



## Intestinal parasites in segregated minority communities of Slovakia: results from a cross-sectional survey in children

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### ABSTRACT

In Slovakia, the Roma population forms the second-largest ethnic minority. A large part of the Roma is semi-nomadic or lives in segregated settlements with poor living standards and limited access to health facilities. More than 40 years ago, a cross-sectional survey revealed a high prevalence of parasitic infections. There is a paucity of recent data, and hence, we designed a study to investigate the current status of intestinal parasitic infections in this population. Overall, 259 children aged 7 months to 18 years from 32 different segregated settlements provided faecal samples for microscopic examination using a sodium acetate-acetic acid-formalin concentration and the Paraprep L technique. Almost 40% of the samples yielded a positive result, with *Ascaris lumbricoides* (27.4%) and *Giardia intestinalis* (9.3%) being the most frequent helminth and intestinal protozoa species, respectively. Many children younger than 2 years were found to be infected, which suggests that community transmission is important. In view of our findings, there is a pressing need for targeted action to improve the health status of this neglected population.

### 1. Introduction

The Roma are one of the largest ethnic minorities in Europe (Foldes and Covaci, 2012). In Slovakia, the Roma form the second-largest ethnic minority after the Hungarians. According to the Atlas of Roma communities (Mušinka et al., 2014), there are 803 Roma settlements in Slovakia with more than 215,000 people. The highest number of Roma communities is found in Košice self-governing region (58.2%), living mostly in segregated settlements with poor living conditions, low personal hygiene and abundant presence of waste, rodents and insects. Electricity is available in 91% of the settlements, but only 42% have a public sewer system (Mušinka et al., 2014). Up to one third of the settlements in this region do not have access to clean drinking water, and hence, water has to be collected from streams or creeks. In some parts, the people live together with domestic animals without proper disposal of animal and human faeces, posing a considerable risk for infection with soil-transmitted helminths and other intestinal parasites (Plutzer et al., 2018; Speich et al., 2016; Strunz et al., 2014).

In a cross-sectional survey conducted in 1978 in the eastern part of Slovakia, 4,840 children aged 0-15 years were examined for a broad range of parasitic infections (Giboda, 1981). High prevalence rates were found for the two helminth species *Trichuris trichiura* (27%) and *Ascaris lumbricoides* (17%) and the intestinal protozoon *Giardia intestinalis* (16%). These infection rates were considerably higher than observed elsewhere in Slovakia.

In view of the preexisting high burden of parasitic infections and the prevailing poor living conditions, there is a pressing need to update the epidemiological situation, so that targeted control measures can be initiated should the situation of this ethnic minority population not have improved markedly. Hence, our aim was to investigate the current status of intestinal parasitic infections among the Roma population in eastern Slovakia and compare the results with data from the late 1970s.

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## 2. Methods

### 2.1. Study area and population

The study was carried out in 32 segregated villages and settlements in Košice and Prešov self-governing regions from September to December 2019. The study population consisted of 259 individuals aged between 7 months and 18 years. During the survey, social workers responsible for the respective villages were present and served as linkage persons between the research team and the villagers. Village elders were informed by the designated social workers about the planned study and were invited to set up a meeting with the villagers the next morning. At these meetings, participants were informed about the purpose and procedure of the study, including potential risks and benefits. Sufficient time was allowed to ask questions in an open manner. Parents or legal guardians were then asked to sign a written informed consent. Participation was voluntary, and, individuals could withdraw from the study at any time without further obligations.

Individuals who agreed to participate received an empty plastic container with a unique identifier number and were instructed on how to collect a small sample (~10–15 g) of their next day early morning stool. The containers filled with stool samples were collected the next morning and transferred to the Institute of Parasitology, Slovak Academy of Sciences (IPSAS; Košice, Slovakia) where they were stored without any preservative in a refrigerator at 4°C and examined within 24–48 hours of collection. Each sample was examined microscopically using two methods: (i) a commercially available kit (Paraprep L; Mondial, France) and (ii) a sodium acetate-acetic acid-formalin (SAF) concentration technique (Utzinger et al., 2010).

