

LABORATORY DIAGNOSIS OF ASCARIS LUMBRICOIDES INFESTATION IN PAEDIATRIC POPULATION IN TWO MUNICIPALITIES IN BOSNIA AND HERZEGOVINA

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Abstract: Introduction: *Ascaris lumbricoides* is a widely spread helminthic infection, predominantly affecting children, making them the most commonly infected population group. The objective of this study is to assess the prevalence of *Ascaris lumbricoides* infestation in two municipalities, Tešanj and Maglaj, and to investigate the occurrence of *Ascaris lumbricoides* infections in the pediatric population, focusing particularly on preschool children.

Materials and Methods: The study involved the collection of 1409 fecal samples from the Tešanj and Maglaj areas, gathered over a 6-month period, spanning from September 2018 to February 2019. The processing of these samples was conducted in the Microbiology Laboratory of Tešanj General Hospital.

Results: Out of the total 1409 samples, 129 (9.16%) tested positive for *Ascaris lumbricoides* infestation. In Tešanj, where 1198 samples were collected, 106 (8.85%) tested positive, while in Maglaj, 211 samples were collected, with 23 (10.9%) testing positive. Notably, the majority of positive cases in both Tešanj and Maglaj were preschool-age children, accounting for 88.68% and 86.96%, respectively. The study did not identify any statistically significant correlation between age and gender distribution among those with positive test results in either Tešanj or Maglaj.

Conclusion: Based on the study results, which have highlighted the infestation of preschool children

with *Ascaris lumbricoides* in two municipalities in our country, it is imperative to implement preventive measures aimed at reducing the incidence of infection.

Keywords: *Ascaris lumbricoides*, Ascariasis, infestation, children.

INTRODUCTION

Ascaris lumbricoides is a parasitic nematode that causes ascariasis (1), the most prevalent helminthic infection affecting over 1.2 billion people globally (2). Its prevalence is particularly concentrated in tropical and subtropical regions with inadequate sanitation conditions, primarily affecting children (3). In 2018, over 676 million school-aged children in endemic countries received antihelminthic medications, covering 53% of children at risk (4).

Transmission occurs through the fecal-oral route, with risk factors including inadequate personal hygiene and sanitation, warm climates, and high humidity (5). Most individuals with intestinal ascariasis are asymptomatic. However, those who do exhibit symptoms commonly experience pulmonary or gastrointestinal issues such as nausea, bloating, reduced appetite, abdominal distension and discomfort, recurrent abdominal pain, and intermittent diarrhea (2). Prolonged infestation with *Ascaris lumbricoides* may lead to poor weight gain or malnutrition, potentially

impairing both physical growth and cognitive development over time (6).

Diagnosing of *A. lumbricoides* involves identifying parasite eggs, larvae, or adult worms. Stool sample examination using light microscopy remains the primary method to identify and quantify *A. lumbricoides* eggs. However, the uneven distribution of eggs in stool samples might yield false-negative results, particularly in cases of low-intensity infections or post-treatment. The standard treatments for ascariasis include albendazole, mebendazole, or ivermectin (7).

Aim

This study aims to evaluate the prevalence of *Ascaris lumbricoides* infestation in two municipalities, Tešanj and Maglaj. Additionally, it aims to investigate *Ascaris lumbricoides* infections among the pediatric population, specifically focusing on preschool children. Our assessment involves examining the infestation occurrence in these areas and analyzing its prevalence concerning gender, age, and place of residence.

MATERIALS AND METHODS

Specimen Collection

This study encompassed the collection of 1409 fecal samples from the Tešanj and Maglaj areas over a 6-month period, spanning September 2018 to February 2019. The samples were obtained and processed at the Microbiology Laboratory of Tešanj General Hospital.

In suspected cases of helminthiasis, it is essential to examine a minimum of 3 fecal samples, collected at intervals of 2 to 3 days. Each sample, weighing 10 grams, was obtained from both the surface and the depth of the fecal material and then delivered to the laboratory in specialized containers. Pertinent information, including the patient's name, surname, and the precise day and time of sample collection, was recorded for each specimen.

Liquid samples were analyzed within 30 minutes, while formed feces were examined within 2-3 hours post-collection. If necessary, formed samples could be studied within 24 hours if refrigerated at 4 °C. For transportation purposes when immediate analysis wasn't feasible, a preservative was added. A 5% buffered aqueous solution of formalin served as the preservative, effectively fixing the *Ascaris lumbricoides* eggs and larvae. There are also various commercially available kits for preserving fecal samples, comprising vials with preservatives (8).

