

***Enterobius vermicularis* infection: a cross-sectional study in preschool and school children in the North-Western part of Slovenia**

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Article info

Received October 6, 2022
Accepted November 15, 2022

Summary

Enterobius vermicularis is a prevalent intestinal nematode. The objective of the research was to study enterobiasis prevalence in symptomatic children <15 years of age attending community health center in North – Western part of Slovenia in years 2017 – 2022. Perianal tape tests were performed on three consecutive days. The overall prevalence was 34.2 % (296 out of 864 children included). The mean age of children positive for *E. vermicularis* was 5.77 (95 % CI: 5.51 – 6.04) and 4.74 (95 % CI: 4.54 – 4.95), $p < 0.001$ for children with negative test results. The positivity rate was not significantly different for boys compared to girls (boys 37.0 %, 95 % CI: 32.4 % – 41.8 %, girls 31.8 %, 95 % CI: 27.6 % – 36.2 %, $p = 0.107$). The number of boys with all three samples positive in a sample set was higher compared to girls (p – value 0.002). Family size affected the positivity rate – the mean number of siblings was higher in positive children. Significant association with *E. vermicularis* infection was proven by the presence of anal pruritus and absence of abdominal discomfort. High *E. vermicularis* warrants careful monitoring of trends and public health response. It is necessary to encourage the use of hygiene measures in schools and empower parents to recognize enterobiasis timely.

Keywords: *Enterobius vermicularis*; Enterobiasis; prevalence; cellulose tape test

Introduction

The most common helminth infection in middle and high income countries, particularly amongst school-aged children, is caused by *E. vermicularis* (pinworm) (Friesen *et al.*, 2019). *E. vermicularis* is distributed worldwide, and it has no association with any particular socioeconomic level, race, or culture (Cook, 1994; Wendt *et al.*, 2019). Transmission of *Enterobius* infestation can be facilitated by certain factors such as poor personal or group hygiene, and overcrowding in preschools, schools, orphanages, and families (Song *et al.*, 2003; Burkhart & Burkhart, 2005).

Enterobiasis is mostly acquired by ingestion of infective eggs. After ingestion, eggs develop to larvae and adult worms which

mainly attach in the lower ileum, caecum and ascending colon (Cook, 1994). At night gravid female worms migrate from the colon through the anus to hatch eggs in perianal and perineal skin. Touching or scratching the perianal area combined with finger sucking causes autoinfection. The eggs contain a thick, outer albuminous layer which adheres to fomites. The environment serves as the route to pass *Enterobius* to others who share the same household or attend kindergarten or school. The eggs survive better at low temperature and high humidity but die-off in a warm dry environment (Cook, 1994).

Socio-demographic risk factors for *E. vermicularis* infestation are lower age, male gender, lower parental education, multiple siblings within the household, and unfavorable living conditions – e.g. shar-

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ing a bed with one person (González-Moreno *et al.*, 2011; Bøås *et al.*, 2012; Steinmann *et al.*, 2010). Tap water and convenient toilet unavailability contribute to an increased risk of parasitotic infection, including enterobiasis (Matthys *et al.*, 2011). The unfavorable living conditions mirror the socio-economic class of children, which is related to a higher rate of *E. vermicularis* infection (Steinmann *et al.*, 2010; Song *et al.*, 2003). Good personal hygiene (hand washing before eating), absence of geophagy and finger sucking, and not eating unwashed raw vegetables lower the change of positive test for *E. vermicularis* (Steinmann *et al.*, 2010; Zukiewicz *et al.*, 2011; Remm & Remm, 2009). Residing in a rural area compared to an urban environment might add to higher risk for infection (Steinmann *et al.*, 2010).

Enterobiasis may remain asymptomatic or cause perianal pruritus, insomnia, restlessness, and irritability, particularly in children (Song *et al.*, 2003). The infection may complicate with superficial cellulitis of the perianal skin. In rare cases, colonic inflammation forms an abscess characterized by eosinophilic infiltrate. Urinary tract infestation generally occurs through aberrant movement or auto-inoculation of *E. vermicularis* from the rectum to urinary tract (Patel *et al.* 2015; Choudhury *et al.* 2017). Migration of adult *E. vermicularis* has also been recognized to cause vulvovaginitis, infection of the uterus, adnexitis, as well as appendicitis (Kashyap *et al.*, 2014; Pigac *et al.*, 2017; Powell *et al.*, 2013; Fleming *et al.*, 2015; Taghipour *et al.*, 2020). An association between *E. vermicularis* infection with the protozoan parasite *Dientamoeba fragilis* has also been reported (Ogren *et al.*, 2015).

