of ten kindergartens, four parks and six public squares were formed and analysed as three separate locations (Figure 1).

Figure 1. Location of sampling sites.



During these two sampling periods, 282 canine faecal samples were collected (141 per sampling) - 20 samples from four kindergartens (in six kindergartens no samples were found), 160 samples from all parks and 102 samples from all public squares. Freshly collected samples were packed in PVC containers with labels on stating the location, the number of the sample and the date. The samples were put in a portable refrigerator and examined in the diagnostic parasitology laboratory of the Scientific Institute of Veterinary Medicine of Serbia, not later than 12 hours after sampling.

Sample inspection

Faeces inspection was conducted by methods of direct smear and flotation. During our examination we always used fresh samples of faeces. For direct smear, a small quantity of faeces is placed on a slide, mixed with a drop of water, spread out and examined directly. At least three slides from different parts of faecal sample should be examined. This method is suitable for very rapid examination, but it will usually fail to detected low-grade infection. Flotation techniques use solutions which have higher specific gravity than the organisms to be floated so that the organisms rise to the top and the debris sinks to the bottom. During our examination we used Zinc sulphate solution with specific gravity 1.36 (700 g $ZnSO_4$ in 1000 ml water). The intensity of infection was measured by McMaster method. The number of eggs per gram can be calculated as follows: count the number of eggs within the grid of each chamber ignoring those outside the squares and then multiply the total by 50 – this gives the number of eggs per gram of faeces (e.p.g.) [24,25]. The determination of parasites was accomplished by morphometric analysis using Euzeby and Anderson's keys [24,26]. The examination of the samples was done with Carl Zeiss microscope AxioLab A1 with the microscope camera AXIOCAM 105 COLOR and ZEN Lite software for morphometric analysis of the size of the eggs and oocysts.

Statistical analysis

The software package SPSS Statistics 21 (IBM, New York, USA) was used for statistical analysis of the data. Attributes are represented through frequencies and percentages. Chi-squared (χ2) test was applied for the analysis of the differences between attributes. A *p*-value less than 0.05 ($p < 0.05$) is considered statistically significant.

Results

78.4% of all the samples (221/282) were positive, of which 17/20 (85.0%) from kindergartens, 125/160 (78.1%) from parks and 79/102 (77.5%) from public squares. Out of the total number of 282 samples, the highest number, 113 (40.1%) were positive for *Ancylostomatidae* spp., followed by 82 (29.1%) positive for *Toxocara canis* 76 (27.0%) for *Dipylidium caninum*, 45 (16.0%) for *Giardia intestinalis*, 44 (15.6%) for *Taenia* spp., 32 (11.3%) for *Amoeba* spp., 19 (6.7%) for *Trichuris vulpis*, 18 (6.4%) for *Toxascaris leonina*, 17 (6.0%) for *Strongyloides stercoralis*, 7 (2.5%) for *Isospora* spp., and 3 (1.1%) for *Cryptosporidium* spp. A significant difference was noticed in the number of positive samples with three or four types of parasites found in kindergartens compared to those found in public squares (χ^2 test; $\chi^2 = 8.627$; $p = 0.003$) and between the positive samples from parks compared to those from public squares (χ^2 test; $\chi^2 = 5.776$; $p = 0.016$), whether there was no significant difference between the number of positive samples in kindergartens and the number of positive samples in parks (χ^2 test; $\chi^2 = 1.767$; $p = 0.184$). There were significantly more samples from kindergartens and parks positive for *D. caninum* compared to the samples from public squares (Table 1).

There were 115/141 (81.6%) positive samples in May and 106/141 (72.5%) positive ones in October. There was a significantly larger number of positive samples in May compared to October (χ^2 test; $\chi^2 = 6.237$; $p = 0.013$) as well as a significantly larger number of samples positive for *S. stercoralis*. No positive sample with three or four different parasites was found in October (Table 2).

