Research Article

Prevalence of Intestinal Parasites in Children Living in Various Living Conditions in Slovakia

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Abstract

The purpose of the present study was to identify the prevalence of intestinal parasites among the children (aged ≤ 18 years) living in various living conditions in the Eastern Slovakia. Their faecal samples were examined applying the flotation concentration technique to verify the presence of helminth eggs and cysts of protozoan parasites. The evidence of *Enterobius vermicularis* pinworm was obtained using the perianal tape test. The diagnostics of the *Cryptosporidium* spp. oocysts was carried out applying the Kinyoun staining technique. The examined faecal samples were collected from 565 children living in Medzev (340 Roma children from the segregated Roma settlement; 123 children from the majority population; 33 children from the orphanage; 35 children from the elementary school; and 34 children from the kindergarten). They were divided into 5 age subgroups. In children from the segregated Roma settlement, the eggs of six helminth species: *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostomatidae* family, *Hymenolepis nana*, *Taenia* spp., *Enterobius vermicularis* and two protozoan species: *Giardia duodenalis* and *Cryptosporidium* spp. were detected. The highest number of positive samples was observed in the group of children aged 6-9 years. In the majority group of children, the eggs of *A. lumbricoides*, *Taenia* spp., *E. vermicularis* and the cysts of *G. duodenalis* were detected. The species detected in children from the orphanage included *A. lumbricoides*, *H. nana*, *E. vermicularis* and the cysts of *G. duodenalis*. Children attending the 3rd year of the elementary school and the kindergarten presented with the eggs of *A. lumbricoides* and *E. vermicularis* and the cysts of *G. duodenalis*. The examination of soil was performed applying the Kazacos' technique and the samples were collected from the area surrounding the houses and drinking water sources in the segregated settlement. It revealed high occurrence of parasites in infective stages. The results presented herein indicate that the environmental factors contribute to the circulation of parasites among children and affect their health.

Keywords: Intestinal Parasites; Children; Soil-transmitted Helminths; Waterborne Disease

1. Introduction

Intestinal parasitosis belong to most frequent causes of parasitic diseases that deteriorate health of the global population. Occurrence of these parasitic diseases is closely related to the socioeconomic status of the population, personal hygiene standards, and drinking water safety. The prevalence of parasitic intestinal infections is higher among children with lower socioeconomic status than among healthy children who live in good conditions [1]. According to the estimates made by the World Health Organization (WHO), the most important soil-transmitted helminths (STHs) include the *Ascaris lumbricoides* roundworm which annually infects over 1.2 million of the global population and the *Trichuris trichiura* whipworm which infects 795 million people [1, 2]. STHs also include the *Ancylostomatidae* hookworm family - a geohelminth with the zoonotic potential, globally afflecting approximately 72.5 million people [1]. *Ascaris lumbricoides* and *Trichuris trichiura* spread primarily via the faecal-oral route, but also indirectly via contaminated food, water, and soil. As a matter of fact, faecal contamination of the environment represents a serious problem in terms of the spread of these helminthoses. As for the *Ancylostomatidae* family (hookworms), the contamination may be transmitted orally and percutaneously. STHs belong to the major causes of malnutrition as well as growth and intellectual retardation in children [2].

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Ascaris lumbricoides mature, copulate, and produce eggs in the human small intestine [3]. The reproductive ability of females is extraordinary; they produce as much as 200,000 eggs daily [4]. After an infective egg is swallowed, the released larva migrates through the intestinal wall into the portal circulation and hepatic veins, then through the heart into the coronary arteries and alveoli, and from there it passes via the airways through epiglottis; when it is swallowed, it penetrates the bowel and becomes sexually mature. During the above described migration route, the larva sheds twice. Larvae mature to an adult male or female worm and can survive in the bowel for 1 year on average [5]. Ascariasis is often asymptomatic or symptomless; some patients may present with mild gastrointestinal symptoms, such as abdominal pain, nausea, vomiting, or diarrhoea. Migration of larvae through the internal organs may induce high temperature, dry cough, or dyspnoe [2, 4]. Migration of adult worms may induce cholecystitis, cholangitis, appendicitis, biliary colic, etc. In children, massive gastrointestinal infections also cause anaemia, growth disorders, and malnutrition [2, 6]. Globally, ascariasis causes 20,000 deaths each year due to complications [7].