In Slovenia, the number of notified *E. vermicularis* cases in the period 2007 – 2016 increased almost seven times along with an upsurge of mebendazole prescriptions (National Institute of Public Health, 2022). Most of the notified cases were not microbiologically confirmed, which may suggest over-diagnosis as symptoms and signs of *E. vermicularis* infection are vague and mostly non-specific. To clarify the unexpected increase in notified cases, we decided to conduct a study in a convenient sample of children in one large health community center. The present investigation attempted to determine *E. vermicularis* prevalence in children with symptoms compatible with pinworm infection.

Patients and Methods

Patients

This study was conducted from April 1, 2017 to June 30, 2022 in the Kranj Community Health Center (CHC), which is located in Kranj municipality (located in the Northern-Western part of Slovenia). There are approx. 57,000 residents in the municipality, with approx. 9,100 (16 % of the total population) children aged less than 15 years. Most of the preschool and primary school children are cared for by their pediatricians in the CHC as there are only two private pediatricians available in the municipality. According to the national statistics, 76 % – 79 % of children aged 1 – 5 years and living in Kranj municipality were registered in the kindergarten from year 2017 to 2021, which does not deviate from the average enrollment in Slovenia.

Prior to commencing the study, we conducted an explanatory meeting with CHC pediatricians concerning the purpose of the project. We provided to pediatricians written material for parents or legal guardians including: an information leaflet about *E. vermicularis* infection and treatment, informed consent, a short questionnaire, detailed instructions how and when to take samples and all material needed for sampling and sending to the laboratory. Parents of children aged under 15 years who had clinical signs of infection or whose parents suspected *E. vermicularis* infection were invited to participate in the study. There were 912 persons included in the 5-year study. After excluding those who were ≥ 15 years of age, did not fill in the questionnaire and did not comply with the three sample rule, 864 (456 girls and 408 boys) were left for the final analysis, which means 2,592 perianal tape samples. In this study we did not follow up participants after obtaining samples, therefore no information on subsequent treatment of participants was available. Treatment, if applied, was prescribed by the participant's pediatrician according to the Slovenian guidelines for the treatment of infection with *Enterobius vermicularis* (National Institute of Public Health, 2017). According to the guidelines children above 1 year of age and adults are treated with mebendazole 100 mg in one dose, or with albendazole 200 mg in one dose for children aged 1 to 2 years and 400 mg in one dose for children older

Table 1. Clinical data in children tested for *E. vermicularis* infection.

| Symptom | No. (%) of positive children with symptom (total No. of children = 296) | No. (%) of negative children with symptom (total No. of children = 568) | p-value |
|-----------------------|---|---|---------|
| Nausea | 26 (8.8%) | 55 (9.7%) | 0.667 |
| Abdominal discomfort | 135 (45.6%) | 300 (52.8%) | 0.044 |
| Diarrhea | 31 (10.5%) | 73 (12.8%) | 0.308 |
| Weight loss | 8 (2.7%) | 29 (5.1%) | 0.098 |
| Insomnia | 38 (12.8%) | 90 (15.8%) | 0.238 |
| Anal pruritus | 129 (43.6%) | 199 (35.0%) | 0.014 |
| Vulvovaginal pruritus | 21* (14.5%) | 29* (9.3%) | 0.1 |

*For calculation of the percentage only girls (No. of positive = 145, No. of negative = 311) were taken into account.

than 2 years and for adults. In all cases the treatment is repeated after 2 weeks. The treatment is simultaneously applied to all family members and is the same as for the infected individual. Also, the patient and family members are educated about preventive practices against the spread of the infection.

Microbiology

Perianal tape tests were performed in the morning by the patients or their parents/legal guardians on three consecutive days. Slides with tapes were sent to the National Laboratory for Health, Food and Environment, Kranj Regional Unit, where they were analyzed microscopically using magnification $\times 100$ first and for confirmation $\times 200$. The results were entered in the laboratory information system. Completed questionnaires were forwarded to the National Institute of Public Health. The laboratory informed parents and the pediatrician of the result.

Statistical analyses

Data analysis was performed using SPSS statistical software (version 27, IBM) and 95 % confidence intervals calculated. Statistical significance was determined by a Pearson Chi-Square or Fisher's exact test (categorical) and independent-samples T test (numerical), and p values < 0.05 were considered significant.