Discussion

This type of research in Serbia was started in Belgrade in 1993 and it has been continuously implemented ever since. Its primary aim was to monitor public areas and parks for contamination with various types of parasitic forms excreted by canine faeces and to invent optimal control measures. This resulted in numerous innovations such as introducing the dog-potty system for disposal of canine faeces, providing parks and shelters for dogs and finally adopting the Strategy for solving the problem of ownerless dogs and

Table 1. Intestinal parasites in canine faeces by locations.

	Location			
	Kindergartens N (%)	**Parks N (%)**	**Squares N (%)**	**Total N (%)**
Examined samples	20 (100.0)	160 (100.0)	102 (100.0)	282 (100.0)
Positive samples	17 (85.0)	125 (78.1)	79 (77.5)	221 (78.4)
with one type	3 (17.6)	17 (13.6)	14 (17.7)	34 (15.4)
with more types	14 (82.4)	108 (86.4)	65 (82.3)	187 (84.6)
2 types	8 (57.1)	80 (74.1)	58 (89.2)	146 (78.1)
3 or 4 types	6 (42.9)**	28 (25.9)*	7(10.8)	41 (21.9)
Ancylostomatidae spp.	9 (45.0)	65 (40.6)	39 (38.2)	113 (40.1)
Toxocara canis	8 (40.0)	46 (28.8)	28 (27.5)	82 (29.1)
Trichuris vulpis	3 (15.0)	10 (6.3)	6 (5.9)	19 (6.7)
Toxascaris leonina	0 (0.0)	11 (6.9)	7 (6.9)	18 (6.4)
Strongyloides stercoralis	1 (5.0)	9 (5.6)	7 (6.9)	17 (6.0)
Dipylidium caninum	7 (35.0)*	51 (31.9)*	18 (17.6)	76 (27.0)
Taenia spp.	4 (20.0)	25 (15.6)	15 (14.7)	44 (15.6)
Giardia intestinalis	3 (15.0)	23 (14.4)	19 (18.6)	45 (16.0)
Amoeba spp.	2 (10.0)	20 (12.5)	10 (9.8)	32 (11.3)
Isospora spp.	0 (0.0)	4 (2.5)	3 (2.9)	7 (2.5)
Cryptosporidium spp.	1 (5.0)	2 (1.3)	0 (0.0)	3 (1.1)

*statistical difference in relation to squares ($p < 0.05$); ** statistical difference in relation to squares ($p < 0.01$).

Table 2. Intestinal parasites in canine faeces by period.

	Period	
	May N (%)	**October N (%)**
Examined samples	141 (100.0)	141 (100.0)
Positive samples	115 (81.6)	106 (72.5)
with one type	11 (9.5)	23 (21.7)*
with more types	104 (90.4)	83 (78.3)
2 types	63 (60.6)	83 (100.0)
3 or 4 types	41 (39.4)	0 (0.0)
Ancylostomatidae spp.	59 (41.8)	54 (38.3)
Toxocara canis	48 (34.0)	34 (24.1)
Trichuris vulpis	13 (9.2)	6 (4.3)
Toxascaris leonina	11 (7.8)	7 (5.0)
Strongyloides stercoralis	15 (10.6)	2 (1.4)*+
Dipylidium caninum	45 (31.9)	31 (22.0)
Taenia spp.	20 (14.2)	24 (17.0)
Giardia intestinalis	26 (18.4)	19 (13.5)
Amoeba spp.	20 (14.2)	12 (8.5)
Isospora spp.	7 (5.0)	0 (0.0)
Cryptosporidium sp.	3 (2.1)	0 (0.0)

* statistical difference in relation to May ($p < 0.05$); ** statistical difference in relation to May ($p < 0.01$).

cats in the territory of Belgrade [27,28]. The positive results were clearly visible. During 2011 and 2012 when dog owners started removing canine faeces using the dog-potty system (dog excrement trash cans) and when parks for dogs (eco zones) inside the actual parks were introduced, contamination in Belgrade was reduced by more than 40% [28]. According to the latest research from 2018, the presence of parasite eggs was detected in 29.68% of examined samples, whereas polyparasitism was detected in 54.61% of samples. *T. canis* eggs were found in 29.68% of samples, *D. caninum* in 25.00%, *Ancylostomatidae* spp. in 17.18 %, *T. vulpis* in 10.93 %, *S. stercoralis* and *T. leonina* in 7.81 % and *Taenia* spp. in 6.25 % [29]. The higher percentage of the presence of parasites in this research compared to the one in Belgrade can be explained by the fact that there is no continuous monitoring of parasite contamination of public areas and that dog waste programs have not come to life.

