

## Prevalence and age distribution of enterobiasis in North-Eastern Bulgaria

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### Article info

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### Summary

Enterobiasis is a worldwide distributed helminthiasis that mainly affects children. Bulgarian health-care legislation mandates annual tests for *E. vermicularis* in large part of the population.

This study aimed to establish the prevalence of enterobiasis in children and adults in Varna district, in North-eastern (NE) Bulgaria.

A retrospective analysis of 71,308 laboratory results from patients tested for *E. vermicularis* between 2009 and 2018 was performed. The overall prevalence of enterobiasis was 0.91 % with a significantly higher rate in children (1.49 %) than in adults (0.25 %). An increased tendency of the annual prevalence rates (0.45 %/2009 – 1.45 %/2018) was demonstrated solely due to the propagation of this disease in children population. Detailed analysis in different groups clustered by age and attendance of children’s collectives showed that the lowest level of enterobiasis (0.36 %) was found in children younger than 36 months and afterwards an increased level (1.58 %) in preschool kids (3 – 6 y) was detected. The highest occurrence (9.57 %) was observed in school-age children (7 – 17 y) where no prophylactic measures by law are required or performed.

The prevalence of the enterobiasis observed in Varna District corresponds with the official reports in the country and reflects the actual situation of this disease in Bulgaria. The regional and countrywide data demonstrate that the levels are significantly lower than those reported in the other European countries. The main reason for that is the implementation of an extensive and effective system for the surveillance, prevention and control of *E. vermicularis*-infection in Bulgaria.

**Keywords:** enterobiasis; *E. vermicularis*; age distribution; Bulgaria; Europe; parasitic diseases

### Introduction

Enterobiasis (pinworm infection) is caused by the small intestinal nematode *E. vermicularis*. It has a worldwide distribution and is probably the most common helminth infection with prevalence rates reaching up to 30 – 50 % (Burkhart & Burkhart, 2005; Wendt *et al.*, 2019). Children older than two years, especially those visiting childcare establishments and primary schools are the most

affected group (Remm, 2006; Wendt *et al.*, 2019). Adults contract enterobiasis usually from infected children by domestic or occupational exposure. Consequently, the prevalence in adults is low and thus seldom researched (Burkhart & Burkhart, 2005).

Hands contamination with the parasite’s eggs plays the most critical role for infection and autoinfection. It is the primary factor for the circulation of the disease in the population (Herrström *et al.*, 2001; Wendt *et al.*, 2019). The facilitated person-to-person transmission

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exempts enterobiasis from the principle that intestinal parasites are uncommon in developed regions. Infection can be observed in children of all strata regardless of socioeconomic level, culture or race (Boas *et al.*, 2012; Wendt *et al.*, 2019).

Despite its wide dissemination on the European continent, enterobiasis is rarely a subject of in-depth epidemiological inquiries. Due to its mild or asymptomatic manifestation, it is sometimes considered more as a nuisance than an actual health problem. The most recent data on prevalence rates of the infection in Europe vary depending on the scope of the research and the investigated population. The reported prevalence rates are 37 % in allergic and 23 % non-allergic children in Sweden (Herrström *et al.*, 2001), 32.4 % in Polish children and adults (Heciak, 2006), 24.4 % in Estonian nurseries (Remm, 2006), 22 % in hospitalised children in Denmark (Lacroix & Sørensen, 2000), 18.2 % in children in Norway (Boas *et al.*, 2012), 17.4 % in the population of Berlin area, Germany (Friesen *et al.*, 2019), 7.73 % in children of central Greece (Patsantara *et al.*, 2016), and 3.59 % in pre-school and school-aged children in the eastern Slovakia (Dudlová *et al.*, 2018).

The aim of the current study was to establish the enterobiasis prevalence and its age distribution in Varna district population, North-eastern (NE) Bulgaria within 2009 – 2018 period and compare the results with the official countrywide data available for the same period.

## Material and Methods

### *Data source, study design and patient population*

Varna District is one of the largest administrative areas in the Republic of Bulgaria. It is located in the North-Eastern region on the Black Sea coast. The administrative centre is Varna-city, and with a population of 336 505 for 2018, it is the third-largest town in Bulgaria (NSI, 2019).

Bulgarian healthcare legislation regulates the monitoring and prevention of local parasitic diseases. Testing for intestinal parasites, including *E. vermicularis*, is mandatory as follows:

- Once yearly in children at age two to seven;
- During the enrollment and after a prolonged leave of absence in nurseries (children 0 – 3 years), in kindergartens and preschools (children 3 to 7 years);
- In adults engaged in professions with an impact on public health.

This retrospective study was conducted in one of the largest Specialized Medical Diagnostic Laboratories in Varna, Bulgaria, where the Parasitology department carries out more than 10,000 diagnostic tests for the detection of human parasites annually.