The developmental cycle of the *Trichuris trichiura* whipworm begins with swallowing an infective egg with a larva. Larvae are released in the small intestine and later they penetrate the glands of the large intestine where they shed. They become sexually mature within 11-12 weeks. Adult worms survive in the bowel for a long period of time - as much as 10 years or longer. Infections caused by some worms are mostly asymptomatic; more massive infections may be manifested with abdominal pain, nausea, vomiting, signs of appendicitis, fever, headache, weight loss, and anaemia [8, 9]. Faeces contain mucus and sometimes also blood. Massive trichuriasis in children is referred to as *Trichuris Dysenteric Syndrome* - TDS, characterised by abdominal pain, tenesmus, and anal prolapse [3].

The recommended therapy of intestinal helminthoses induced by *A. lumbricoides*, *T. trichiura*, and family *Ancylostomatidae* is the administration of mebendazole or albendazole in the dose of 100 mg twice daily (in the morning and in the evening) for three consecutive days. The recommended therapy of *E. vermicularis* is the administration of a single 100 mg dose repeated after 2 to 4 weeks.

Protozoan parasitosis also cause serious health problems all over the world. *Giardia duodenalis* and *Cryptosporidium* spp. rank among opportunistic pathogens afflicting mostly young people with immature immune system, in immunosuppressed individuals, or as a secondary infection [10]. They may cause serious infections with significant clinical manifestations, especially in immunosuppressed individuals [11]. They have been detected in most surface waters all over the world and their concentration depends on the degree of water contamination with faeces [12]. *Giardia* is a ubiquitous intestinal protozoan parasite and one of the major intestinal pathogens in humans which is present in tropical as well as temperate zones [13]. Infectious cysts can be transmitted directly through the faecal-oral route, but also via contaminated food and water. They induce water-borne and food-borne diseases [14, 15]. The most vulnerable individuals include children younger than 2 years [16]. The *Cryptosporidium* genus includes the globally-spread monocellular and intracellular extracytoplasmic parasites that infect epithelial cells of the gastrointestinal tract of many vertebrates, including humans [15].

Cryptosporidiosis is a parasitic disease clinically manifested by the asymptomatic form or acute diarrhoea, sometimes even as the malabsorption syndrome. In September 2004, WHO included cryptosporidiosis, together with giardiasis, into the ″Neglected Diseases Initiative″ [11, 17]. *Giardia duodenalis* and *Cryptosporidium* spp. were detected in most surface waters all over the world and their concentration depends on the degree of water contamination with faeces [12, 18]. The recommended therapy of *G. duodenalis* is the administration of 5-nitroimidazole derivates (metronidazole, tinidazole) and for pregnant/breastfeeding women the recommended therapy is the administration of paromomycin. No particular reliable therapy of *Cryptosporidium* infections has been determined yet. Nitazoxanide is recommended to immunocompetent patients with severe diarrhoea. As for the immunosuppressed patients, positive outcomes were observed with the antibiotic therapy with paromomycin and azithromycin [3, 19]. The symptomatic therapy includes oral rehydration and infusion of electrolyte solutions. Also, there are a few reports on the success of the antiretroviral therapy, especially in patients with AIDS [19, 20].

Even in 21st century, parasitic infections represent a serious health problem in developing as well as developed countries. The most vulnerable populations are the isolated communities where various infections develop, depending on the socioeconomic status, poverty, water supply, poor or absent sewage system, and low hygiene standards [21]. Such infections typically affect marginalised communities, such as the homeless, Romani people living in segregated settlements, international immigrants and refugees living in refugee camps or asylum-seeker camps, as well as people living in poor hygiene conditions and of low socioeconomic status. From the epidemiology point of view, the international migration of the populations, especially from developing countries with high incidence of parasitic diseases to developed countries, has serious consequences [22-25]. In poor socioeconomic and hygiene conditions, people of all age categories experience cycles of recurrent infections, primarily the children [26].