Ethical Approval and/or Informed Consent

For this study, research related to human use complied with all the relevant national regulations, institutional policies and was in accordance with the tenets of the Helsinki Declaration. This study was approved by the Commission of the Republic of Slovenia for Medical Ethics (No. 0120-137/2017/7). Informed consent was obtained and signed by all parents/guardians of children in this study. The parents or guardians consented to fulfill the questionnaire and to collect perianal tapes on three consecutive days.

Results

The five-years (2017 – 2022) overall positivity rate in children < 15 years of age was 34.2 % (296 out of 864 children) with prevalence 35.8 %, 35.0 %, 41.6 %, 29.5 %, 25.7 % and 26.5 % in 2017, 2018, 2019, 2020, 2021 and 2022, respectively. The highest number of samples analyzed was in the last pre-pandemic year. The number of children tested for *E. vermicularis* halved during the COVID-19 pandemic, rising again to pre-pandemic levels in 2022.

There were 589 samples positive for ova of *E. vermicularis* out of 2592 samples collected. *Enterobius vermicularis* was found in 190 samples taken on day one, 192 samples taken on day two and in 207 samples taken on day three. Of the positive sample sets, only 64.1 % were positive on the first slide. One-hundred and six (35.8 %) children were positive in one sample only, 87 (29.4 %) in two samples and 103 (34.8 %) in all three samples.

The mean age of children positive for *E. vermicularis* was 5.77

(95 % CI: 5.51 – 6.04) and 4.74 (95 % CI: 4.54 – 4.95), $p < 0.001$, for children with negative test results. The positivity rate was not significantly different for boys compared to girls (boys 37.0 %, 95 % CI: 32.4 % – 41.8 %, girls 31.8 %, 95 % CI: 27.6 % – 36.2 %, $p = 0.107$). Parental education level stratified in three categories (finished primary school, secondary school or graduated from university had no impact on the percentage of positive children ($p = 0.504$).

Children who had at least one positive sample for *E. vermicularis* had on the average statistically significant higher number of siblings compared to children with negative testing result (1.35, 95 % CI: 1.25 – 1.45 and 1.11, 95 % CI: 1.04 – 1.17, respectively, $p < 0.001$).

There were 615 preschool children included in the study. The majority of preschool children were enrolled in kindergarten (528, 85.8 %). The positivity rate in preschool children who were not enrolled in kindergarten was not statistically significantly lower compared to those who were attending kindergarten (20.7 %, 95 % CI: 13.2 – 30.1 % and 30.9 %, 95 % CI: 27.0 – 34.9 %, respectively, $p = 0.057$). A comparison of preschool kindergarteners to school-children revealed a significant difference in positivity rate (30.9 %, 95 % CI: 27.0 – 34.9 % and 46.2 %, 95 % CI: 40.1 – 52.4 %, respectively, $p < 0.001$).

The frequency of health problems in patients with samples taken for *E. vermicularis* infection are presented in Table 1. With the exception of anal itching in positive children and abdominal discomfort in children with a negative result for *E. vermicularis*, there were no statistically significant differences found. Patients or their parents rarely reported the presence of parasites observed in the stool (4.4 % and 4.7 % in patients with positive or negative testing result, respectively, $p = 0.81$).

The characteristics of children with only one positive sample out of three were compared to children with all three positive samples in the sample set. There were no statistically significant differences found for parental education level, symptoms or presence of parasite in stool (Table 2). Children with all three positive samples had a statistically significant higher mean age (p -value 0.004) and were of male gender (p -value 0.002).

Discussion

Findings from this study indicate a very high prevalence of *E. vermicularis* infection in children who consulted their pediatricians in Kranj Community Health Center. The yearly prevalence in preschool and primary school children with clinical suspicion of *E. vermicularis* infection varied from 25.7 % to 41.6 % in the period 2017 – 2022. Wide range prevalence has been reported in different studies, depending on the geographical area, age group included, observational period, presence or absence of symptoms and type of sample. In economically privileged countries, *E. vermicularis* prevalence decreased in the second half of the 20th century due to better hygiene, general upgrade in living standards,

Table 2. Socio-demographic characteristic and clinical data in children with only one or all three positive samples for *E. vermicularis* in a three-sample set.