The investigation encompassed 71 308 patients examined for *E. vermicularis* between 01/01/2009 and 12/31/2018. The primary criterion for inclusion was a current residence within the municipality of Varna. Patients outside of those parameters were excluded from the research.

Of all 71 308 samples, 37 886 (53.1 %) belonged to children (<18 years of age), and 33 422 (46.9 %) to adults. Male to female ratio

was 39.2 % (27 916) to 60.8 % (43 392).

Patient's data were grouped by age in 2 categories – adults and children. The children's group was further stratified according to age and child care facilities attendance:

- Group 1: adults – all patients at the age of 18 years and older;
- Group 2: infants attending nurseries – at age 1 – 3 years;
- Group 3: preschool children between 3 and 6 years, attending kindergartens and mandatory (at five years) preschool education;
- Group 4: school attending children and adolescents at age 7 – 17 years.

### *Sample collection and microscopic examination*

*E. vermicularis* eggs in the perianal area were detected microscopically using Graham's adhesive-tape test in concordance with the standards for parasitological testing in Bulgaria. The patients or their parents/legal guardians executed the perianal tests following the explicit instructions from the laboratory personnel. All samples were collected and screened microscopically within 48 hours of submission. Tests containing *E. vermicularis* ova or adult parasites were considered positive and entered as such in the laboratory information system. Due to the low sensitivity of the adhesive-tape test (Boas *et al.*, 2012; Friesen *et al.*, 2019; Wendt *et al.*, 2019) the patients (especially in cases with strong clinical suspicion, direct contact with enterobiasis or during treatment monitoring) were advised to submit at least three probes from 3 consecutive days. All results from a single patient (irrespective of the number examined slides) were treated as a unique data record in the analysis. All patients diagnosed with enterobiasis received standardized etiological therapy, followed by control tests at day 10 and 20. All family members and the close contacts were invited for testing and treated if needed.

### *Data analysis*

The data were processed and analysed with the R language and environment for statistical computing (R Core Team, 2018; Devleeschauwer *et al.*, 2015). All observations are reported within the 99.9 % confidence interval (CI) calculated by the Clopper-Pearson's exact method for binomial proportions. Groups were compared using Pearson's  $\chi^2$ -test (in 2-by-2 analyses) or likelihood ratio  $\chi^2$ -test (in binomial logit models). The size of the observed differences is represented by odds ratios (OR). Each OR is accompanied by its 99.9 % asymptotic CI and the value of the exact (in 2by2 analyses) or asymptotic (in binomial logit model) probability (p-value) for the OR being equal to 1.

## Ethical Approval and/or Informed Consent

The study was designed, conducted and reported in agreement with the Declaration of Helsinki (rev. 2013). Each patient or their legal representative gave informed consent for the laboratory testing as it is mandatory for all tests performed on an outpatient basis in clinical laboratories in Bulgaria. All data regarding the testing

Table 1. Enterobiasis prevalence and distribution by age and sex, Varna District, NE Bulgaria, 2009 – 2018

Age	Sex	Positive	Negative	Prevalence % (CI)	OR (CI)	p
Children	Female	277	18 057	1.51 (1.23 – 1.83)	1	–
Children	Male	289	19 263	1.48 (1.21 – 1.78)	0.98 <sup>#</sup> (0.74 – 1.29)	0.80
<b>Children</b>	<b>Total</b>	<b>566</b>	<b>37 320</b>	<b>1.49 (1.30 – 1.71)</b>	<b>5.95<sup>*</sup> (4.05 – 8.73)</b>	<b>2.2x10<sup>-16</sup></b>
Adults	Female	58	25 000	0.23 (0.14 – 0.35)	1	–
Adults	Male	27	8 337	0.32 (0.16 – 0.58)	1.40 <sup>#</sup> (0.65 – 3.01)	0.17
<b>Adults</b>	<b>Total</b>	<b>85</b>	<b>33 337</b>	<b>0.25 (0.17 – 0.36)</b>	<b>1</b>	
<b>Total</b>	<b>Female</b>	<b>335</b>	<b>43 057</b>	<b>0.77 (0.64 – 0.92)</b>	<b>1</b>	<b>–</b>
<b>Total</b>	<b>Male</b>	<b>316</b>	<b>27 600</b>	<b>1.13 (0.94 – 1.36)</b>	<b>1.47<sup>#</sup> (1.13 – 1.90)</b>	<b>1.21x10<sup>-6</sup></b>

<sup>#</sup> OR male vs female; <sup>\*</sup> OR children vs adults

and detection of *E. vermicularis* performed between January 2009 and December 2018 were extracted retrospectively from the laboratory information system. Only study-relevant patient information (age, sex, residence, year of sample reception) was collected for the purpose of this research. The data was presented in the form of frequency tables containing combinations of factor levels and

the corresponding number of positive and negative results. This data format makes it impossible to reconstruct the initial source completely and to identify the patients. Given the retrospective nature of the study and the preserved anonymity, no further ethical approval was required and requested.