Methods

The flotation technique and sedimentation methods belong to the category of concentrated methods.

The main task of these methods is to separate the parasites from other elements in the fecal sample and to concentrate them, which enables easier detection. The flotation technique concentrates the parasites on the surface of the concentration solution, while the other elements of the fecal sample settle to the bottom of the test tube, precisely because of the differences in specific weights between the parasites and the solution used for concentration. There are two types of flotation techniques, with or without centrifugation, and zinc sulphate ($ZnSO_4$) and saturated salt solution (NaCl) can be used as flotation solutions.

In our study, the flotation method saturated with salt solution (NaCl) without centrifugation was used. After a sample of feces is taken from the examinee, a small amount is taken with a wooden stick in several places and dissolved in a saturated salt solution (NaCl), the specific gravity of which is 1.20.

Procedure

1. Emulsify 0.5 grams of feces in 2 to 3 ml of saturated NaCl solution within a test tube with a 15 mm inner diameter.
2. Fill the test tube with NaCl solution and mix well. Place the test tube in a vertical position in the test tube rack.
3. Add additional NaCl solution to fill the test tube to its brim.
4. Carefully cover the test tube with a cover glass.
5. Allow the solution to stand at room temperature for 30-45 minutes, allowing sufficient time for *Ascaris lumbricoides* eggs to rise to the surface.
6. Carefully lift the cover glass using tweezers, transfer it onto a microscope slide, and examine under a microscope.

The disadvantage of this method is that after 60 minutes the eggs of *Ascaris lumbricoides* begin to settle at the bottom of the test tube, so it is necessary to make the slides within 30-45 minutes.

The results were processed using standard statistical methods using the SPSS Statistical Package for Social Sciences version 13.0 (Chicago, IL, USA). The results for independent continuous variables are expressed as absolute N and percentage values (%). The analysis of categorical variables' differences among observed groups employed either the Chi-square or Fisher's exact test. The value of $p < 0.05$ was taken as statistically significant.

RESULTS

Out of the 1409 samples analyzed, 129 (9.16%) yielded a positive result for *Ascaris lumbricoides* infestation, while the remaining 1280 (90.84%) were

negative. This difference was found to be statistically significant ($p < 0.001$).

Among the 1198 samples collected in Tešanj, 106 (8.85%) tested positive for *Ascaris lumbricoides* infestation. Within this group, 42.45% were males, and the remaining 57.55% were females. The majority of the samples 91.15%, yielded a negative result. In Maglaj, a total of 211 samples were collected, and 10.9% of these samples tested positive for *Ascaris lumbricoides* infestation. Among those with positive results, 73.91% were males, while the remaining 26.09% were females. 188 samples (89.1%) showed a negative result for *Ascaris lumbricoides* infestation. The determined difference in the gender distribution of respondents with a positive finding in relation to the surveyed municipalities was statistically significant ($p = 0.01$) (Figure 1).

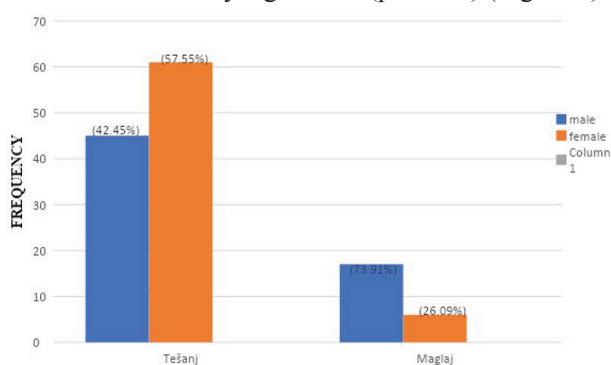


Figure 1. Gender distribution of respondents with a positive findings in relation to the investigated municipalities

Among the samples from Tešanj with a positive result for *Ascaris lumbricoides* infestation, 11.32% of them were of school-age, and the remaining 88.68% were preschool-age children. In the case of the 23 samples from Maglaj that tested positive, 13.04% of them belonged to the school-age group, while the remaining 86.96% were preschool-age children (Figure 2).

