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A Study on the Epidemiology of Intestinal Parasitic Infections in Autistic Children in Zella - Libya

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معدل انتشار الطفيليات المعوية بين الاطفال المصابين باضطراب طيف التوحد في مدينة زلة - ليبيا

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Abstract:
This study aims to investigate the prevalence of intestinal parasites among children diagnosed with Autism Spectrum Disorder (ASD) in the city of Zella, located in the Al-Jufra municipality in central Libya. The study was conducted between March and October 2024 and included a sample of 15 children aged between 3 and 15 years, selected from Al-Amal Center for Special Needs in the city. Descriptive Statistics analysis was used in this study. Following the necessary ethical approval, demographic data were collected through questionnaires directed to the children's parents. These questionnaires covered aspects related to the children's medical history, characteristics of autism, and gastrointestinal symptoms. Stool samples were collected from the autistic children and examined in the laboratory using direct microscopic examination techniques to detect common intestinal parasites at the central hospital laboratory in Zella. The findings revealed that the overall prevalence of intestinal parasitic infections among autistic children was 80%. Infection rates were higher among males (90%) compared to females (60%). The distribution of infections by parasite type was as follows: *Entamoeba histolytica* (68%), *Giardia lamblia* and *Ascaris lumbricoides* (17% each), and *Entamoeba coli* (8%). Additionally, 92% of the cases involved multiple infections, with more than one parasite found in the same autistic child. Analysis indicated that children aged 6 to 8 years were the most affected, with a percentage of 58%. A strong association was observed between intestinal parasitic infections and hyperactivity, recorded in 80% of the cases, while chronic diarrhea was reported in only 8% of the studied sample.

Keywords: North Africa, Children, Autism spectrum, Libya, Intestinal Parasites, Zella.

الملخص
تهدف هذه الدراسة إلى تقصي معدل انتشار الطفيليات المعوية لدى الأطفال المصابين باضطراب طيف التوحد في مدينة زلة، التابعة لبلدية الجفرة في وسط ليبيا. نُفذت الدراسة خلال الفترة الممتدة من مارس حتى أكتوبر 2024، وشملت عينة مكونة من 15 طفلًا تتراوح أعمارهم بين 3 و15 عامًا، تم اختيارهم من مركز الأمل لذوي الاحتياجات الخاصة في المدينة. تم استخدام منهج الاحصاء.. الوصفي في هذه الدراسة. بعد الحصول على الموافقة الأخلاقية اللازمة، تم جمع البيانات الديموغرافية من خلال استبيانات وُجهت إلى أولياء الأمور، وتناولت جوانب تتعلق بالتاريخ المرضي للأطفال، وخصائص اضطراب التوحد، والأعراض المرتبطة بالجهاز الهضمي. كما جُمعت عينات برازية من الأطفال، وتم فحصها مخبريًا باستخدام تقنيات الفحص المباشر للكشف عن الطفيليات المعوية، وذلك في مختبر مستشفى زلة المركزي.. أظهرت نتائج الدراسة أن معدل الإصابة الكلي بالطفيليات المعوية بين الأطفال المصابين بالتوحد بلغ 80%. وكانت النسبة أعلى لدى الذكور (90%) مقارنة بالإناث (60%). وتوزعت الإصابات بحسب نوع الطفيل كما يلي: *Entamoeba histolytica* بنسبة 68%، *Giardia lamblia* و*Ascaris lumbricoides* بنسبة 17% لكل منهما، و *Entamoeba coli* بنسبة 8%. كما بينت النتائج أن 92% من الحالات كانت إصابات متعددة، حيث وُجد أكثر من نوع واحد

من الطفيليات لدى نفس الطفل. أشارت التحليلات إلى أن الأطفال في الفئة العمرية من 6 إلى 8 سنوات كانوا الأكثر عرضة للإصابة. كما لوحظ وجود علاقة قوية بين الإصابة بالطفيليات المعوية وفرط الحركة، حيث سُجلت هذه العلاقة في 80% من الحالات، في حين ظهرت أعراض الإسهال المزمن لدى 8% فقط من العينة المدروسة.

الكلمات المفتاحية: شمال افريقيا، الاطفال، طيف التوحد، الطفيليات المعوية، ليبيا، زلة.