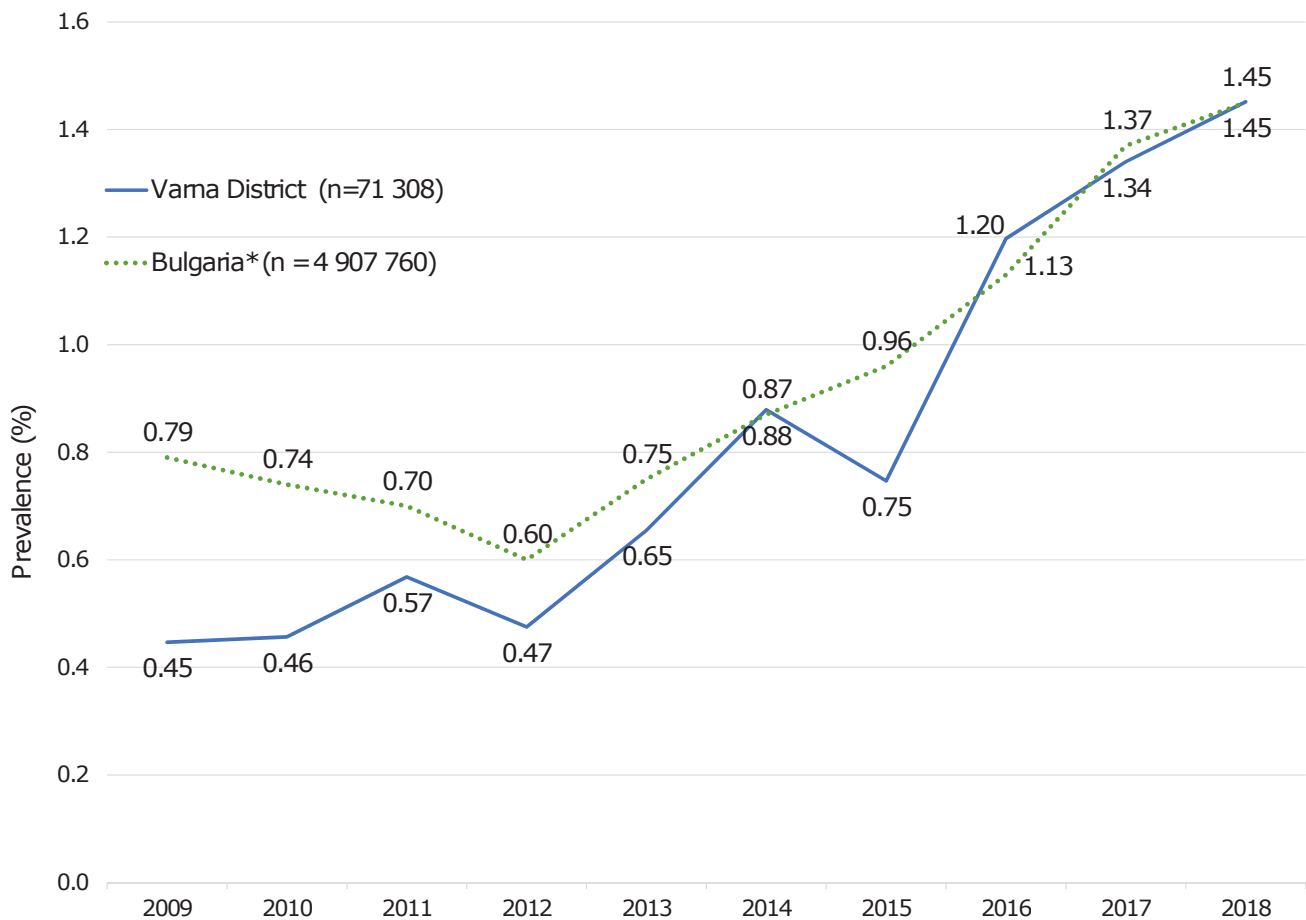


Fig. 1. Annual prevalence of enterobiasis in Varna District and Bulgaria, 2009 – 2018. Overall annual prevalence of enterobiasis in Varna district (blue line) compared to observed levels for the entire country (green dotted line). \* Data from the official analyses of the NCIPD (Rajnova *et al.*, 2018, 2019; Stoyanova, 2019).

