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PARASITE SCREENING IN STOOL WITH NATIVE-LUGOL, RAPID DIAGNOSTIC TESTS, AND CELLOPHANE BAND METHOD IN CHILDREN WITH DEVELOPMENTAL DELAY

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Keywords

Developmental delay

Intestinal parasites

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ABSTRACT

Intestinal parasites have a widespread distribution globally. While it is known that approximately 3.5 billion people are infected with intestinal parasites, the majority of this distribution is comprised of children. Intestinal parasites in children can lead to various detrimental outcomes, ranging from physical growth retardation to intellectual development issues, particularly during the developmental stages. The study aimed to investigate intestinal parasites in children diagnosed with developmental delay who sought medical attention at Hatay Mustafa Kemal University Hospital. Fifty-six children aged 0-15 years diagnosed with developmental delay, who applied to the Pediatric Clinic of Hatay Mustafa Kemal University Faculty of Medicine, were included in the study. Stool and cellophane band samples were collected from these children. For the diagnosis of parasitic infections, stool samples were examined using native-lugol, acid-fast, and trichrome staining methods. And commercial rapid diagnostic tests for Cryptosporidium parvum, Giardia intestinalis, and Entamoeba histolytica/dispar were also utilized. The collected samples with cellophane band method were evaluated for the presence of Enterobius vermicularis. Parasites were identified in 30.36% of 56 children diagnosed with developmental delay. Among the stool samples, Giardia intestinalis was detected in two cases (3.57%), Strongyloides stercoralis in two cases (3.57%), and Blastocystis hominis in 10 cases (17.86%). A positive band was also obtained in rapid diagnostic kits in two of the samples in which Giardia intestinalis was detected. In cellophane band samples, Enterobius vermicularis was found in three cases (5.36%). The study concluded that intestinal parasites should be investigated more widely in children diagnosed with developmental delay, parasitic control should not be ignored in these patient groups, and regular screenings should be performed.Intestinal parasite-induced

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INTRODUCTION

Infections can be prevented and treated. Despite this, it continues to be seen as an important public health problem in developing countries [1]. It is reported that approximately 3.5 billion people worldwide are infected with intestinal parasites, with the majority being children [2]. More than 267 million preschool children and over 568 million school-age children are at risk in countries where intestinal parasites are endemic [3]. In Turkey, the prevalence of intestinal parasites in children is reported to be between 13-66% [4].

Due to factors such as the incomplete development of children's immune systems, insufficient attention to personal hygiene, and playing with soil and water, intestinal parasites are more frequently observed in children [5].

Parasitic infections tend to progress more slowly and chronically compared to viral and bacterial infections. Generally, they cause asymptomatic or nonspecific mild

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clinical symptoms [6]. In symptomatic cases, intestinal parasites in children can lead to nutritional and digestive disorders, malabsorption, diarrhea, anemia, protein-energy malnutrition, deficiencies in nutrients and vitamins as well as developmental delays [7-9].

Developmental delay occurs when a child fails to reach expected developmental milestones in a timely manner compared to peers of the same age group. It can manifest in various areas, including the development of physical, mental, and motor skills, as well as in emotional and social domains [10]. Intestinal parasites in children, along with developmental delay, can lead to deficiencies in micronutrients such as iron and even behavioral disorders. Moreover, they are thought to disrupt the balance of the intestinal microbiota, potentially contributing to neurodevelopmental problems [11].

The connection between parasitic diseases and developmental delay has not been fully elucidated. However, it is believed that systemic responses in the body to parasitic infections, coupled with impaired intestinal absorption and anemia, may contribute to developmental delay in children [12].

The study aimed to investigate intestinal parasites in children diagnosed with developmental delay aged 0-15 years.

METHOD

Ethical Approval

In our study, written consent was obtained from all the cases participating in our study, in accordance with the Declaration of Helsinki. Ethics Committee permission was obtained from Hatay Mustafa Kemal University Faculty of Tayfur Ata Sökmen Medicine Clinical Research Ethics Committee with the protocol code for the research: 2020/85

Data Collection

A total of 56 patients diagnosed with developmental delay, aged between 0-15 years, who applied to the Pediatric Clinic of Hatay Mustafa Kemal University Faculty of Medicine, were included in the study. Necessary information about the study was provided to the parents of the children, and informed consent was obtained. A questionnaire was prepared regarding socioeconomic status, parents' education, living conditions, water usage, and animal feeding status, and parents were asked to fill it out.