Introduction

Autism, also called autism spectrum disorder (ASD), is a behavioral illness that can be categorized as a heterogeneous neurodevelopmental disorder and is diagnosed after the child's second year of life. About 1 in 44 children suffer from autism [1]. Social contact, communication, and the ability to respond to environmental cues are all impacted by this illness in both adults and children. Furthermore, repetitive behavior patterns, delayed language development, and challenges with social imagination and interaction are also associated with autism [2]. Parasite infection can also indirectly affect the behavior of their hosts by disturbing their metabolism, development, or immunity. Parasitic castrators drastically modify their hosts' metabolism and reproduction, sometimes by secreting castrating hormones, changing their behavior and physiology to benefit the parasite [3]

According to the World Health Organization (WHO), Neglected tropical diseases (NTDs) are a broad category of infectious diseases that primarily affect 149 tropical and subtropical regions. NTDs impact low-income populations that lack access to adequate healthcare, clean water, and hygienic environments. (Mitra & Mawson [4]. Since the expenses of diagnosis, prevention, and treatment infection NTDs cause losses for a country that affect both the health and economic sectors [5,6]. NTDs are currently seen in European countries as well, due to a combination of increased international travel and climate change. However, the worldwide objectives to combat NTDs were once again overlooked as the COVID-19 pandemic [7, 8].

Among the neglected tropical diseases, intestinal parasite infection is a significant issue that affects many countries. For example, more than 30–807 million people are infected with the soil-transmitted nematodes, and 40–207 million people are infected with Platyhelminthes worldwide [9]. It has been reported that infection with some parasites led to complicated health issues in particularly if the infection interacts with the host immune response, which might contribute to human carcinogenesis and death if unrecognized. For example, the fish-borne trematode O. viverrini is classified by the International Agency for Research on Cancer (IARC) as a group 1 carcinogenic to humans for cholangiocarcinoma [10]. In addition, some studies have reported that parasite infection affects human behavior and concentration. For example, a study in Turkey has revealed that drivers who have been infected with *Toxoplasma gondii* in the past were involved more in traffic accidents compared to age-matched non-infected drivers [11]. Some studies have reported that there is a possible relationship between *T. gondii* infections and intelligence, education, and memory [12,13] were found to be spurious when all confounding factors were taken into account [14].

Even though the immune system is the main system involved in the communication between the periphery and the CNS, neurons and glial cells can also signal to the immune system upon exposure to pathogens or injury and communicate back to the neurons, a network now called the neuro-immune axis, with inflammation being a common point between behavioral disorders such as autism and immune-related diseases. In recent years, there has been growing scientific interest in the role of gastrointestinal health, particularly the gut microbiome, in influencing behavior and neurological function. Studies have shown that children with autism suffer from a greater degree of gastrointestinal problems, such as constipation or chronic diarrhea, compared to children without autism [15, 16].

Among the factors that may contribute to these gastrointestinal disorders are infections with intestinal parasites, such as *Giardia lamblia* and *Entamoeba histolytica*, which can negatively impact nutrient absorption and lead to chronic inflammation, which may impact brain development or behavior [15, 17]. The current study aims to estimate the prevalence of intestinal parasites in children with autism spectrum disorder, identify common parasite species, and examine the potential relationship between parasite presence and certain clinical or behavioral factors associated with autism. The study findings provide a scientific basis for developing interventional programs targeting gastrointestinal health as part of integrated care for this population.

Material and methods

A structured questionnaire was designed to collect data about the prevalence of intestinal parasites among autistic children. The questionnaire consisted of 13 items divided into three sections. Section A included demographic questions, while Section B focused on Symptoms of autism, and Section C included Digestive problems in autistic children. Stool samples were collected from participants enrolled in the study conducted between March to October 2024 in Zella city, Al-Jufra Municipality, Libya. Each participant was provided with a clean, dry, wide-mouthed, screw-capped plastic container labeled with a unique identifier. Participants were instructed to collect approximately 5–10 grams of fresh stool and

to avoid contamination with urine or water. Samples were transported to the Zella Central Laboratory within 2 hours of collection and stored at 4°C when immediate examination was not possible.

Macroscopic and Microscopic Examination

All analyses were conducted at Zella Central Hospital Laboratory. Each stool specimen was first examined macroscopically to assess its consistency (formed, semi-formed, or loose), color, and the presence of mucus, blood, or visible adult parasites. For the Microscopic examination, all specimens were subjected to direct wet mount examination using both normal saline and Lugol's iodine preparations to detect motile trophozoites, cysts, ova, and larvae of intestinal parasites. To ensure accuracy and reproducibility, samples were re-examined by a second experienced parasitologist. Microscopic examination was carried out using 10x and 40x objectives. Ethical approval for the study was obtained from the Board of Directors of Al-Amal Center for People with Special Needs, and written informed consent was obtained from all participants or their legal guardians before sample collection and questionnaire filling.