| Variable | No. (%) of children with only one positive sample (106 children) | No. (%) of children with all three positive samples (103 children) | p-value |
|-----------------------|--|--|---------|
| Mean age | 5.43 | 6.41 | 0.004 |
| Gender | | | |
| No. of boys | 43 (40.6%) | 64 (62.1%) | 0.002 |
| No. of girls | 63 (59.4%) | 39 (37.9%) | |
| Parental education | | | |
| Primary school | 10 (9.8%) | 3 (3.0%) | 0.126 |
| Secondary school | 26 (25.5%) | 25 (24.8%) | |
| University | 66 (64.7%) | 73 (72.3%) | |
| No. of siblings | 1.24 | 1.4 | 0.241 |
| Symptoms | | | |
| Nausea | 11 (10.4%) | 11 (10.7%) | 0.943 |
| Abdominal discomfort | 50 (47.2%) | 51 (49.1%) | 0.735 |
| Diarrhea | 12 (11.3%) | 12 (11.6%) | 0.94 |
| Weight loss | 2 (1.9%) | 3 (2.9%) | 0.628 |
| Insomnia | 16 (15.1%) | 11 (10.7%) | 0.341 |
| Anal pruritus | 48 (45.2%) | 45 (43.6%) | 0.817 |
| Vulvovaginal pruritus | 9* (14.2%) | 5* (12.8%) | 0.834 |

*For calculation of the percentage only girls (No. of one positive sample = 63, No. of three positive samples = 39) were taken into account.

healthcare and use of antihelminthic therapy (Kubiak *et al.*, 2017). This study found a higher prevalence compared to studies from EU countries (Friesen *et al.*, 2019; Bøås *et al.*, 2012; Dudlová *et al.*, 2018). The lower prevalence in recently published EU studies may reflect better control of enterobiasis. Additionally, it might be explained by the different designs of the studies or a convenient sample tested for other common parasites not targeting *E. vermicularis* (Tomaso *et al.*, 2001; Bartolini *et al.*, 2017; Masucci *et al.*, 2011; Zukiewicz *et al.*, 2011; González-Moreno *et al.*, 2011). Some studies included children with and without symptoms which are usually connected with enterobiasis, e.g. anal pruritus (Bøås *et al.*, 2012; Dudlová *et al.*, 2018). Studies where only one sample was obtained from each participant might underestimate the true prevalence of enterobiasis. Taking only one sample per patient might carry the risk of missing infested patient due to suboptimal sample collection by parent or patient. Repeated adhesive tape tests remain so far the method of choice for diagnosing *E. vermicularis* (Jeandron *et al.*, 2010; Remm & Remm, 2009).

One of the recent studies from Berlin, Germany, found an increasing prevalence within a 10-year period of investigation. On average, 17.4 % of patients were positive, with the highest positivity rate in the 4 – 10-years age group and with higher positivity rate in male than female patients (Friesen *et al.*, 2019). Slight male predominance was found in a study from Gran Canaria, Spain, with

overall prevalence of 11.4 % (Carrillo-Quintero *et al.*, 2016). In a Norwegian study using a set of three tape samples the prevalence reached 18 %, which was still a little bit less than half the positivity rate confirmed in our study (Bøås *et al.*, 2012). In this study by Bøås *et al.* (2012) the prevalence was higher in those children who carried the human Leukocyte Antigen (HLA) genotype conferring the high risk for type 1 diabetes (Bøås *et al.*, 2012). The authors expressed the opinion that studies using one sample for each participant underestimated true prevalence by approx. 40 %. Seasonal variation has been observed in some but not in all studies (Friesen *et al.*, 2019; Bøås *et al.*, 2012).

A considerable range of *E. vermicularis* prevalence was found in the eastern part of the European Union. Epidemiological studies from Poland and Slovakia found prevalence of ≤5 % to 12.5 %, and 22.8 % in Estonia (Dudlová *et al.*, 2018; Zukiewicz *et al.*, 2011; Bitkowska *et al.*, 2004; Remm & Remm, 2008). The study by Dudlová *et al.* (2018) from Eastern Slovakia revealed a 3.6 % prevalence rate in preschool and schoolchildren but only one sample was tested per participant. The same low prevalence (3.3 %) was found in the Polish study by Zukiewicz *et al.* (2011) despite children and adolescents being tested due to symptoms resembling parasite infection. The prevalence of *E. vermicularis* was also low in an Italian study with possible underestimation, as the scotch tape test was not requested in the majority of included patients

(Bartolini *et al.*, 2017). In the present study, only 64.1 % of participants were positive on the first slide – not collecting tapes on two more consecutive days would cause a significant downgrading in the overall positivity rate.