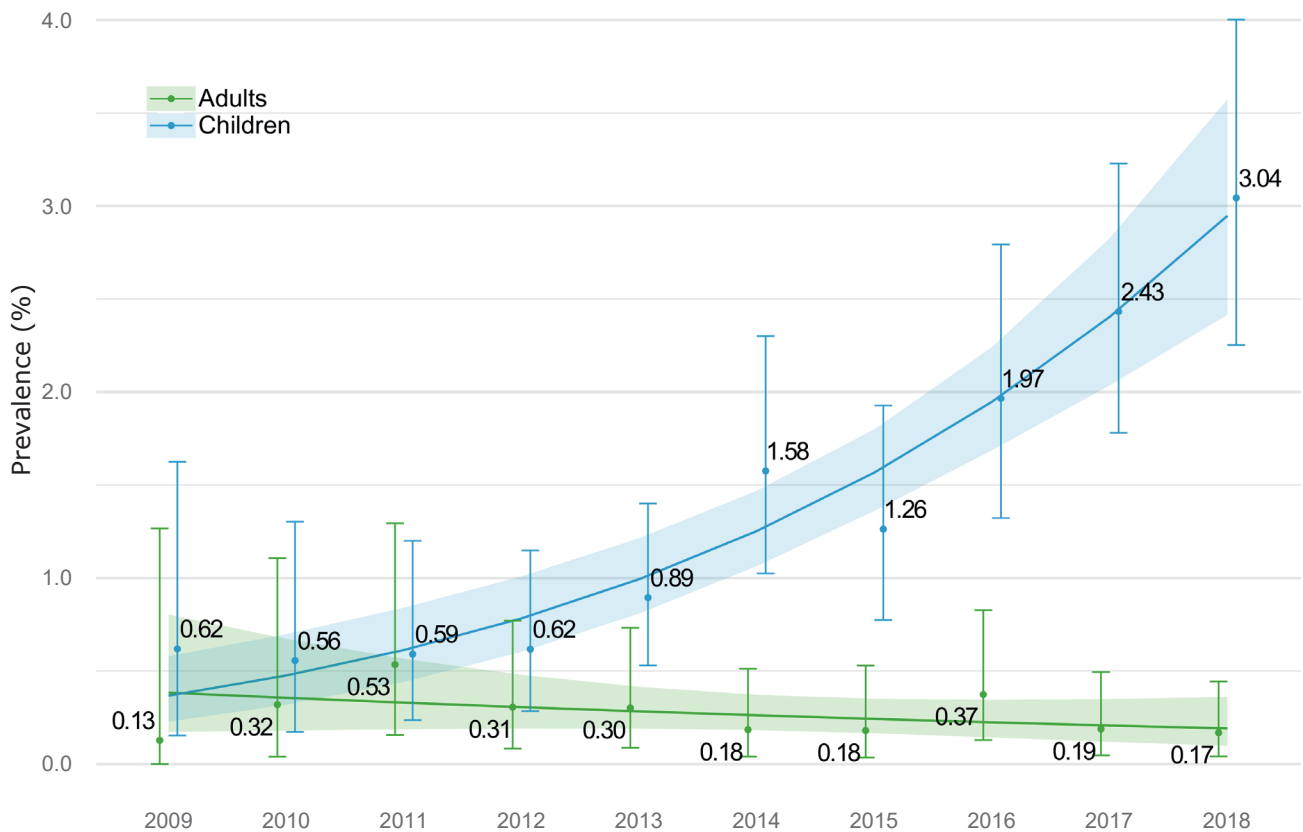


Fig. 2. Annual dynamics of the prevalence of enterobiasis in children and adults in Varna District, NE Bulgaria, 2009 – 2018. The observed annual prevalence (labelled points) of enterobiasis in children (blue) and adults (green) is depicted within the exact Clopper-Pearson CI (error bars). The predictions of the binomial linear model and CI are presented by continuous lines and shaded areas correspondingly. The model confirms the observed significant 5 – 6-fold increase in the prevalence of enterobiasis in children during the observed period as compared to the relatively constant levels in adults.

## Results

### *The overall prevalence of enterobiasis*

From the total number of 71 308 individuals, examined with Graham's adhesive test, eggs and/or adult forms of *E. vermicularis* were detected in 651 patients. The youngest diagnosed person was a 16 months old infant, and the oldest was a 73-years old male. In all cases, the patient or the patient's legal representative received personalised advice from a specialist in clinical parasitology regarding the treatment, personal hygiene, and the obligatory follow up of the treatment effectiveness.

The overall prevalence of enterobiasis for the period 2009 – 2018 was 0.91 % (CI:0.80 – 1.04). In children enterobiasis was significantly more prevalent – 1.49 % (CI:1.30-1.71), than in adults – 0.25 % (CI:0.17-0.36), with OR=5.95 (CI:4.05-8.73;  $p < 2.2 \cdot 10^{-16}$ ).

### *Distribution of enterobiasis by sex*

Table 1 demonstrates the distribution of enterobiasis by sex and

age. The disease was more prevalent in males – 1.13 % (CI:0.94-1.36) than in females – 0.77 % (CI:0.64-0.92). The difference between the sexes, however, becomes negligible when the prevalence was analysed in children and adults separately.