The purpose of our research was to document the prevalence of intestinal parasites in the children living in various

living conditions and point out higher incidence of parasitic infections among the Roma children living in segregated Roma settlements as well as non-Roma groups of children in the Košice Region, the Eastern Slovakia.

2. Materials

2.1 Sample collection

The monitoring was aimed at the incidence of giardiasis in various groups of children in the town of Medzev located 36 km west of Košice. In 2016, the town’s population was 3,988 inhabitants; out of them, 1,250 people lived in the Roma settlement. In Medzev, the Romani people live in a segregated settlement where the personal and community hygiene standards are low. As for the children attending the local elementary school, the Roma school children represented 58%. The local orphanage and the kindergarten are located in the centre of the town. The examinations were carried out with 565 samples collected from the children living in various living conditions and they were divided into five groups. Group 1 consisted of the children from the Roma segregated settlement (340); Group 2 consisted of the children from the majority population (123). The examined children were divided into five age groups (Table 1). Group 3 consisted of the children from the orphanage where we collected the faecal samples from 33 Roma children aged 3-14 years. The hygiene standards in the facility were good. On working days, the children (mostly the Roma children) are provided the all-day-long care by their caregivers and teachers.

Group 4 of the examined children consisted of 8-year-old children attending two classes of the 3rd grade in the local elementary school. Out of the total number of 35 children, 11 were the Roma children and 24 children were from the majority population. Group 5 consisted of the children attending the kindergarten (34) aged 4-6 years; out of them, 24 children were from the majority group and 10 children were from the minority group (Roma children) of the population.

3. Methods

3.1 Examination of faecal samples

The samples were collected in cooperation with the paediatric out-patient clinic in the Healthcare Centre in Medzev. The faecal samples were examined applying the flotation method according to Kozák/Magrova (specific gravity of 1.24 $g.cm^{-3}$) and Faust (specific gravity of 1.18 $g.cm^{-3}$) for the presence of helminth eggs and protozoan cysts (*G. duodenalis*), respectively [27, 28]. The diagnostics of *Cryptosporidium* oocysts was carried out applying the Kinyoun staining method [28].

3.2 Perianal tape test

In order to obtain the microscopic evidence of the *Enterobius vermicularis* eggs, the samples were collected applying the perianal and anal tape tests. The tests were performed by the parents who were instructed to collect a sample in the morning, best as soon as their children wake up, and not to wash the anal area in the previous evening. The results of this methodology depend on the correct execution of the tape test as incorrectly performed tape tests confirm false-negative results.

3.2 Examination of soil

The Kazacos' technique (1983), aimed at obtaining evidence of the presence of helminth eggs in the soil samples, was applied to examine 14 soil samples collected from various locations in the segregated settlement in Medzev [29].

4. Results

4.1 Faecal samples and perianal tape test

4.1.1 Group 1: Out of 340 examined stool samples collected from the children in the Roma settlement, a monoinfection caused by a single parasite species was detected in 64.4% of the children. The detected eggs included the eggs of six helminth species: *Ascaris lumbricoides* (49.4% of samples); *Trichuris trichiura* (14.1%); the *Ancylostomatidae* spp. family (0.3%); *Hymenolepis nana* (0.6%); *Taenia* spp. (0.3%); and two protozoan species: *Giardia duodenalis* (14.1%) and *Cryptosporidium* spp. (1.2%) (Table 2). The eggs of *Enterobius vermicularis* were present in 16.5% of the examined tape test samples. The total prevalence of enterobiasis was calculated on the basis of incorrectly performed tape tests (Table 3). The highest number of positive samples was observed in the group of children aged 6-9 years. The diagnostics was carried out for mixed infections in 23.2% (79/340) (Table 4).

4.1.2 Group 2: In the majority group of children, we detected the eggs of *A. lumbricoides*, *Taenia* spp. in one case; *E. vermicularis* in 14.7% of the samples; and *Giardia duodenalis* 1.6% (Table 2). Mixed infections were only observed in two cases - *G. duodenalis* + *E. vermicularis* in one case and *A. lumbricoides* + *G. duodenalis* + *E. vermicularis* in the other case. The highest number of positive samples was observed in the group of children aged 6-9 years.