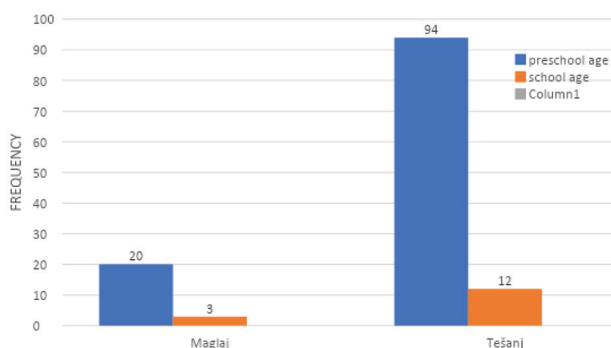


Figure 2. Age distribution of respondents with a positive findings in relation to the investigated municipalities

Among 106 examinees with a positive result from Tešanj, 20.75% were from urban areas, while the other 79.25% were from rural areas.

Out of a total of 106 respondents with a positive result from Tešanj, the frequency of male examinees of school age was 6.6%, and of preschool age 35.85%. The frequency of female examinees of school age was 4.72%, and of preschool age 52.83%. There was no statistically significant dependence between the age distribution and gender of examinees with a positive test in Tešanj ($p = 0.353$).

Among 23 samples with a positive result from Maglaj, the frequency of male examinees of school age was 8.7%, and 65.22% of preschool age. The frequency of female examinees of school age was 4.72%, and of preschool age 21.74%. There was no statistically significant dependence between age distribution and gender in examinees with a positive test in Maglaj ($p = 1.0$).

Out of the 106 samples with a positive result from Tešanj, the frequency of male examinees from urban areas was 8.49%, while from rural areas, it was 12.26%. For female examinees, the frequency from urban areas was 33.96%, and from rural areas, it was 45.28%. There was no statistically significant dependence between the regional distribution and gender of examinees with a positive test in Tešanj ($p = 0.896$).

The frequency of school-age examinees from urban areas among 106 positive samples from Tešanj, was 1.89%, and from rural areas 9.43%. The frequency of preschool examinees from urban areas was 18.87% and from rural areas 69.81%. No statistically significant dependence was found between age and regional distribution in examinees with a positive test in Tešanj ($p = 0.340$).

DISCUSSION

Parasitosis is prevalent in tropical countries, with the highest number of cases reported in China, South-east Asia, and the coastal regions of West and Central Africa. Annually, it affects more than one billion people globally, and over 60,000 cases result in fatalities (9). School-aged children are more susceptible to infestations compared to the adult population. Consequently, the World Health Organization has initiated control programs in endemic countries with a high prevalence of infestations. These programs are designed to decrease the number of infected individuals and minimize morbidity. Additionally, the World Health Organization has incorporated preschool children as a high-risk population in these programs because 10-20% of children in that age group are infested with *Ascaris lumbricoides* or other helminthes (10, 11).

In endemic countries, it is the most common cause of malabsorption and malnutrition in children, and it can also lead to disorders of growth, development, and

cognitive dysfunction. Given that *Ascaris* disrupts the absorption of vitamin A, in preschool children, this interference can result in clinical symptoms of vitamin A deficiency, increasing morbidity and mortality (12). A study conducted in Nepal revealed that the prevalence of xerophthalmia was three times greater in children between the ages of 6 and 120 months who were infested with *Ascaris lumbricoides*, in comparison to children who were not infested (13, 14).

In our study, 9.16% of the samples tested positive for *Ascaris lumbricoides*, and all individuals with positive fecal findings were either school-aged or preschool-aged children. Similar research conducted in South Asia reported similar findings (15).

In Tešanj and Maglaj, there were more positive findings of infestation with *Ascaris lumbricoides* among preschool-age children than among school-age children. A study conducted in Slovakia reported similar results (16). Conversely, somewhat different results were obtained by studies conducted in Sri Lanka and India, showing almost an equal number of infected individuals in both age groups (17, 18). By observing the increased prevalence of infestations in preschool children in our research, we can conclude that age affects the frequency of infestations.

A study conducted in Slovakia and China shows that infestations with *Ascaris lumbricoides* are more common in male examinees, correlating with the results of our research in the area of Maglaj compared to the data in the area of Tešanj (16, 19). Conversely, studies conducted in Sri Lanka and India showed that the prevalence of infestations was higher in female children (17, 18). The variations in gender distribution among the individuals who were infested can be attributed to inadequate personal hygiene in some subjects, as well as differences in the dietary habits of household members. Additionally, the significance of the water supply should not be discounted, as individual households are typically responsible for their own water sources, often relying on their private wells.