Parasitological Evaluation

Stool and cellophane band samples were collected from children diagnosed with developmental delay to investigate intestinal parasites. Parents were explained how to apply the cellophane band. Cellophane band samples were evaluated for the presence of Enterobius vermicularis under a light microscope with 10x and 40x objectives.

Stool samples were macroscopically examined for the blood, mucus, color, consistency, and presence of adult helminths or segments. All samples were evaluated under a light microscope with 10x and 40x objectives using the native-lugol method. Preparations prepared from stool samples were examined by staining with Kinyoun Acid Fast and Trichrome staining methods.

Rapid Diagnostic Test

Four rapid diagnostic tests were applied to stool samples using commercial kits for Cryptosporidium parvum (CerTest Spain), Giardia intestinalis (CerTest Spain), and Entamoeba histolytica/dispar (CerTest Spain), as well as a commercially prepared combo test for the simultaneous detection of the three parasites (CerTest Crypto + Giardia + Entamoeba one step combo card test, Spain). These tests were performed according to the protocols provided within the kits.

FINDINGS

Survey Data

A total of 56 patients diagnosed with developmental delay, aged between 0-15 years, who applied to the Pediatric Clinic of Hatay Mustafa Kemal University Faculty of Medicine, were included in the study. Among the patients, 26 were male, and 30 were female. It was found that the majority resided in various districts, predominantly in Antakya, including Kırıkhan, Reyhanlı, Samandağ, Yayladağ. The patients presented with different clinical symptoms such as abdominal pain, nausea, diarrhea, perianal itching, and excessive salivation. Information regarding the educational status of parents, gender, living area, water usage, and animal feeding status of the patients was recorded (Table 1, 2).

Table 1: Distribution of the number and percentage of patients based on the education status of parents

	Mother		Father	
	n	%	n	%
Education Status				
İlliterate	1	1,79	2	3,57
Literate	4	7,14	7	12,5
Primary School	13	23,21	12	21,43
Middle School	16	28,57	17	30,36
High School	14	25,00	11	19,64
University	8	14,29	7	12,5
Total	56	100	56	100

Table 2: Distribution of the number and percentage of patients based on gender, living area, water usage, and animal feeding status

	n	%
Gender		
Female	30	53.57
Male	26	45.43
Living area		
Rural	25	44.64
Urban	31	55.36
Water usage		
Prepared water	19	33.93
City water	35	62.5
Prepared water + City water	2	3.57
Animal feeding		
Yes	18	32.14
No	38	67.86
Total	**56**	**100**

Prevalence of Intestinal Parasites

Intestinal parasites were found in 30.36% of the 56 patients diagnosed with developmental delay, and all patients were infected with a single species of parasite. Among the stool samples, 14 children (25%) tested positive for parasites, with two cases (3.57%) of Giardia intestinalis, two cases (3.57%) of Strongyloides stercoralis, and 10 cases (17.86%) of Blastocystis hominis. (Figure 1, 2, 3, 4).