4.1.3 Group 3: Out of 33 examined faecal samples collected from the children in the orphanage, the eggs of *A. lumbricoides* were present in 9.1% and of *H. nana* in 3.0%; one species of protozoan parasite, *G. duodenalis*, was detected in 57.6% (Table 2). *E. vermicularis* was present in 21.1% of the examined tape test samples (Table 3).

Age group	**Boys**		**Girls**		**Total**	
	Roma	**non-Roma**	**Roma**	**non-Roma**	**Roma**	**non-Roma**
< 1	4	0	5	1	9	1
1-5	78	26	58	35	136	61
6-9	61	18	46	18	107	36
10-14	30	6	36	9	66	15
> 15	9	3	13	7	22	10
TOTAL	**182**	**53**	**158**	**70**	**340**	**123**

Table 1: Number of examined children from the Roma segregated settlement (340) and children from the majority population (123) in Medzev by ethnicity, age, and gender.

Examined samples	**Roma**		**non-Roma**		**Orphanage**	
	340		**123**		**33**	
Number of positive samples (%)	N	%	N	%	N	%
Ascaris lumbricoides	168	49.4	1	0.8	3	9.1
Trichuris trichiura	48	14.1	-	-	-	-
Ancylostomatidae	1	0.3	-	-	-	-
Hymenolepis nana	2	0.6	-	-	1	3,0
Taenia spp.	1	0.3	1	0.8	-	-
Giardia duodenalis	48	14.1	2	1.6	19	57.6
Cryptosporidium spp.	4	1.2	-	-	-	-

Table 2: Intestinal parasitosis in children from the Roma and non-Roma background, and from the orphanage in Medzev.

Examined samples	**Roma**		**non-Roma**		**Orphanage**	
	97		**95**		**19**	
Number of positive samples (%)	N	%	N	%	N	%
E. vermicularis	16	16.5	14	14.7	4	21.1

Table 3: *Enterobius vermicularis* in children from the Roma and non-Roma background, and from the orphanage in Medzev.

Mix infection	**Number of children with mixed infections**	**Age range of children with mixed infections**
A.lumbricoides + *T. trichiura*	44	1-17
A.lumbricoides + *T. trichiura* + *G. duodenalis* + *E. vermicularis*	2	7-8
A.lumbricoides + *T. trichiura* + *G. duodenalis*	7	2-11
A.lumbricoides + *T. trichiura* + *E. vermicularis*	1	9
A.lumbricoides + *T. trichiura* + *Taenia* spp.	1	5
A.lumbricoides + *G. duodenalis* + *E. vermicularis*	2	9-10
A.lumbricoides + *G. duodenalis*	14	2-11
A.lumbricoides + *E. vermicularis*	3	2-10
G. duodenalis + *T. trichiura*	1	6
G. duodenalis + *E. vermicularis*	3	9-12
G. duodenalis + *H. nana*	1	9
Total	**79**	

Table 4: Number of faecal samples with mixed infections of the children from the segregated Roma settlement.

4.1.4 Group 4: The examinations were also performed with 35 faecal samples and tape test samples collected from the 3-graders in the elementary school in Medzev. Two Roma pupils presented with the eggs of *E. vermicularis*. In one boy, the mixed infection of *E. vermicularis*, *A. lumbricoides*, and *G. duodenalis* was observed. Six pupils (6/24) from the majority group presented with the eggs of *E. vermicularis*. The faecal samples of two teachers were negative.

4.1.5 Group 5: Out of 34 samples collected from the kindergarten children, two Roma children (2/10) were positive. A 6-year-old girl presented with a mixed infection *G. duodenalis* and *E. vermicularis*, and a 5-year-old boy was positive for *A. lumbricoides*. In the majority group, *E. vermicularis* was detected in one child (1/24). All ten examined employees were negative (teachers, the cook, and the school janitor).