In this research a high representation of intestinal parasites was discovered in canine faeces and when compared to Europe the result is the most in accordance with the prevalence of 75.7% in Albania [30]. According to some research worldwide, results vary from 8.6% in Italy [31] to 98.0% in Mexico [32]. The differences in research results worldwide can be associated with various factors such as different diagnostic techniques [33], the socioeconomic status of the countries where the research was conducted (veterinary control and animal care, hygienic standards) [4,34], dog age [35], the estimated dogs (ownerless or owned), etc. [36,37].

During this research, we concluded that the most common parasites were *Ancylostomatidae* spp., *T. canis*, *D. caninum*, *G. intestinalis* and *Taenia* spp. If we look at the studies conducted worldwide, we can see that these types are globally most frequent in dogs and that they are the most common contaminants of the urban areas [1,4,5,35,37-40].

In Europe, *Ancylostom caninum* and *Uncinaria stenocephala* from Ancylostomatidae family are found in dogs [35,41], and since their eggs are very difficult to identify morphologically they are often referred to as *Ancylostomatidae* spp. [41]. In our research, the representation of *Ancylostomatidae* spp. was the highest and at the global level it was similar to the prevalence in dogs in central Italy, Mexico and in South American countries [32,33,37,38]. As opposite to our results**,** a very low prevalence of these parasites was reported in owned dogs in Italy and Russia [39,42].

T. canis is one of the most frequently found parasites in canine faeces and it is certainly a globally significant cause of human infections (human toxocariasis syndrome or larva migrans syndrome) [2,6,7,10]. In comparison with our results, a significantly higher prevalence of *T. canis* was reported in Albania [30] and in stray dogs in Iran [43]. Approximately the same prevalence of this parasite was reported in a research concerning the canine faeces from public areas in the north of Poland [44], in stray dogs in central Italy [38] and in Libya in owned dogs where the lack of treatment and dogs' age were risk factors for infections in dogs [45]. As opposite to our research, a lower prevalence was found in Moscow [39], the Czech Republic [5] and Germany [35]. In Italy, a low prevalence of *T. canis* in canine faeces is a negligible source of contamination of public areas which minimizes the risk of zoonotic infections [31]. The studies conducted in Poland over the period of 20 years provided an insight into the distribution of *Toxocara* spp. eggs in different types of soil, seasonal and perennial dynamics of soil contamination where the level of contamination was higher in towns [46].

A significantly high percentage of *D. caninum in* canine faeces in kindergartens and parks indicates two facts – the presence of dogs in these places and their infection with the vectors of this tapeworm - fleas. The presence of *D. caninum* in the faeces (of owned and ownerless dogs) and the correlation with fleas were proven in various studies such as the ones in Bulgaria or Brazil which confirm fleas are an ever-present problem in the urban areas [3,37,47-49]. These results also indicate the possibility of humans being infected with this tapeworm accidentally by ingesting fleas which was confirmed in the central part of China and in the south of the USA [19,20].