### *Annual dynamics of enterobiasis*

In the investigated period, we observed a steady, substantial increase in absolute numbers of positive samples through the years –  $n=10$  in 2009 to  $n=140$  in 2018. The total increment corresponded with the growing trend in the overall yearly prevalence. Fig. 1 compares the results in Varna District with the annual prevalence rate of enterobiasis in Bulgaria as reported by the National Centre of Infectious and Parasitic Diseases (NCIPD) (Harizanov *et al.*, 2020; Rainova *et al.*, 2019, 2018).

Initially, the prevalence of the disease followed a relatively flat trend. Starting from 2012, however, a steady increase was evident in both datasets. For the additional investigation, we compared the average rates for the periods – 2009 – 2012 and 2013 – 2018. The

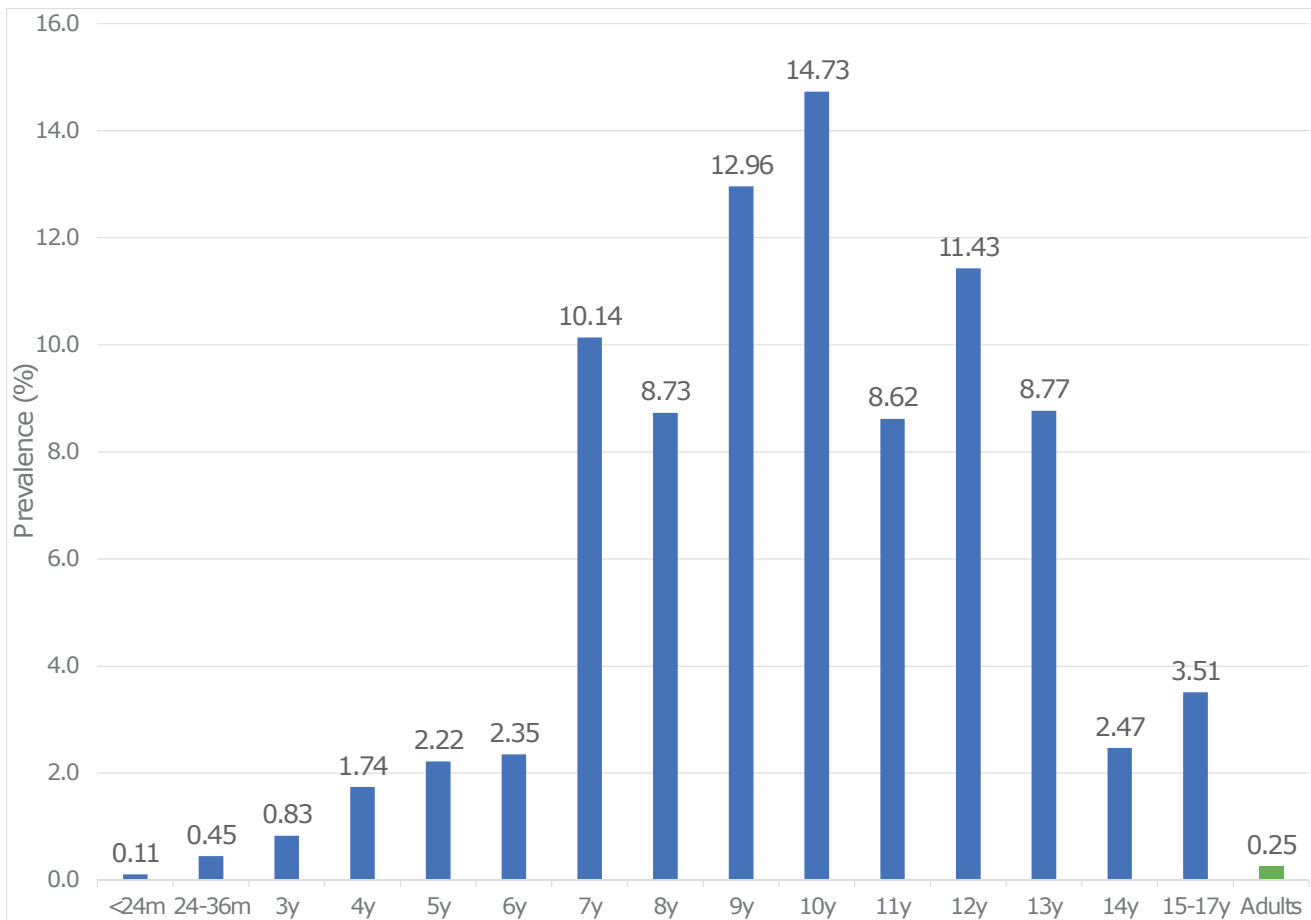


Fig. 3. Average ten-year prevalence of enterobiasis distributed by age, Varna District, NE Bulgaria, 2009 – 2018.

total prevalence before 2012 was 0.49 % (CI:0.34-0.69) whereas after 2012 it rised to 1.06 % (CI:0.92-1.21). This observation corresponded to an approximately two-fold increase in the odds of detecting the parasite in the studied population (OR=2.15; CI:1.72-2.69;  $\chi^2=47.55$ ;  $p=3.3 \times 10^{-13}$ ).