4.2 Soil

The examination of the soil near the dwellings and the drinking water sources located in the settlement revealed a high number of parasite eggs, cysts, and larvae. 13 positive samples were observed (92.9%) with a wide range of endoparasites: the eggs of *Ancylostomatidae (Ancylostoma/Uncinaria* spp.), *A. lumbricoides*, *Toxocara* spp., *Toxascaris leonina*, *Trichuris vulpis*, *T. trichiura*, and *Hymenolepis diminuta*. The results also revealed a high number of *Ancylostoma/Uncinaria* spp. larvae in 57.1% of the 14 samples. *Strongyloides stercoralis* larvae were observed in two samples (14.3%); one sample came was collected outdoors and one inside a dwelling with the earthen floor [30].

5. Discussion

The results of the investigation facilitated the identification of the prevalence of intestinal parasites in the minority and majority groups of children living in various living conditions in the Kosice Region.

In Slovakia, according to the Statistical Office of the Slovak Republic, the proportion of Roma nationals, out of the total population of 5,435,343 inhabitants, was 2,03% (110,261 inhabitants) (The Statistical Yearbook of the Slovak Republic 2017). However, the estimated number of Roma citizens in Slovakia ranges from 350,000 to 400,000 [*31, 32*]. Currently the most detailed and most accurate publication is the updated "Atlas of Romani Communities in Slovakia 2013" which states that there are 402,840 Romani inhabitants in Slovakia [33]. Considering the method of the data collection, such estimate is regarded as very well-founded. According to the performed research projects and the key relevant documents, the Romani people in Slovakia are regularly included in the groups of people at the highest risk for poverty, social exclusion, and discrimination. This population group possesses multiple disadvantages: they live in poverty resulting from unemployment, poverty caused by insufficient education or associated with demographic conditions and discrimination. The worst situation was observed for those members of the Romani population who live in segregated settlements. The key indicators within the monitoring of living conditions of the Romani people include the settlement type and location as well as availability and use of utility networks. The average number of people living in a single dwelling is 7.3. This figure varies, depending on the dwelling type (flats-residential houses with flats; individual dwellings-wooden houses, shacks, container homes, and caravans). The average number of people who live in a single shack is 7.6; when considering the number of shacks and the number of people, this figure is rather high.

As much as 30.5% of all segregated settlements lack the access to public water supply system; 23.7% of dwellings use their own wells as the sources of drinking water; and 15% use other sources of drinking water (natural springs, streams, etc.). In 76.0% of dwellings located in segregated settlements there is no public sewage system. In Slovakia, as much as 76.0% of dwellings in segregated Roma settlements have no public sewage system [33].

In Slovakia, Königová et al. (2010) summarised the data published over the last 50 years by various authors regarding the prevalence of ascariasis and trichuriasis in children who live in various areas and in various conditions across Slovakia [34]. According to such data, the prevalence of *A. lumbricoides* has been observed in various groups of children, representing 0.3-16.8% while the prevalence of *T. trichiura* was 0.4-28.0% [6]. Rudohradská et al. (2012) confirmed the presence of helminth eggs in 19.7% of faecal samples collected from children living in the environments with poor hygiene standards in the Eastern Slovakia [35]. The highest prevalence was observed for *A. lumbricoides* (24.7%) and *T. trichiura* (17.3%). No helminth eggs were detected in the group of children living in the environments with normal hygiene standards. They performed examinations of the soil at poor-hygiene locations and concluded that the most frequently occurring were the eggs of *A. lumbricoides* (50.0%), *Toxocar*a spp. (36.3%), and *Trichuris* spp. (31.6%) [5].

Globally, the highest prevalence of STHs was observed in poor countries of tropical and subtropical regions. In the largest region of Romania called Timis, the average annual incidence of intestinal parasitosis over the period of 14 years, from 1993 to 2006, was as follows: ascariasis - 194 cases per 100,000 inhabitants; enterobiasis - 777 cases per 100,000 inhabitants; and giardiasis - 1,076 cases per 100,000 inhabitants [6]. Zukiewicz et al. (2011) examined the children (0-18 years of age) living in the north-east of Poland and identified the prevalence of ascariasis of as much as

55.83% and the prevalence of enterobiasis of 3.33% [36].

