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Original Article

Contamination of Soil and Vegetation with Developmental Forms of Parasites in the Area of the Federation of Bosnia and Herzegovina

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Abstract

Background: According to the WHO (2019), more than 1.5 billion people worldwide are infected with soil-transmitted parasites. Previous research in the Federation of Bosnia and Herzegovina (FB&H) was mainly conducted in the area of the Sarajevo Canton. Therefore, the aim of the research was to explore contamination of soil and vegetation with developmental forms of parasites in the other cantons of FB&H.

Methods: Between Apr and Oct 2018, a total of 1,618 soil and vegetation samples were taken from 386 different locations in the 9 cantons of the FB&H.

Results: Positive samples were observed, 65/66 (98.48%) municipalities/cities and on 239/386 (61.92%) locations. Out of 1,618 samples taken in total (1,263 soil samples and 355 vegetation samples), 357 (22.06%) were positive, out of which 337 (26.68%) and 20 (5.63%) were soil and plant samples, respectively. In total, the following adult and developmental forms were identified: Taeniidae eggs (7.30%), *Toxocara* spp. eggs (62.08%), Ancylostomatidae eggs (25.00%), *Trichuris* spp. eggs (9.55%), *Capillaria* spp. eggs (3.37%), *Toxascaris leonina* eggs (1.40%), Nematodes larvae (19.38%), *Giardia duodenalis* cysts (5.06%), *Cryptosporidium* spp. oocysts (1.4%), oocysts and cysts of different species of Protozoa (3.93%).

Conclusion: The identified developmental forms of parasites pose a permanent threat to human health. It is necessary to carry out measures to reduce the contamination of soil and vegetation in coordination with systematic solutions (legislation), parallelly with contribution of animal owners, veterinarians, physicians, ecologists, parents and all the others involved in this issue.

Introduction



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Soil is the habitat for a large number of microorganisms (approximately 25% of animal species), which generally are not a threat to human health. Through various interactions, soil microorganisms play a significant role in the ecosystem in maintaining soil fertility, water filtration, providing compounds useful for the preparation of antibiotics and other. Although people use different components from the soil to fight diseases, the soil also contains microorganisms that are capable of causing human and animal diseases (1). The connection between soil and human health has been recognized for thousands of years, and geophagy (soil consumption) is one of the ways of introducing various microorganisms and has been documented in animals and humans. Even Hippocrates (460-380 BC) mentions geophagy in his writings, while Mesopotamians and ancient Egyptians watered their wounds with mud and ate soil to treat various diseases, especially intestinal ones. Some indigenous peoples in America used the soil as a spice, and geophagy was a common practice in Europe until the 19th century (2).

It has been estimated that 24% of the world's population, or more than 1.5 billion people, are infected with soil-transmitted helminths worldwide, which can be a threat to human health (3). Helminths, mainly species from the Nematode class, are the only group for which there is currently a specific focal point within the WHO collaboration network for pathogens or parasites transmitted via soil. According to CDC (Centers for Disease Control), approximately 807-1.121 million people are infected with *Ascaris lumbricoides*; 604-795 million people with *Trichuris trichiura*, and 576-740 million people with *Ancylostoma duodenale* and *Necator americanus* (4).

In addition to the mentioned species for which humans are the final hosts, carnivores are also particularly important in soil and

vegetation contamination. Dogs and cats can host approximately 300 species of parasites (5), while there are more than 40 of such species in B&H out of which more than half can parasitize in humans (6). If invasive larvae of dog parasites are introduced into the intestinal tract of humans as non-specific carriers (*T. canis*, *T. vulpis*, etc.) or by the skin-penetrating larvae (*A. caninum*, *U. stenocephala*, etc.), they cause pathogenic activity in individual organs or tissues, although they do not develop into adults. The disease manifests as "syndrome of visceral, cutaneous or ocular larvae migrans" (VLM, CLM, OLM, respectively). Clinical signs of illness in humans vary, where various symptoms may occur, such as fever, cough, abdominal and muscle pain, liver enlargement, anemia, eosinophilia, and others. The disease can result in tissue damage to one or both eyes, causing the development of granuloma, which can lead to the loss of vision. The action of larvae migrans in humans was first recognized by Beaver et al by detection of larvae of *Toxocara* spp. in eosinophilic granulomas in three children at laparotomy (7). Previously, Wilder (8) also described granulomatous and eosinophilic abscesses in the eyes of some patients with suspected retinoblastoma, and the larvae were observed by histological sections of the granules, which Nichols identified as second-stage larvae of *T. canis* (9).