### 2.2. Diagnostic techniques

Paraprep L is a single-use, disposable device offering prevention against cross-contamination. The tangential hexagonal filter provides a reliable filtration of the sample. The preparation was carried out according to the manufacturer's instruction. Briefly, 0.5 g of faeces is placed on the filter and mixed in a tube with 2 ml of ethyl acetate and 6 ml of 10% formalin. Next, the tube is connected to a conical collection tube with the filter in between. After 24 hours incubation at room temperature, the tube is centrifuged at 500 x g for 1 min, the supernatant discarded and the sediment examined microscopically with a Leica DM 5000B light microscope (Leica Microsystems; Wetzlar, Germany) at 100x and 400x magnification.

The SAF concentration technique is widely used in European reference laboratories for the detection of helminths and intestinal protozoa. In brief, ~1 g of faeces was used for examination. The SAF-fixed stool sample were re-suspended and strained through a medical gauze into a centrifuge tube. After centrifugation at 500 x g for 1 min, the supernatant was decanted and the sediment re-suspended in 7 ml of 0.85% NaCl. After addition of 2–3 ml diethyl ether, the tube was closed with a rubber stopper, shaken vigorously for ~30 sec and then centrifuged at 500 x g for 5 min. The three top layers were discarded, and the resulting sediment was examined microscopically with a Leica DM 5000B light microscope (Leica Microsystems; Wetzlar, Germany) at 100x and 400x magnification for the presence of helminths and intestinal protozoa.

### 2.3. Statistical analysis

The data were entered into an Excel spreadsheet and a number of internal consistency checks were carried out before analysis. We employed EpiData version 3.1 (EpiData Association; Odense, Denmark) for comparing sensitivity of the two diagnostic methods, using McNemar's  $\chi^2$  test.

## 3. Results

A total of 259 children were included in the study and examined microscopically for the presence of intestinal parasites. Overall, 101 children (39.0%) were found to be infected with at least one species of intestinal parasites. The predominant intestinal parasite was the soil-transmitted helminth *A. lumbricoides* (71 positive samples, prevalence 27.4%). Cysts of the intestinal protozoan *G. intestinalis* were detected in 24 of the participants (9.3%). Eggs of the soil-transmitted helminth *T. trichiura* were found in six participants (2.3%). *Hymenolepis nana* eggs were detected in 5 participants (1.9%), *Enterobius vermicularis* eggs were found in 4 participants (1.5%) (Table 1) and *Blastocystis hominis* in only 1 participant.

Most infections were single-species infections; only seven of 101 infected children (6.9%) showed double infections (all of them concurrently infected with *A. lumbricoides* and *G. intestinalis*) and 1 child (1%) had a triple infection (*A. lumbricoides*, *T. trichiura* and *H. nana*).

When analysing different age groups, preschool children (aged ≤5 years) were found to be most frequently infected, whereas the infection rates steadily decreased in children above 10 years of age (Table 2).

Stool samples were subjected to both Paraprep L and SAF concentration techniques. When comparing the respective sensitivities of the two methods, we found some differences for *A. lumbricoides* and *G. intestinalis*. Paraprep L detected significantly more eggs of *A. lumbricoides* (71 vs. 63; McNemar's  $\chi^2$  test,  $p < 0.005$ ), while the SAF method yielded more *G. intestinalis* (22 vs. 16), although this was just above the level of statistical significance (Table 3).

## 4. Discussion

The prevalence of intestinal parasites in Slovakia in the general population is relatively low, which is explained by environmental characteristics (i.e. the country is situated in temperate and continental climate zones with cold, cloudy and humid winters) and overall good hygiene conditions. A recent study found prevalence rates for *A. lumbricoides*, *T. trichiura* and *G. intestinalis* of 3.8%, 0.8% and 0.7%, respectively in the general population (Dudlová et al., 2016). However, in socially disadvantaged people in marginalized communities, this may look different. According to government-released strategy papers, the group most at risk, living in poverty and being socially isolated and discriminated, is represented by the Roma population. Living in shelters on the outskirts of towns and villages, these settlements are often poorly kept and open disposal of animal excrements and human faeces leads to a considerable risk for infection with soil-transmitted helminths and other intestinal parasites (Plutzer et al., 2018; Speich et al., 2016; Strunz et al. 2014). There is often an accumulation of waste around public waste containers, where it can become a source of food for rodents, birds, foxes and stray cats and dogs, further increasing the risk of transmission of infectious diseases.