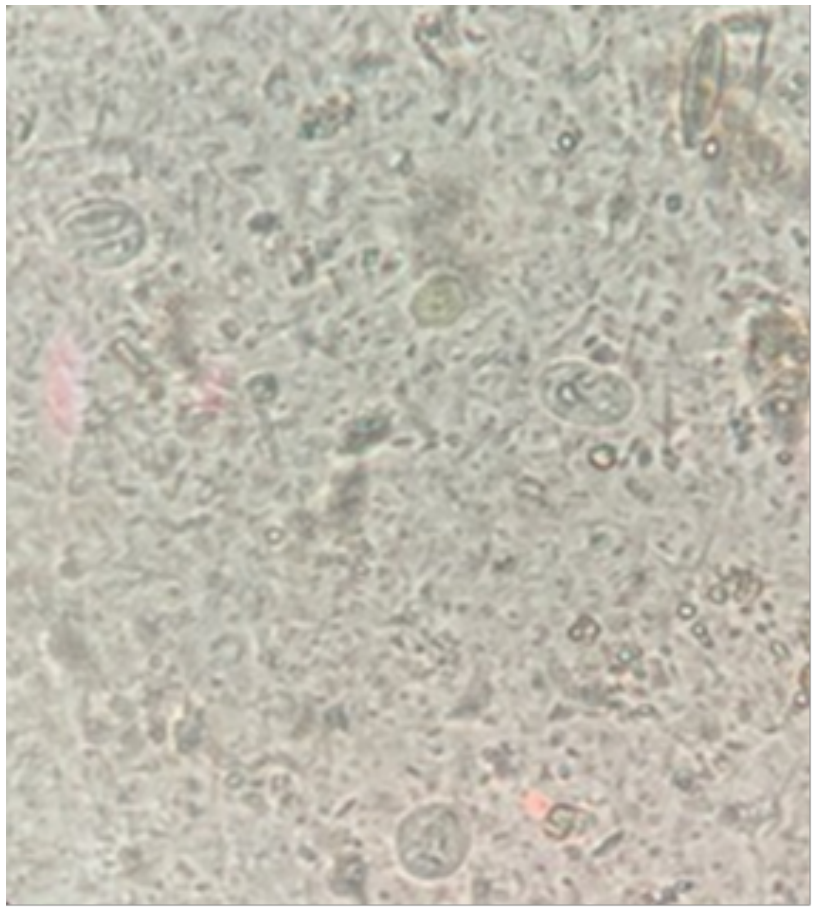


Figure 1: *Giardia intestinalis* cysts in direct microscopic examination

In rapid diagnostic tests prepared with antigens specific to Cryptosporidium parvum (CerTest Spain) and Entamoeba histolytica/dispar (CerTest Spain), no bands were observed. For Giardia intestinalis (CerTest Spain) and the combo test (CerTest Crypto + Giardia + Entamoeba one step combo card test, Spain), two samples tested positive for Giardia intestinalis in both rapid diagnostic tests, and parallel results were obtained through the direct microscopic examination method.

Enterobius vermicularis was detected in three (5.36%) of the collected cellophane band samples (Figure 5).