There have been few studies published from non-EU European countries showing diverse *E. vermicularis* prevalence – 19.3 %, 2.5 % and >50 % positivity rate was confirmed in schoolchildren from Kyrgyzstan, Tajikistan and Uzbekistan, respectively (Steinmann *et al.*, 2010; Matthys *et al.*, 2011; (Gungoren *et al.*, 2007).

In the present study, the mean age of children with at least one positive tape for eggs of *E. vermicularis* was higher than in the negative group. Schoolchildren usually take care of their personal hygiene after using the toilet and before meals. We can speculate that schoolchildren do not always comply with the best hygienic practices. Non-compliance with hygienic measures carries the risk of *E. vermicularis* infection in this age group. Hygienic measures in toddlers are done by their parents which might explain the lower prevalence in pre-school children. The results of our study are in accordance with previous studies – the lowest prevalence was found in children >3 years and the highest in children aged from 6 – 10 years usually attending first classes of primary education (Friesen *et al.*, 2019; Song *et al.*, 2003). Dudlová *et al.* (2018) found the highest prevalence in 4 – 5 year-old preschool children. The age related prevalence reflects organizational differences of day care and age at entry into the school system (Friesen *et al.*, 2019). Moreover, environmental factors such as overcrowding and level of hygiene in the education system must be taken into consideration. In an Italian study there were more immigrants with positive samples compared to the Italian population (Bartolini *et al.*, 2017). Two major risk factors were identified in the immigrant population: inadequate housing (living in shacks) and cohabitation with other families (Manganelli *et al.*, 2012; Masucci *et al.*, 2011). In our study, enterobiasis was not more frequent in boys than in girls as some reports have shown (Friesen *et al.*, 2019) but was not confirmed in others (Bøås *et al.*, 2012; Song *et al.*, 2003). Interestingly, the number of boys with all three samples positive in a sample set, was significantly higher compared to girls. One could hypothesize that boys have a higher quantity of eggs or eggs are not washed away when passing urine. Family size affected the positivity rate – the mean number of siblings was higher in positive children as was also observed in previous studies (Bøås *et al.*, 2012).

In this study, significant association with *E. vermicularis* infection was proven by the presence of anal pruritus and absence of abdominal discomfort. *Enterobius vermicularis* might be present without any or with few symptoms and is usually considered to be a nuisance rather than a cause of serious disease (Cook, 1994). However, a serious presentation with *E. vermicularis* infection is also possible (Hasan *et al.*, 2020). Hasan *et al.* (2020) documented an association between *Enterobius* infection and a picture of severe abdominal pain and acute appendicitis.

A hypothesis was developed that *E. vermicularis* infection has an

immunomodulatory effect that protects against the development of immune-mediated disorders including diabetes and asthma (Gale, 2002). A large, population-based cohort study from Denmark showed that enterobiasis did not reduce the risk for asthma, type 1 diabetes, arthritis, or inflammatory bowel disease (Bager *et al.*, 2012).

The number of children tested dropped in the first pandemic year, which may reflect a decline in the number of cases or less testing and consequent increase in the under-identification of cases. Non-pharmaceutical measures intended to reduce the number of COVID-19 cases included the closure of kindergartens, distance learning and the creation of the so-called “social bubbles” – socializing with a limited number of people. There was a decline in numerous notified communicable diseases in the lock-down period and *E. vermicularis* seems not to be an exemption. Intensive hygiene recommendations may also have had a certain effect on the spread of *E. vermicularis*. The emphasis on frequent hand washing and disinfection to counteract the spread of SARS-CoV-2 in educational facilities, may have had a favorable effect and prevented the spread of *E. vermicularis*.

The study limitation is that it was conducted in one community health center only in the North-Western part of Slovenia. There are no data available about how many parents were invited to participate but declined to complete the questionnaire and take samples from their children. We assume, according to our experience with similar studies, that the proportion of non-responding parents was low.

Conclusions

This study verified that infestation with *E. vermicularis* remains a public health challenge. The prevalence in preschool and school children is high and demands continuous surveillance, and testing of potential cases on at least three consecutive days to confirm or exclude *E. vermicularis* infection. Hygiene promotion should be integrated into the national concept of schools and kindergartens for health to improve hygiene standards, with an impact on other communicable disease.

Conflict of Interest

Authors have no potential conflict of interest pertaining to this submission to Helminthologia.

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