In the cross-sectional survey presented here, we found that the most affected group are young children, who are exposed to both, environmental and anthropogenic risk factors. In Slovakia, the most frequent

**Table 1**

Prevalence of intestinal parasites among 259 Roma children, aged between 7 months and 18 years in a cross-sectional survey in 2019 in eastern Slovakia.

Intestinal parasite	Positive cases	%
Helminth species		
<i>Ascaris lumbricoides</i>	71	27.4
<i>Trichuris trichiura</i>	6	2.3
<i>Hymenolepis nana</i>	5	1.9
<i>Enterobius vermicularis</i>	4	1.5
Protozoa species		
<i>Giardia intestinalis</i>	24	9.3
Overall prevalence		39.0

**Table 2**

The occurrence of intestinal parasites in Roma children in a cross-sectional survey in eastern Slovakia in 2019, stratified by age group.

Intestinal parasite	Age (years)				
	0-2 (n=45)	3-5 (n=71)	6-8 (n=57)	9-11 (n=29)	12-18 (n=57)
<i>Ascaris lumbricoides</i>	11 24.4%	26 36.6%	17 29.8%	8 27.6%	9 15.8%
<i>Trichuris trichiura</i>	0 0%	3 4.2%	2 3.5%	1 3.4%	0 0%
<i>Hymenolepis nana</i>	1 2.2%	3 4.2%	1 1.8%	0 0%	0 0%
<i>Enterobius vermicularis</i>	0 0%	2 2.8%	2 3.5%	0 0%	0 0%
<i>Giardia intestinalis</i>	5 11.1%	10 14.1%	4 7.0%	3 10.3%	2 3.5%

n = number of samples examined

**Table 3**

Comparison of SAF concentration technique and Paraprep L for the detection of intestinal parasites in stool samples.

	Paraprep L Positive samples	SAF Positive samples	McNemar
<i>Ascaris lumbricoides</i>	71	63	p < 0.005
<i>Giardia intestinalis</i>	16	22	n.s.

n.s. = not significant

soil-transmitted helminths are *A. lumbricoides* and *T. trichiura*, while hookworm infections are absent (Pipiková et al., 2017a). Our study corroborates these prior observations. However, while more than a quarter of the participants were infected with *A. lumbricoides*, *T. trichiura* was found only rarely, as was *H. nana*, the only tapeworm present. More frequent were infections with *G. intestinalis* (9.3%), a flagellate often found in areas where there is no safe water supply (Mekonnen and Ekubagewargies, 2019; Shrestha et al., 2018). In the absence of molecular diagnostic tools in our setting, the genotypes and therefore the origins of *Giardia* could not be determined. Our results, however, correspond with those of Rudohradská et al. (2012) and Pipiková et al. (2017a, 2017b), but are in contrast to results reported as part of Giboda's PhD thesis in the late 1970s, when a considerably higher infection prevalence of *A. lumbricoides* and a lower infection prevalence of *T. trichiura* were observed among Roma. Of note, the methods used in these studies were less suitable to detect certain other helminths that were reported elsewhere to be of relevance in neglected populations in eastern Slovakia, e.g. the soil-transmitted nematode *Strongyloides stercoralis* (Strkolcova et al. 2017, Giboda & Maloveska 1982), yet, Giboda (1981) was still able to detect 17 cases of *S. stercoralis*. Our study is further limited by the examination of only one stool sample per participant, which likely has led to an underestimation of the 'true' prevalence (Sayasone et al. 2015). However, our findings underscore that the parasite representation and distribution have shifted in Slovakia over the past 40 years.

We conclude that intestinal infections still present a public health problem in Slovakia, especially in young children of poor and segregated communities. Therefore, further studies assessing the epidemiology, clinical relevance, prevention and prophylaxis of intestinal parasitic infections in these population groups are strongly encouraged.

## Declarations

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**Conflicts of interest/competing interests:** None

**Ethics approval:** The proposal was reviewed and approved by the ethical commission of Košice self-governing region (document no. 5436/2019/ODDZ-25820)

**Consent to participate:** Participation was voluntarily. Participants signed a written informed consent after having been informed about the purpose and procedures of the study, including potential risks and benefits

**Consent for publication:** All authors have signed a statement agreeing to publish the study

**Availability of data and material:** These are available from the first author and the corresponding author upon reasonable request

## Author statement

All authors have read and approved the revised version of the manuscript.

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