The results of our research show a high representation of *Taenia* spp. in the samples of canine faeces which opposes the significantly lower reported prevalence in owned dogs in Moscow [39], a three-year research in the urban and rural area of Prague [5], in public areas in the north of Poland [44]. In comparison with our research, a significantly higher representation of *Taenia* spp. in stray dogs was reported in a research in South Africa [50]. The mere presence of the eggs of family Taeniidae can be considered potentially positive for the genus *Echinococcus* as it is not possible to differentiate between the eggs of genus *Taenia* and the eggs of genus *Echinococcus* by coprological examination [44,51]. According to the available data for Kruševac (obtained from an infectious disease doctor and veterinary inspection), the percentage of echinococcosis and hydatidosis does not present a serious health problem in this part of the country, but a high representation of *Taenia* spp. eggs in our research may present a health risk, as concluded by I. Pavlović *(personal communication, January 17, 2019).* Epidemiological studies conducted in Serbia indicate occurrences of echinococcosis/hydatiosis in particular districts, but the percentage is within the European values and there are no significant deviations [17].

Visiting parks and urban areas increases the risk of dog infections and protozoa, most commonly *G. intestinalis* and *Cryptosporidium* spp. that are also zoonoses [52,53]. As regards the prevalence of *G. intestinalis*, results similar to ours were obtained in studies that inspected parks in Spain [1], public areas in Brazil [37] and during an eight-year research in Germany [35].

A high representation of intestinal parasites in canine faeces indicates a severe soil pollution of public areas. This opposes the results of a research conducted in urban Florence where stray dogs are practically non-existent and the majority of dog owners adhere to the municipal law on faeces collection [31]. It is a fact that the rate of infection is significantly higher in stray dogs in comparison with pets [36]. A significantly lower prevalence was noticed in dogs that had been treated with antihelminthics [45]. In some countries where dog owners are not aware of the transmission of zoonotic diseases, the importance of pet health care and environmental pollution, there is a high infection rate with a high percentage of multiple infections [36,40].

A significantly larger number of samples with three or four parasites taken from kindergartens and parks in Kruševac can be explained by the very location of certain kindergartens and parks and their surroundings, which is the primary reason for the fact that ownerless dogs can be seen there. For example, owing to the fact that the most popular park is close to the centre of the town there is a larger number of people and their pets there. Besides, there are two local cemeteries nearby, where people tend to leave food due to certain religious customs, so stray dogs are frequently seen there. The fact that tradition can contribute to the increase of the rate of canine parasitic infections is confirmed by the results of a research conducted in Kosovo [54]. Another equally popular park is situated close to a Roma neighbourhood with poor hygiene, so stray dogs and owned dogs with inadequate health care can be seen there; this is in accordance with a research conducted in Slovakia [34].

Seasonal distribution of canine parasitic infections is higher in spring than in autumn [31,55], which was confirmed by our research as well. Depending on the climatic conditions of the investigated area, in Spain the rate of canine parasitic infections was slightly higher in autumn [56]. The estimated prevalence of canine infections was significantly higher during the wet season in comparison with the dry season [57,58]. Vin our research, very warm and wet weather in May can be associated with a significantly larger number of samples with three or four parasites and samples positive for *S. stercoralis* in comparison with October when the weather was dry. A high prevalence of this parasite is typical for tropical and subtropical countries with hot and wet climate and poor inhabitants who live in inadequate sanitary conditions [40,59]. It was proven in eastern Slovakia that this parasite survives and spreads among animals and humans in poor hygiene [60].

Conclusions

The results of this research indicate that canine faeces from public areas (parks, kindergartens and public squares) in Kruševac is probably the cause of high environmental contamination with the eggs of canine intestinal parasites. A high prevalence of parasites in canine faeces indicates the presence of stray dogs, a low level of veterinary control of owned dogs, poor legislation and the lack of awareness among the human population concerning the harmfulness of contaminated soil. So, it would be essential for public health to take measures to eliminate these causes of infection. It is also important to further examine the soil of the public areas with high concentration of people, especially children, and to develop the database for monitoring people infected with canine intestinal parasites. Having conjoint local information would be of great importance for local governments as it would encourage them to take measures to protect the environment from pollution, which would consequently reduce the potential risk of zoonoses. For developing countries such as Serbia, taking measures for reducing the soil contamination of public areas with the eggs of canine intestinal parasites would not demand large financial investments and it would certainly contribute to a healthier environment.

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