Results

The obtained data from Al-Amal Center for People with Special Needs records, affiliated with the Libyan State Solidarity Authority, revealed that 21 children at the center were confirmed to have autism. The male-to-female ratio was 76% and 24%, respectively, as illustrated in Figure 1. After obtaining ethical approval for the study, 15 of the 21 families agreed to participate in the study, representing a percentage of 71%.

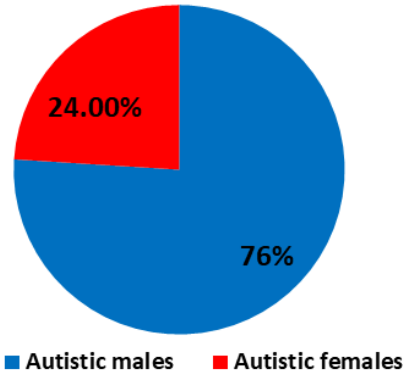

Figure 1: The percentage of autism in the study sample.

The incidence of intestinal parasites in children with autism

The results of the current study showed that intestinal parasite infection in autistic children was 80%, compared to 20% of the uninfected cases. The results of Table 1 showed that the autistic males were more likely to be infected with intestinal parasites compared to autistic females, since the percentage of infected autistic males was 90%, and 60% of autistic females were infected with intestinal parasites. The findings of this study also revealed a strong association between intestinal parasitic infections and hyperactivity among children with autism spectrum disorder (ASD). 83% of autistic children who tested positive for intestinal parasites also exhibited symptoms of hyperactivity. This suggests a potential link between parasitic infections and increased activity in this population. On the other hand, no significant association was observed between parasitic infections and the occurrence of recurrent intestinal diarrhea, as only 8% of infected children experienced frequent episodes of diarrhea. This indicates that diarrhea may not be a prominent or consistent clinical symptom of parasitic infections in autistic children.

Table (1): The relationship between gender and infection with intestinal parasites.

Gender	Number of samples tested	Number of infected samples	Percentage (%)
autistic Females	5	3	60%
autistic Males	10	9	90%
Total	15	12	80%

The relationship between age and parasitic infection in children with autism

The results of the current study showed that intestinal parasite infection in children with autism varied according to the child's age. The highest rate of parasitic infection was in the 6-8-year age group, at 58%, followed by the 3-5-year age group and the 9-11-year age group, at 17%. The lowest rate of infection was in the 15-17-year age group, at 8%, as shown in Table (2).

Table (2): The relationship between age group and parasitic infection in autistic children.

Age Group	05-03	08-06	11-09	14-12	17-15
Percentage (%)	%17	%58	%17	0	%08

Types of intestinal parasites in autistic children in Zella city

The macroscopic examination illustrated that Most of the samples were semi-formed, and they had a yellow-brownish color. There is no presence of mucus or worms in the collected samples. Parasites were identified based on their morphological characteristics following standard parasitological identification keys and WHO diagnostic guidelines. Microscopic examination was carried out using 10x and 40x objectives. The results of the current study (Table 3) showed the presence of four types of intestinal parasites in autistic children, with infection rate varying according to the parasite type as follows*: E. histolytica* (68%), *Entameba coli* (8%), G. lamblia (17%), and *Ascaris lumbricoides* (17%). The results of the current study also showed that 92% of infections were double infections, meaning the autistic child was infected with more than one type of parasite mentioned above.

Table (3): Type of intestinal parasite infections.

Type of infection	N	%	Parasite species	N	%
Single parasite infection	11	92%	*E. histolytica*	8	67%
			G. lamblia	1	08%
			E. coli	1	08%
			A. lumbricoides	2	17%
Mixed parasite infection	1	8%	*E. histolytica* + *A. lumbricoides*	-	-

Discussion

The present study investigated the prevalence of intestinal parasitic infections (IPIs) among children diagnosed with autism spectrum disorder (ASD). The results revealed a notably high prevalence of intestinal parasites within this group. In our study, the most identified parasites were *E. histolytica*, *A. lumbricoides,* and *G. lamblia,* which are typically transmitted via the fecal–oral route. These findings align with regional epidemiological evidence: for example, a large hospital-based survey in northern Jordan (2009–2013) found *G. lamblia* (41 %) and *E. histolytica* (31 %) to be predominant, with *A. lumbricoides* relatively rare (1 %) [18]. Similarly, a cross-sectional study of children in Egypt observed high prevalence (12.7 %) of both *E. histolytica* and *A. lumbricoides*, and *G. lamblia* (7.1 %), all strongly linked to poor hygiene practices, unsafe water, and low socioeconomic status [19].