Since there is a significant difference in the rates between children and adults, where the age has the potential to play a major confounding role, we stratified the analysis further. We fitted the data for *E. vermicularis*-infection to a binomial logit model with age category (“children” or “adults”), year of detection and their interaction as independent predictors.

Fig. 2 shows the model’s predictions and the observed annual point prevalence. The model was significantly better at explaining the observed data than the hypothetical model of random variance ( $\chi^2=513.24$ ;  $p<2.2 \times 10^{-16}$ ) and the model without interaction ( $\chi^2=42.3$ ;  $p=7.85 \times 10^{-11}$ ). The rates of *E. vermicularis* in adults remain relatively constant throughout the entire decade. In children, the model predicted 1.26-fold (CI:1.18-1.34) annual increment in the odds of contracting enterobiasis. This finding confirmed that the observed significant increase in enterobiasis between 2012 and 2018 is predominantly due to the facilitated disease circulation

in children’s population.

Age distribution of enterobiasis is demonstrated on Fig. 3. The lowest levels were recorded in children younger than 24 months (0.11 %; CI:0.01-0.50;  $n=3/2794$ ) with an subsequent increase (0.45 %; CI:0.24-0.75;  $n=36/8000$ ) at age of 24 – 36 months. The prevalence continued to grow throughout the preschool age until it peaked in group of 6-year old children (2.34 %; CI:1.63-3.25;  $n=92/3925$ ). At school age, we observed a sudden jump in the prevalence rates which reached the highest values in 9- (12.96 %; CI:5.81-23.64;  $n=21/162$ ) and 10-year old (14.73 %; CI:6.32-27.34;  $n=19/129$ ) children.

To reduce the bias introduced through the unbalanced number of test subjects, we stratified the children in the groups above and compared the average prevalence in each group with the adult population (Fig. 4). The prevalence in 0 – 2-years old kids was the lowest (0.36 %; CI:0.20-0.59) and comparable with the rate found in the adults (OR=1.42; CI:0.73-2.64;  $p=0.07$ ). In children at kindergarten and preschool age enterobiasis affected 1.58 % (CI:1.34-1.86) of the population (OR=6.32; CI:4.33-9.53;  $p=2.2 \times 10^{-16}$ ) while in 7 – 18 years old children reached its peak at 9.57 % (CI:7.01-12.63) with OR=41.49; CI:25.78-67.54;  $p=2.2 \times 10^{-16}$ ).