In addition to direct contact, humans can also be infested with parasites via contaminated soil or vegetation, introducing the developmental forms of parasites directly by food, water or geophagy. Developmental forms of parasites can be found in soil and on vegetation in high concentrations, close to places of movement or housing of dogs, with the ability to survive for long periods of time under adverse environmental conditions (10). Gillespie claims that human toxocariasis is most prevalent in children and associated with geophagy (11). Furthermore, Glickman and

Schantz pointed out that 50.0% of UK patients with clinical toxocariasis have never owned dogs or have been in direct contact with dogs, which indicates that soil contamination also plays an important role in the transmission of parasites and human diseases (12).

It is important to point out that the globalization of the food and touristic market, the increase in the world population, the constant demographic changes, as well as numerous sensitive populations (elderly, immunocompromised persons), malnutrition and other chronic disorders are some of the factors that contributed to the increased frequency of parasitic diseases and consequently a growing interest in their research. For example, in the past, the risk of infecting humans with parasites in Europe has generally been considered restricted to remote geographical areas due to unfavorable conditions for survival of parasites in this continent (climate, vectors, hosts), but these barriers are slowly disappearing (13).

Some of the studies reported high frequencies of parasites, with no exception of B&H, where high prevalence values of contamination of soil and vegetation were observed. In the surveys conducted in 2015 and 2016 in the Sarajevo Canton area, of the 55 sites and 300 samples examined, developmental forms of parasites were established at 60.00% of locations and in 23.66% of samples. The following parasites were identified: Taeniidae eggs, *Toxocara canis* eggs, *Trichuris* spp. eggs, Ancylostomatidae eggs and Nematoda larvae (6).

Bearing in mind these findings and the fact that the number of dogs, especially stray ones, has largely increased in the FB&H, as well as that climate conditions allow long-term survival of the developmental forms of parasites, the aim of the study was to research

contamination of soil and vegetation by parasites and parasite developmental forms in 9 cantons of the FB&H and propose relevant remediation measures.

Materials and Methods

Study area

Bosnia and Herzegovina (B&H) is located in the western part of the Balkan Peninsula. The capital is Sarajevo, and BiH is administratively divided into the Federation of Bosnia and Herzegovina (FB&H), Republika Srpska (RS) and the Brcko District. The FB&H consists of ten cantons (Fig. 1) and has a population of approximately 2,219,220 (2013).

Almost the entire FB&H (26,110.5 km²) is located in the Dinarides. It is mostly forested except the plains of the Posavina region of the Pannonian Plain. Moderately continental climate with warm summers and cold winters prevails in FB&H, except southern Herzegovina where Mediterranean climate with mild rainy winters and hot summers prevails.

Sample collection and testing

Between Apr and Oct 2018, a total of 1,618 soil and vegetation samples were taken from 386 different locations in the 9 cantons of the FB&H. The soil sampling was carried out in the yards of kindergartens (n=85), primary schools (n=541), highschools (n=260), public playgrounds (n=89), parks (n=413), public places (n=162) and at other locations (n=68). The plant samples were taken at the same locations. Using the metal spatula, four soil samples from each site were taken from a surface measuring 25x25cm and 5 cm in depth, each weighting approximately more than 100 g (6, 13). At each sampling site covered with vegetation, four plant samples from a 25x25cm surface were obtained using scissors.



Fig. 1: Map of administrative division of the Federation of Bosnia and Herzegovina (34). 1. Una-Sana Canton, 2. Posavina Canton, 3. Tuzla Canton, 4. Zenica-Doboj Canton, 5. Bosnian Podrinje Canton, 6. Central Bosnia Canton, 7. Herzegovina-Neretva Canton, 8. West Herzegovina Canton, 9. Sarajevo Canton, 10. Canton 10

The samples were taken from the sites without grass to avoid intensified drainage on grassy soil. The samples were packed in sterile plastic bags and delivered in a portable refrigerator at 4 °C to the Laboratory for Parasitology of the Veterinary Faculty, University of Sarajevo, accredited according to the requirements of BAS EN ISO/IEC 17025: 2006. Parasitological testing was carried out using standard techniques (14). All the samples were also examined for the presence of *Giardia* spp. cysts and *Cryptosporidium* spp. oocyst by the direct immunofluorescence method described previously using the MERIFLUOR *Cryptosporidium*/*Giardia* DFA test® (Meridian Bioscience, Inc.) (15).

Parasitological determination was based on the assessment of morphological characteristics and measurement of diameter of parasite specimens under microscope CH20 BIMF200®, (Olympus) and fluorescence microscope BH-2-RFCA® (Olympus).