The eggs of six helminth species, *A. lumbricoides*, *T. trichiura*, *Ancylostomatidae* spp. family, *H. nana*, *Taenia* spp., *E. vermicularis,* and two protozoan species, *Giardia duodenalis* and *Cryptosporidium* spp., were detected in the children from the segregated Roma settlement. The highest number of positive samples was observed among the children attending the lower stage of primary schools. In this group, mixed infections were detected in 23.2% of the examined samples.

In children from the orphanage, the eggs of *A. lumbricoides* were detected in 9.1%; *H. nana* in 3.0%; *E. vermicularis* in 21.1%; and one species of protozoan parasite, *G. duodenalis* was detected in 57.6% of the children. In the majority group of children, the results confirmed the eggs of *A. lumbricoides*, *Taenia* spp., *E. vermicularis,* and *G. duodenalis.* Mixed infections were only observed in two cases. The examinations carried out at schools (elementary school, kindergarten) confirmed the occurrence of *A. lumbricoides*, *E. vermicularis,* and *G. duodenalis.*

The examinations of the soil near the dwellings and near the sources of drinking water located in the settlement revealed a high number of parasite eggs, e.g., *A. lumbricoides*, *Ancylostomatidae* family, *Toxocara* spp., *Toxascaris leonina*, *Trichuris vulpis, T. trichiura* and *Hymenolepis diminuta* and a high number of larvae of *Ancylostoma*/*Uncinaria* spp. and *Strongyloides stercoralis*. These results indicate that the soil in the settlement is excessively contaminated with human and canine parasites. It is a known fact that dogs represent an inseparable part of all Roma settlements in Slovakia; therefore, contamination with canine faeces and transmission of zoonotic species may represent a serious epizootological problem.

Repeated examinations of the samples indicated the same, sometimes even stronger infections caused by *A. lumbricoides*; this proves the fact that if a child undergoes a therapy and subsequently returns back to the same conditions, without improving their hygiene habits, the infection recurs within 3-4 months. The exemplary case is the case of a 2-year-old boy from the settlement who was diagnosed with advanced *A. lumbricoides* in the age of 1.3 years. Repeated examinations carried out when he was 2 years old revealed high-intensity ascariasis. Such cases of ascariasis were also observed in other children younger than 5 years, often as the coinfection with *T. trichiura*. Obviously, the mental development and physical growth of such children was delayed. Frequent malnutrition and anaemia were the most frequent symptoms reported by parents who brought their children to the paediatric out-patient clinic. Our results prove the occurrence of repeated infections with *A. lumbricoides* among children from the settlement. We assume that also other parasite species similarly circulate among the settlement inhabitants, in the soil, and among the animals that live there.

In the poor socioeconomic and hygiene conditions, the cycles of recurrent infections develop in people of all age categories, but mostly in children [26]. Several studies have proven that soil represents an important source of infection, significantly affecting the public health [37].

6. Conclusion

The results of our research indicate that the environment in the above specified Romani settlement is contaminated with parasites. Children who underwent the anti-parasite therapy subsequently returned to the highly exposed environment where they are repeatedly afflicted by infections. We recommend taking preventive measures aimed at reducing the environmental contamination with parasites as the best prevention against the spread of gastrointestinal parasites. The most reliable precaution is to combine multiple measures while emphasising thorough personal hygiene, public sanitation, health education, and sanitation of the environment.

Great attention should be paid to improving the social behaviour and hygiene habits of the local population, building latrines in the settlement, providing regular disinfection, training and increasing the quantity of educated social workers, and providing cooperation in raising awareness among the Roma mothers. Without these improvements, there will always be a tendency of parasitic infections to return to the levels observed prior to the therapy initiation, within a few months of the last deworming [1]. Climate changes in individual countries (especially elevated average temperatures of the air and of the Earth's surface) might particularly prolong the viability and infectability of parasites in the outdoor environment. Hygiene, adequate prevention and therapies, as well as targeted control programmes are inevitable in order to reduce the number of infected people.

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