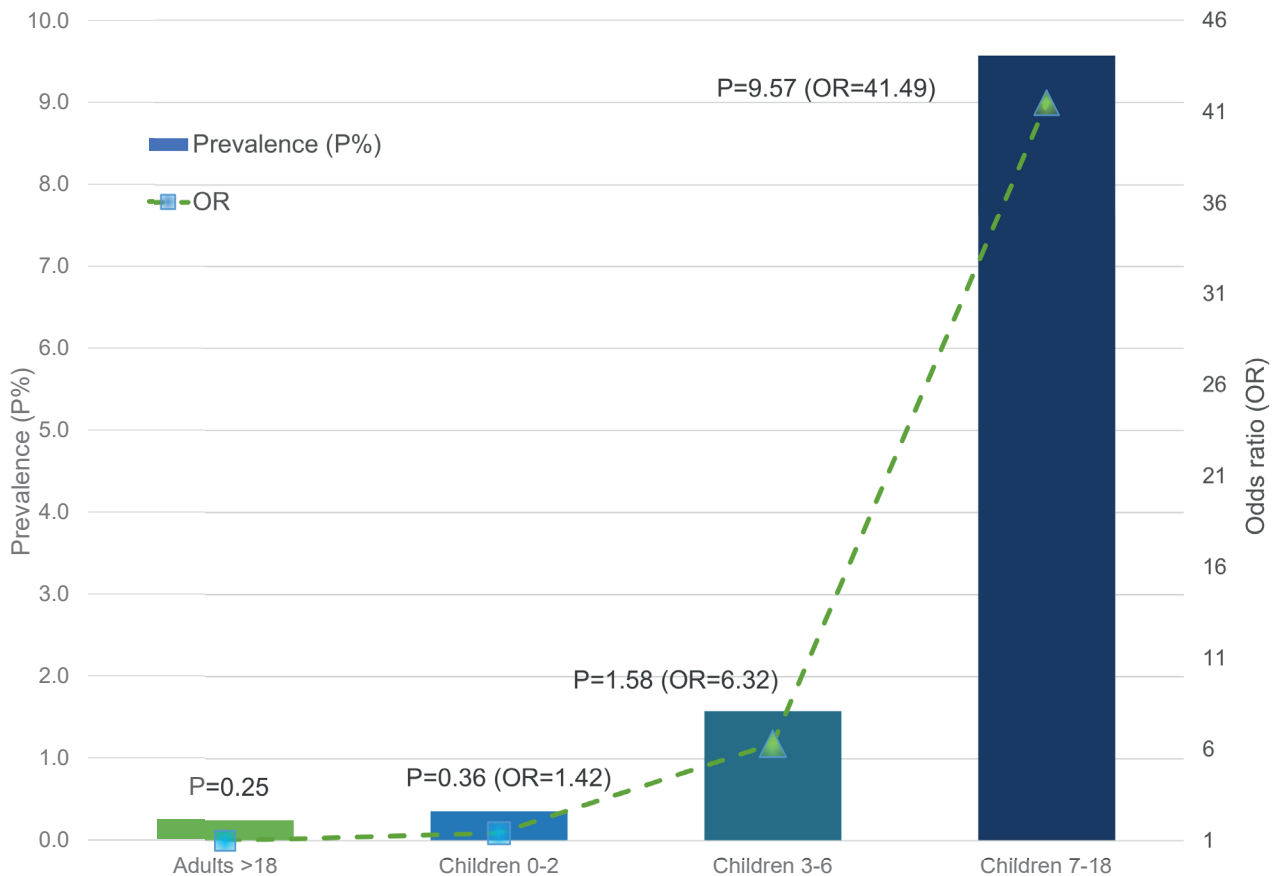


Fig. 4. Average ten-year prevalence and odds ratio of enterobiasis in different age groups, Varna District, NE Bulgaria, 2009 – 2018. Average prevalence (columns; left axis) for the observed decade in adults and children 0 – 2, 2 – 6 and 7 – 18-year-old. Odds ratios comparing the relative risk of infection to the risk in the adult population are presented with labelled dots and projected on the right axis. Odds ratios significantly different from 1 are presented with a triangular shape.

## Discussion

With an average prevalence of 0.91 %, *E. vermicularis* was the most common intestinal parasite in Varna District for the last decade when compared with all other intestinal helminths and protozoa during an overlapping period (2007 – 2016) (Stoyanova, 2019). The rates in the NE region are equivalent to the average prevalence of enterobiasis in Bulgaria (0.90 %; n=4 4115/4 907 760) as reported by NCIPD (Harizanov *et al.*, 2020; Rainova *et al.*, 2018, 2018). It is important to note that the real prevalence might be even higher. The Bulgarian healthcare regulations do not require the adhesive tape tests to be carried out by a specialist personal, and this might increase the rate of false-negative results. Our results about the sex distribution of enterobiasis showed higher prevalence in males than females. Although the overall odds ratio suggested elevated risk in males (OR=1.47), further analysis demonstrated that sex differences are insignificant in adults (OR=1.40) as well as in children (OR=0.98). Thus, similarly with other researchers (Boas *et al.*, 2012; Herrström *et al.*, 2001), we

conclude that the observed small discrepancies are not part of a regular pattern but arise from random variance and inhomogeneities within the tested populations.

We have found an alarming tendency for rising of enterobiasis after 2012 (Fig. 1). The average prevalence in Varna District tripled in the decade under observation and in 2018 (1.45 %) exceeded levels detected in the region at the end of the last century (1.11 % in 1989 and 1.20 % in 1990) (Ruseva & Popova, 1992). A similar pattern was observed throughout the whole country, as reported by NCIPD (Fig. 1).

The statistical models of the annual prevalence rates in children and adults (Fig. 2) clearly demonstrated that the increase in enterobiasis is specific and solely due to the elevated infection rates in childhood. The annual differences in prevalence were typical for the entire 10-year interval but did rise sharply in the second half of the decade.

Several factors may have contributed to this tendency. Regionally and countrywide, there is continuous overcrowding of child care facilities accompanied by a deficit of qualified and skilled person-

nel (Stoyanova, 2019; Stoyanova & Cvetkova, 2018). Additionally, during various periods in 2014, 2015 and 2016, anthelmintic drugs were absent from the pharmacy network in Bulgaria. This fluctuation in the availability of the medication and the complete absence of an appropriate administration form for younger children (suspension or syrup) lead to a delay of etiological therapy and prolonged the period in which the infected persons were epidemiologically active sources of infection (Stoyanova, 2019; Stoyanova *et al.*, 2017).