Statistical analysis

Statistical analysis was performed using Minitab® 17 Statistical Software (Minitab Inc., USA). Chi-square test was used to assess the

differences in numbers of positive findings among the studied municipalities and locations. *P*-values less than 0.05 were considered statistically significant.

Results

Laboratory testing of soil and vegetation from 9 cantons of the FB&H, which included the samples taken from 386 locations from 66 municipalities/cities, revealed positive samples from all the 9 cantons surveyed, or at 65 (98.48%) municipalities/cities and at 239 (61.92%) locations. Out of 1,618 samples taken (1,263 soil samples and 355 vegetation samples), in total 357 (22.06%) samples were positive, out of which 337 (26.68%) soil samples and 20 (5.63%) vegetation samples (Table 1) (Fig 2). The frequency of positive findings among municipalities ($P = 0.011$) and among vegetation samples ($P < 0.001$) differed significantly, as well as among all samples ($P = 0.023$). In contrast, there were no statistically significant differences between the sampled locations and soil samples.

Table 1: Contamination of soil and vegetation by developmental forms of parasites in 9 cantons of the Federation of Bosnia and Herzegovina

Canton	Municipalities			Locations			Soil samples			Vegetation samples			All samples		
	N	+	%	N	+	%	N	+	%	N	+	%	N	+	%
USC	8	8	100	55	35	63.64	176	50	29.55	55	3	5.45	231	53	23.38
PC	2	2	100	9	6	66.67	36	7	19.44	6	0	0.00	42	7	16.67
TC	13	13	100	73	47	64.38	236	69	29.24	65	6	7.69	301	75	24.92
ZDC	11	11	100	64	41	64.06	203	57	27.59	61	4	6.56	264	60	21.97
BPC	3	2	66.67	11	7	63.64	38	16	42.11	10	4	40.00	48	20	41.67
CBC	12	12	100	55	35	63.64	168	47	27.98	53	2	3.77	221	52	23.98
HNC	7	7	100	56	30	53.57	198	41	20.20	48	1	2.08	246	42	16.67
WHC	4	4	100	21	10	47.62	63	15	20.63	21	0	0.00	84	15	17.86
K10	6	6	100	42	28	66.67	145	35	24.14	36	0	0.00	181	33	18.23
Total	66	65	98.48	386	239	61.92	1,263	337	26.68	355	20	5.63	1,618	357	22.06
<i>P-value</i>	0.011			0.881			0.253			<0.001			0.023		

Legend: + - number of positive, % - percentage of positive;

USC – Una-Sana Canton, PC – Posavina Canton, TC - Tuzla Canton, ZDC - Zenica-Doboj Canton, BPC – Bosnian Podrinje Canton, SBC – Central Bosnia Canton, HNC – Herzegovina-Neretva Canton, WHC – West Herzegovina Canton, K10 – Canton 10



Fig. 2: The helminths eggs recovered from soil and vegetation samples the area of the Federation of Bosnia and Herzegovina (magnification, 400 x). (A) *Toxocara* spp., (B) Taeniid, and (C) *Capillaria* spp.

By examining the contamination of soil and vegetation samples by parasites and parasite developmental forms, the following were identified: Taeniidae eggs (7.30%), *Toxocara* spp. eggs (62.08%), Ancylostomatidae eggs (25.00%), *Trichuris* spp. eggs (9.55%), *Capillaria* spp. eggs (3.37%), *Toxascaris leonina* eggs (1.40%), Nematodes larvae (19.38%), *Giardia duodenalis* cysts (5.06%), *Cryptosporidium* spp. oocysts (1.4%), oocysts and cysts of different species of Protozoa (3.93%).

The highest contamination was found in parks (85.57%) and playgrounds (77.27%).

The contamination among sampled locations was statistically significantly different ($P < 0.001$). The contamination among soil samples taken at various locations was also significantly different ($P < 0.001$), as was the overall contamination among all the tested samples of soil and vegetation ($P < 0.001$). However, when considered separately, the vegetation samples were not significantly differently contaminated by sampling locations ($P = 0.177$) (Table 2).