Regarding the total number of examinees with infestation caused by *Ascaris lumbricoides* from Tešanj, most of them were from rural areas compared to subjects from urban areas ($p < 0.001$). These results do not coincide with the results of research from Nicaragua, where there is a statistically significant difference in regional distribution, but in favor of respondents living in urban areas ($p = 0.004$) (20). The results of the regional distribution of Tešanj may differ from the results of other studies due to different systems of collection and removal of sanitary-fecal wastewater, where some households still use septic tanks while others are

connected to the sewage network, which prevents the spread of infectious diseases.

Analyzing the collected data from the area of both municipalities, we conclude that there is no statistically significant difference in the frequency of infestations, nor is there a difference in the age distribution of subjects infested with *Ascaris lumbricoides*, in contrast to the gender distribution where a statistically significant difference is observed. The research confirmed that the prevalence of infestations in both municipalities is higher in the pediatric population of preschool-age compared to the school population, as well as that the prevalence of infestations is higher in rural areas of Tešanj compared to urban ones.

Understanding the factors contributing to the occurrence of infestations is crucial for implementing measures to reduce the number of infections and prevent complications, especially in children who are more vulnerable than the adult population. The preventive actions that should be undertaken include: enhancing sanitation conditions, improving access to clean water, educating the public about parasitic infections, emphasizing the significance of maintaining personal and communal hygiene, and discouraging the use of human feces in agriculture.

CONCLUSION

Ascariasis stands as a significant public health concern, demanding focused attention on high-risk groups and ensuring access to improved water, sanitation, and hygiene facilities. Given that the findings of our study have revealed the infestation of preschool children with *Ascaris lumbricoides*, it is essential to implement preventive measures aimed at reducing the incidence of infection. In conclusion, heightened awareness and knowledge about *A. lumbricoides* infection, among both healthcare professionals and the general public, are imperative. This will enhance the clinical detection and management of cases, as well as public health control measures.

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Sažetak

LABORATORIJSKA DIJAGNOSTIKA ASCARIS LUMBRICOIDES INFESTACIJE U PEDIJATRIJSKOJ POPULACIJI U DVE OPŠTINE U BOSNI I HERCEGOVINI

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Uvod: *Ascaris lumbricoides* je najčešći uzrok helmintičkih infekcija u svetu, a prvenstveno pogađa decu, što ih čini najčešće zaraženom populacionom skupinom. Cilj ovog istraživanja je proceniti prevalenciju infestacije *Ascaris lumbricoides* u dve opštine, Tešanj i Maglaj, te istražiti pojavu infekcija *Ascaris lumbricoides* u pedijatrijskoj populaciji, sa posebnim fokusom na decu predškolskog uzrasta.

Materijali i metode: Istraživanjem je prikupljeno 1409 uzoraka stolice sa područja Tešnja i Maglaja. Ovi uzorci su prikupljeni u periodu od 6 meseci, od septembra 2018. do februara 2019. godine. Obrada uzoraka obavljena je u Mikrobiološkoj laboratoriji Opšte bolnice Tešanj.

Rezultati: Od ukupno 1409 uzoraka, 129 (9,16%) je bilo pozitivno na infestaciju *Ascaris lumbricoides*-

-om. U Tešnju, gde je prikupljeno 1198 uzoraka, pozitivno je bilo 106 (8,85%). U Maglaju je prikupljeno 211 uzoraka, a 23 (10,9%) su imala pozitivan nalaz. Većina pozitivnih uzoraka u Tešnju i Maglaju bila su deca predškolskog uzrasta, 88,68%, odnosno 86,96% respektivno. Studija nije utvrdila nikakvu statistički značajnu korelaciju između dobne i polne distribucije među onima sa pozitivnim rezultatima testa niti u Tešnju, ni u Maglaju.

Zaključak: U svetlu rezultata našeg istraživanja, koji su ukazali na infestaciju dece predškolskog uzrasta *Ascaris lumbricoides*-om u dve opštine u našoj zemlji, neophodno je sprovesti preventivne mere s ciljem smanjenja incidencije infekcije.

Cljučne reči: *Ascaris lumbricoides*, Ascariasis, infestacija, deca.

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