A recent meta-analysis across Egypt also confirmed high pooled rates of *Entamoeba sp* and *Giardia sp*, with clear associations with young age, rural residence, low income, and inadequate sanitation [20]. Together, these data support the role of environmental exposure and hygiene behavior in driving transmission in similar socio-environmental settings. Multiple meta-analyses and reviews have established that gastrointestinal symptoms are significantly more common in children with ASD than in neurotypical peers, with prevalence estimates averaging around 33%. Moreover, up to 46%–84% of autistic children may experience GI-related issues such as diarrhea, constipation, reflux, and food sensitivities, highlighting chronic gut-immune dysfunction in ASD [21].

A case report from the United States described a child with ASD diagnosed with Baylisascaris procyonis (raccoon roundworm) infection following pica behavior and environmental exposure near a raccoon latrine. This case underlines the elevated risk in ASD children who exhibit pica or geophagia and engage in high-risk environmental exposures [22]. In Iraq, a small comparative study found 13.3% IgG seropositivity to Toxoplasma gondii among autistic children versus very low rates in controls ($p \leq 0.01$), suggesting an association between ASD and Toxoplasma exposure, possibly via environmental or foodborne routes [23]. In non-ASD child cohorts, parasite prevalence can vary widely depending on region and context. For example, preschool children in Saudi Arabia showed 18.4% prevalence, with *G. lamblia* (14.5%) most common, followed by *E. histolytica* and *E. vermicularis* [24]. Another study in Ethiopia (ages 6–59 months) reported an overall prevalence of 48.7%, with giardiasis in 10% and various helminths present in combination [25].

These figures suggest that high parasitic prevalence is endemic in certain low-resource settings, so ASD-specific rates would need to be contextualized accordingly. Behavioral and environmental factors common in ASD, such as pica, coprophagia, geophagia, and reduced hygiene awareness, may elevate exposure to parasitic pathogens, especially in areas with raccoon or soil contamination. These behaviors were directly linked in the *B. procyonis* case [26]. Furthermore, altered gut microbiota in ASD may compromise mucosal immune defenses. Dysbiosis is consistently observed in autistic children

across bacteria, fungi, archaea, and viruses, potentially increasing susceptibility to infection and promoting inflammatory responses via the gut-immune-brain axis.

The overlap between GI symptoms and ASD may lead to diagnostic overshadowing, wherein treatable conditions such as parasitic infection are overlooked because behavioral issues are attributed to autism rather than medical causes. Untreated parasitic infections can exacerbate nutritional deficiencies, anemia, and immune activation, potentially worsening growth and cognitive outcomes in ASD children. Given this context, routine screening for IPIs in ASD populations, particularly those with GI symptoms, pica, or known exposure risks, may be justified. Early detection and treatment of protozoa infection could mitigate systemic inflammation and improve the overall well-being of autistic children.

Conclusion

The study highlights a notably high prevalence of intestinal parasitic infections among children with Autism Spectrum Disorder (ASD) in Zella City, with a particularly high rate among males and those aged 6 to 8 years. The predominance of *E. histolytica* and the frequent occurrence of multiple infections suggest a need for improved hygiene practices and routine screening in this vulnerable population. Moreover, the observed link between parasitic infections and hyperactivity underscores the potential impact of gastrointestinal health on behavioral symptoms in autistic children. These findings emphasize the importance of integrating parasitological evaluation into the clinical management and care strategies for children with ASD in similar settings.

Recommendations

Promote better hygiene practices among children, and staff at special needs centers through targeted health education programs focusing on hand-washing, food safety, and clean water usage. In addition, conduct awareness campaigns and workshops for parents of children with ASD to inform them about the risks, symptoms, and prevention of intestinal parasitic infections .Further Research and more comprehensive studies with larger sample sizes across different regions to better understand the relationship between parasitic infections and autism-related behaviors and Collaboration with Public Health Authorities.

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