Table 2: Contamination of soil and vegetation by developmental forms of parasites on different sampling sites in 9 cantons of the Federation of Bosnia and Herzegovina

Sampling site	Locations			Soil samples			Vegetation sam- ples			All samples		
	N	+	%	N	+	%	N	+	%	N	+	%
Kindergartens	21	3	14,29	67	6	8,96	18	0	0,00	85	6	7,06
Primary schools	129	69	53,49	418	97	23,21	123	4	3,25	541	100	18,48
Highschools	62	28	45,16	201	37	18,41	59	2	3,39	260	39	15,00
Playgrounds	22	17	77,27	68	21	30,88	21	3	14,29	89	24	26,97
Parks	97	83	85,57	323	122	37,77	90	6	6,67	413	129	31,23
Public areas	38	26	68,42	129	38	29,46	33	4	12,12	162	42	25,93
Other	17	13	62,50	57	16	27,78	11	1	30,0	68	17	26,56
Total	386	239	61,92	1.263	337	26,68	355	20	5,63	1.618	357	22,06
*P=	<0,001			<0,001			0,177			<0,001		

Legend: + - number of positive, % - percentage of positive;

*the row "Total" was not taken into account when calculating the P values

Discussion

A previous study reported more positive locations in Herzegovina region than this study found in the FB&H (16). Higher numbers of contaminated locations were also identified in other countries, including UK (17), Chile, Japan, and Turkey (10). Lower frequencies of B&H locations contaminated with parasites were previously identified in the Sarajevo Canton (6, 18). Likewise, lower prevalences of environmental parasitic contamination were also reported worldwide, such as in Poland (19), Serbia (20), Niger, USA, Argentina, Brazil, Thailand, Turkey, Ireland, Italy, Czech Republic and Slovak Republic (10).

Similar results are reported for the samples collected in Perth, Australia (21), Dublin, Ireland (22) and Zagreb, Croatia (23). On the other hand, smaller proportions of the parasite-positive samples were reported in London, UK (24, 25), Leeds, UK (26), Milan, Italy (27), Philadelphia, USA (28), Brisbane, Australia (13), Cairo, Egypt (29) and Pula, Croatia (30).

Consistent with some earlier studies in B&H (6, 18, 31), the present study also identified parks and playgrounds as the locations with

the highest parasite contamination of soil and vegetation. Outdoor facilities, such as public playgrounds and parks, include large areas available for both, owned and stray dogs. Unlike the studies conducted in B&H, high parasite contamination of public playgrounds and parks were not reported in other countries (13, 14, 24, 27).

Presence of some eggs showed the range and intensity of contamination with canine faeces on some areas. The most prevalent parasite species in this study was *T. canis*, being detected in 62,08% of positive samples, which is in line with the previous study of contamination of soil and vegetation in the Sarajevo Canton (6), where the most frequent species was *T. canis* with a prevalence estimated at 70.42%. This finding is in agreement with the results from other previous studies on canine parasites in the area of Sarajevo (32) and in the Bosnian Podrinje Canton (33), which also observed a high prevalence of *T. canis* infection. In addition, *T. canis* was also described as the most common parasite in dogs worldwide (10, 19, 22, 24, 26, 27, 31, 34)). Nevertheless, other species (Taeniidae, Ancylostomatidae, *Trichuris* spp., *Giardia* spp., etc.) have also been identified.

They might have great zoonotic potential (13, 18, 21, 31).

Conclusion

The developmental forms of the potentially zoonotic animal parasites identified in this study pose a permanent human health hazard and should be considered as an exceptional public health problem, especially due to the high contamination of parks and playgrounds where children are predominantly exposed. Therefore, measures should be taken to reduce the contamination of soil and vegetation, first of all by adopting and effectively implementing legislation regulating the protection, conditions and manner of keeping animals as well as obligations of pet owners when bringing animals to public areas. Protection of dogs and humans should be based on the diagnostic examination, treatment, and control of the diseased animals. In addition, the care of abandoned animals, as well as the harmless disposal of dog droppings from homes and gardens, and restricting access for infected dogs to public areas, parks and other open spaces should be also strictly imposed and monitored. Furthermore, it is extremely important to educate the owners and breeders of animals and to familiarize them with the various pathogens, as well as the symptoms and changes they can cause in animals. It is also of great importance to educate children and adults about the importance of personal hygiene, especially after contact with pets and any outdoor activities, and before the food consumption (washing raw fruits and vegetables before eating). The education can be provided through media, workshops, lectures in kindergartens, schools, public forums and other means. Persons who own or care for animals have an obligation to provide them with sufficient food and water, adequate housing and space for contact with other animals. Owners must be aware of preventive, diagnostic, hygienic, therapeutic and other measures in order to protect the health and welfare of animals, i.e., to prevent their in-

juries, diseases, pain, suffering, fear and stress. The role of veterinarians is essential, and it is emphasized through regular activities in the prevention and protection of animal health, and continuous personal and animal owner education as well.

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Conflict of interest

The authors declare that there is no conflict of interest.

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