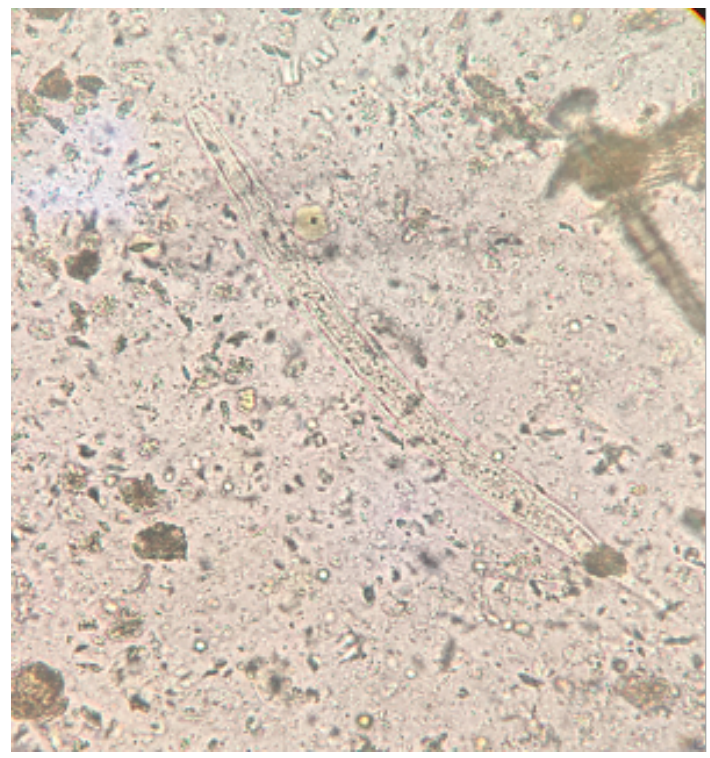


Figure 2: *Strongyloides stercoralis* larvae in direct

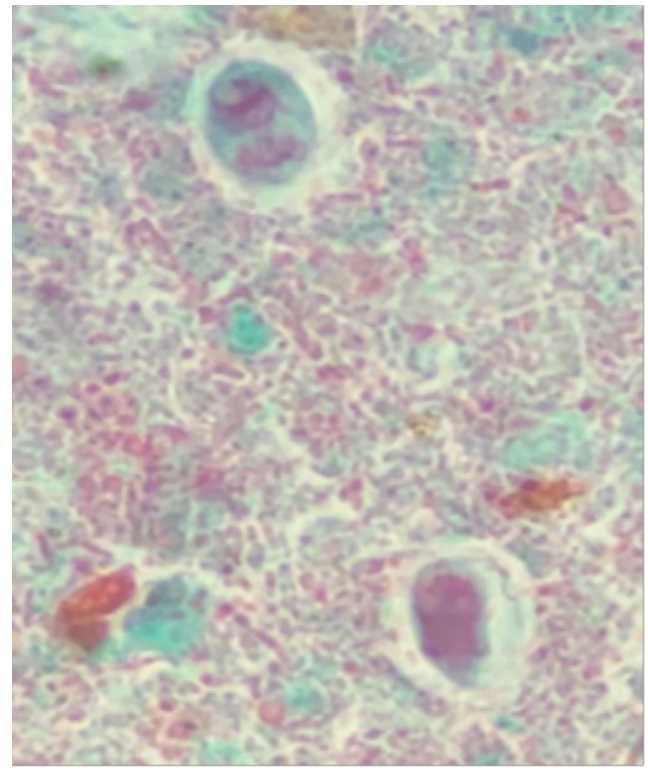


Figure 3: *Giardia intestinalis* cysts (Trichrome Staining Method)

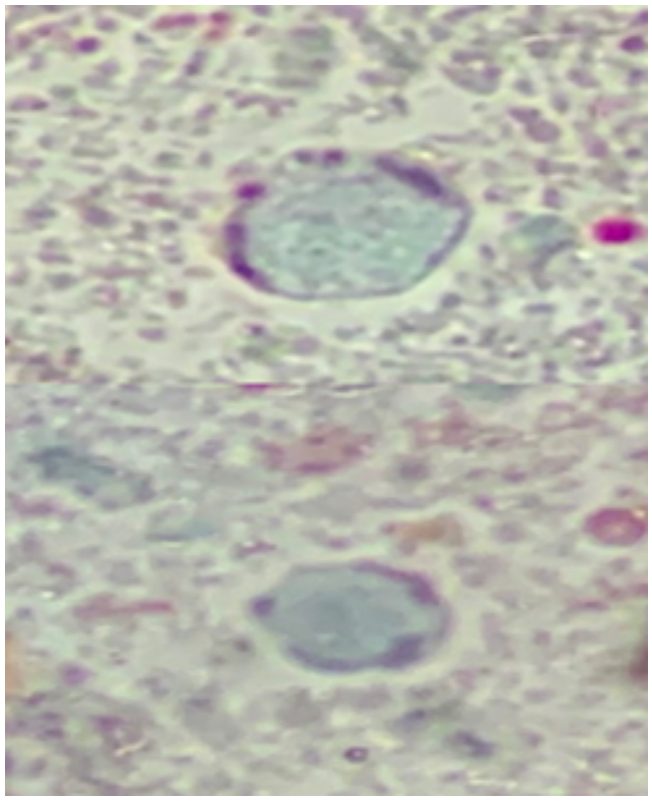


Figure 4: *Blastocytis hominis* (Trichrome Staining Method)

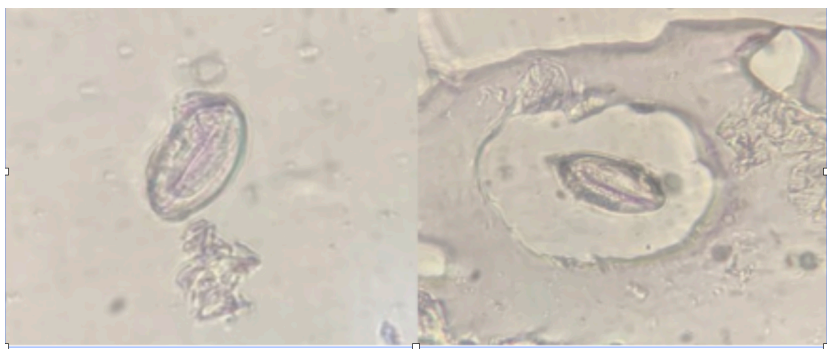


Figure 5: *Enterobius vermicularis* in the cellophane band samples

DISCUSSION

Intestinal parasites are a significant public health problem more commonly encountered in children than adults, which can lead to mental and physical developmental delays. The prevalence of parasitic infections in children is higher due to factors such as playing with soil, eating without washing hands, consuming contaminated water, vegetables, and fruits without proper attention, and inadequate adherence to hygiene rules in toilet habits and cleanliness [13]. Additionally, factors like insufficient parental knowledge on the subject, low socio-economic status, crowded family environments, and contact with pets contribute to an increased incidence of parasitic infections in children [14].