Since the last wide-scale examination in Varna District, the levels of enterobiasis in children dropped approximately ten times from 12.4 % in 1975 – 1977 (Kovchazov, 1979) to 1.49 %. The same did not apply for the adult population, where the average prevalence diminished only by half – from 0.50 % (1975 – 1977) to 0.25 % (2009 – 2018). The prevalence levels for the adults have not changed substantially in the last 40 years as concurred by the annual model (Fig. 2). This finding indicates that there is a stable background level of enterobiasis in the adult population, and it can be used as a base level for comparison with other age groups.

The enterobiasis in adults is rarely researched in other European countries. One epidemiological study in Poland reported 2.59 % prevalence in children and 0.43 % in adults (Kasprzak *et al.*, 2017). Another older survey in Lithuania revealed rates of 18.4 % and 1.3 % in children and adults, respectively (Mazhilene, 1991).

Fig. 3 demonstrates the growing rate of enterobiasis in children by age which is facilitated by increased exposure. In the older adolescent (>14 years), the level dropped and reached the background rates observed in the adult population. Despite the significantly higher prevalence, similar distributions are reported in different European countries. Investigation in healthy Norwegian children shows 2 – 3 years old kids as least affected (7.3 %), what is followed by 4 – 5 years-old group (14.1 %), and the highest prevalence (34.4 %) was observed in 5 – 14 years old children (Boas *et al.*, 2012). Parallel age dependency was observed in Estonia where the prevalence was 16.2 % in 1 – 3 years-old kids, 16.0 % in 4-year-olds, 29.9 % in 5 and 6-year-olds and 42.0 % in 7 – 8 years old group (Remm, 2006).

The attendance in children's collectives is a significant risk factor for the propagation of enterobiasis in Bulgaria (Genov *et al.*, 1975; Rainova *et al.*, 2018; Ruseva & Popova, 1992; Stoyanova, 2019). The age-dependent partitioning of the prevalence that was apparent in Fig. 3 also corresponded with the main types of facilities for child care and education. Infants attending nurseries (0 – 2-years old) are less exposed and less affected. Their primary contact with the disease is probably via infected family member – parent or older sibling, and thus the average level of the infection (0.36 %) is similar and insignificantly higher than in adults.

The disease rate increased substantially to 1.58 % in the age group attending kindergartens and preschool (2 – 6-year-olds) as the odds of contracting the disease here are about six times higher than in adults (OR=6.32).

These results also showed the encouraging trend that over the

past 40 years, the enterobiasis prevalence in the childcare facilities in Varna District has decreased about 3 times in both age groups – from 1.1 % (1971 – 1975) (Kovchazov, 1979) to 0.36 % (2009 – 2018) in the nurseries and from 3.6 % to 1.58 % in the kindergartens.

Almost the same rate in reduction of enterobiasis was observed in the children in school age. It decreased from 35.6 % (1971 – 1975) (Kovchazov, 1979) to 9.57 % in our current study. Nevertheless, students remain the most affected by enterobiasis. The significant differences from younger age-groups can be attributed to the absence of any preventive measures that should focus on the children or on their close environment (schools). In contrast, there is an effective system for the prevention in nurseries, kindergartens and preschools. The higher prevalence detected at school-age must be interpreted cautiously. This group is not subjected to preventative screening and is comprised of a population tested for *E. vermicularis* either on clinical (manifested symptoms) or on epidemiological (contact with an infected) ground. Therefore, the observed rates are biased upwards, and the actual prevalence is probably lower. Still, there is a six-fold increase in odds as compared with kindergarten age group (OR=6.57; CI:4.59-9.41) and 41-fold in comparison with adults. This finding requires detailed proactive surveillance in this group and the implementation of focused preventive measures to limit disease circulation in schools. Similarly to our findings, higher rates of enterobiasis in older children were found in Denmark (29 % in 5 – 12 years old kids) (Lacroix & Sørensen, 2000), and in children from neighbouring Turkey where 10.1 % of the pupils in the primary schools in South-Western (Aksoy *et al.*, 2007) and 18.2 % of the 7 – 14 year-old students in Western region (Okyay *et al.*, 2004) were affected.

## Conclusion

This research identifies an alarming upward trend in the annual enterobiasis prevalence in children within NE Bulgaria. The infection is most frequent in preadolescent and early adolescent age where targeted preventive measures should be introduced and enforced.

Our results about the prevalence of enterobiasis in Varna District and its rates in children and adults reflected the overall epidemic process of this helminthiasis in Bulgaria. The levels of *E. vermicularis* infection (regionally and countrywide) were significantly lower than the prevalence data reported from most of the European countries. Two factors were responsible for the observed discrepancy. Our pool of subjects included a vast number of children and adults, tested annually on prophylactic indications which rarely are an object of epidemiological studies available. More importantly, the Bulgarian proactive surveillance system for intestinal parasites serves as an effective approach to screen for such mild or generally asymptomatic infections. Consequently, the majority of the 'hidden' disease carriers are revealed, and subsequently treated and eradicated as potential sources of further transmission.

## Conflict of Interest

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

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