Parasitic infections in children lead to developmental delays associated with inadequate nutrition [15]. Especially *Enterobius* vermicularis is commonly observed in primary school and elementary school-aged children, and it is among the most common factors contributing to developmental delays in children. [16]. In 2004, a study found *Enterobius vermicularis* in 63 out of 185 children examined using the cellophane band method in Child Protection and Training Institutions in Hatay Province [25]. In another study conducted in Hatay in 2019, among 167 children aged 6-14, 45 (26.8%) were found to be positive for *Enterobius vermicularis* [26]. In this study, *Enterobius vermicularis* were found in three children (5.36%) diagnosed with developmental delay, living in rural areas, and experiencing perianal itching.

Another intestinal parasite that can cause developmental delay and is commonly seen in children is *Giardia intestinalis*. While it can be asymptomatic, it can also cause digestive system clinical symptoms such as abdominal pain, diarrhea, and weight loss [17]. In a cross-sectional study conducted by Şimşek et al. with 160 children aged 0-5 years, they reported that 80 children were infected with at least one intestinal parasite, with *Giardia intestinalis* being the most frequently detected. The study found that *Giardia intestinalis* infection had a negative impact on growth and psychomotor development in children [20]. In the current study, two children diagnosed with developmental delay were found to be positive for *Giardia intestinalis*.

While *Strongyloides stercoralis* can lead to a severe clinical condition, especially hyperinfection in immunocompromised individuals, its exact impact on developmental delay in children is not fully understood [18]. In the study, *Strongyloides stercoralis* was detected in two out of the 56 patients who had received a preliminary diagnosis of developmental delay. It is necessary to evaluate other children who are physically and mentally behind their peers in terms of intestinal parasites.

In children, it is reported that the acute loss of blood due to the systemic invasion of intestinal parasites and the depletion of protein stores can lead to developmental delay [19]. In a study conducted in Şanlıurfa investigating the relationship between intestinal parasites and growth and psychomotor development retardation in children aged 6 and below, they found 42.53% *Giardia intestinalis*, 27.58% *Enterobius vermicularis*, 18.39% *Ascaris lumbricoides*, 5.75% *Hymenolepis nana*, 3.45% Trichuris trichiura, 1.15% *Entamoeba coli*, and 1.15% *Blastocystis spp*. In children infected with intestinal parasites, they found a higher prevalence of psychomotor development delay and growth retardation. [21]. In the current study, *Giardia intestinalis* was found in two stool samples of children diagnosed with developmental delay, *Strongyloides stercoralis* in two samples, *Blastocystis hominis* in ten samples, and *Enterobius vermicularis* in three cellophane band samples.

No existing information was found regarding a study on intestinal parasites and developmental delay in children conducted in Hatay. In studies investigating intestinal parasites in children, with gastrointestinal and anal itching complaints ranging from ages 0 to 14, a total of 602 children were evaluated, and 561 stool and 534

cellophane band samples were assessed. In the stool samples, they found one or more intestinal parasites in 104 cases. Out of the 534 cellophane band samples, they reported that 85 (15.91%) were positive for *Enterobius vermicularis* [22]. In 2005, in four private day care centers and nurseries, out of a total of 86 fecal samples, *Giardia intestinalis* was found in four, *Blastocystis hominis* in 12, E*ntamoeba coli* in four, and *Hymenolepis nana* in one, making a total of 18 cases (20.93%). Additionally, out of 109 cellophane tape samples, *Enterobius vermicularis* was detected in eight cases (7.40%) [23]. In 2009, among a total of 177 children residing reformatory in Hatay, they found one or more parasites in 87 (49.2%), including 57 with *Enterobius vermicularis*, 14 with *Giardia intestinalis*, 11 with *Ascaris lumbricoides*, 5 with *Taenia saginata* [24].

In the current study, among a total of 56 patients aged 0-15, including 26 boys and 30 girls, intestinal parasites were found in 14 stool samples. For patients identified as positive for *Giardia intestinalis* with rapid diagnostic tests, both Giardia intestinalis (CerTest Spain) and the combo test showed positive bands in their stool samples.

CONCLUSION

The conclusion drawn is that comprehensive studies with larger sample groups in children with developmental delay, as well as regular screenings, are necessary. It is essential to provide education to children and their families about the ways of transmission, possible clinical symptoms, and the importance of early diagnosis and treatment in asymptomatic cases of intestinal parasites. Additionally, teaching personal hygiene rules to children is deemed crucial.

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Confict of interest statement

The authors have no conflict of interest regarding this study.

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