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ORIGINAL ARTICLE

Study Of Parasitic Infections Among Autistic Children In Outpatient Clinics

Sara A. Abdel-Rahman [1], Eman M. Mostafa[1], Aya I. Mohamed[2], Asmaa M. Darwish[1], Sara Maged Yousef[1], Shaimaa E. Ashoush[1*]

1 Medical Parasitology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
2 Psychiatry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

***Corresponding author**:
Shaimaa Elsayed Ashoush

Email:
shaimaaashoush@gmail.com;
sheashoush@zu.edu.eg

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ABSTRACT

Background: Children with autism spectrum disorder (ASD) often present with a variety of comorbidities, including gastrointestinal disturbances, altered behavior and immunity dysfunction, which may increase their susceptibility to pathogenic infections. In autistic children, gastrointestinal symptoms are frequently reported, but their potential link to parasitic infections is underexplored. So, we aimed to identify the frequency of parasitic infections in autistic children in Sharkia Governorate.

Methods: The present work was carried out on 40 ASD cases and another 40 non-autistic children as control group, aged 2–18 years. They were selected from Outpatient Pediatric Clinics and Faculty of disability sciences and rehabilitation in Zagazig University. From each individual, blood samples were collected for detecting anti-*Toxoplasma gondii* IgG antibodies. Additionally, stool samples were collected and examined by microscopy and antigen detection assays to identify various intestinal parasites.

Results: There was a significant difference between the two groups regarding anti-*T. gondii* IgG antibodies. Furthermore, the prevalence of overall intestinal parasitic infections was a significantly higher in the autistic group compared to non-autistic children using both microscopy and antigen detecting immunochromatographic test (ICT). *Cryptosporidium parvum* was the most detected intestinal parasite in both groups. ICT showed better performance than microscopy in detecting some intestinal protozoa, reinforcing its value in epidemiological studies and routine screening in children.

Conclusion: Children with ASD appear to be more susceptible to parasitic infections, particularly intestinal protozoa, than children without ASD. Moreover, the higher detection of anti- *T. gondii* antibodies among autistic children suggests a possible association between ASD and toxoplasmosis.

Keywords: Autism spectrum disorder; Toxoplasmosis; Intestinal parasites; Gastrointestinal symptoms; Pediatric comorbidities

INTRODUCTION

Parasitic diseases are public health issues across the world, with an estimated 230 million (43%) cases in the poor world. It has been identified as one of the causes of undernutrition in preschool-aged children globally [1]. Parasitic infections are well known for causing a significant burden of disease amongst the inhabitants of the tropic and subtropic regions of the globe and can be transmitted in several ways. The commonest methods of infection spread include contaminated food, water, soil and blood, or through transmission via infected insects that act as a vector or carrier of the disease [2].

Children, in particular, are more susceptible to parasitic infections and reinfection. WHO estimated that more than 270 million preschool children and more than 600 million of school children reside in regions where the parasites are highly prevalent and intensively transmitted [3].Children with parasitic infections are more likely to experience stunting, wasting, underweight, and undernutrition. Severe infections can lead to neurological and mental disorders as well as significant disability [2].

Autism spectrum disorder (ASD), is a broad category of neurodevelopmental conditions characterized by social communication deficits and repetitive behaviors. Its cause is likely multifactorial including genetics and environmental factors. It affects about 1 in 100 children with rising global prevalence [4]. Children with ASD are picky eaters and show aversions to certain food characteristics as texture, smell, color, and temperature, which can adversely affect diet quality. Notably, a greater incidence of pica was observed among those children [5], which could increase the risk of soil transmitted parasitic infections. Furthermore, individuals with cognitive or physical limitations affecting adequate hand washing are susceptible to fecal-oral transmission of infections. Children with ASD have shown altered personal hygiene, and inadequate hand hygiene put them at risk for infectious disease transmission [6].

In recent years, researchers have been looking at the potential link between parasite infections and autism, specifically how these infections may impact immune responses, gut-brain axis, and brain development. These alterations may subsequently influence neurodevelopmental processes and possibly contribute to symptoms of ASD [7].

Therefore, the present study investigated the prevalence of parasitic infections in autistic children versus non-autistic children, aiming to reduce the morbidity and mortality rates, improve quality of life and lessen the economic burden on patients and government. For our knowledge this is the first study of its kind in Sharkia governorate and so far, there are only a few studies in this point in Egypt.

METHODS

Ethical approval

The IRB committee at Faculty of Medicne, Zagazig University approved this study (IRB#:10740-3-5-2023).

Sample size and inclusion and exclusion criteria

This was a case-control study conducted between May 2023 and September 2024. Forty patients of autistic spectrum disorder (both male and female) with various gastrointestinal issues and ages ranging from 2 to 18 years were selected from Zagazig University's Outpatient Paediatric Clinics and Faculty of Disability Sciences and Rehabilitation. Additionally, 40 non-autistic children in the same age range as the patients were taken into consideration as a control group. Individuals were included in the study if their parents were willing to participate and signed informed consent. While participants whose guardians refused to participate, or were outside the target age range, and/or recently received anti-parasitic treatment were excluded.

For every patient, a case history was taken including age, sex, residence, clinical presentation (GIT disturbances as diarrhoea, abdominal colic, vomiting and constipation), history of contact with animals and history of taking any immunosuppressive therapy and/or receiving any antidiarrheal drugs.

Laboratory investigations

Blood and faecal samples were collected from all participants and transported to Medical Parasitology Department, Faculty of Medicine, Zagazig University for examination.

Blood samples collection and examination for anti-*Toxoplasma gondii* IgG

Blood samples were gathered from all participants and serum was separated by centrifugation at 1000 r.p.m. and stored at -20°C until use. Serum samples were analysed using the enzyme-linked immunosorbent assay (ELISA). The test was carried out according to the technique described in the manufacturer's kit [8]. Enzyme immunoassay test kit for Toxoplasma IgG (no. 10234 by PerkinElmer Health sciences, Inc., Hayward CA, USA) was used.

Microwells were coated with purified *T. gondii* antigen. Diluted patient serum was added to

wells. If *T. gondii* IgG specific antibodies were present in serum, they bound to the antigens. Unbound components were then washed away. Then the enzyme conjugate was added which binds to the antibody-antigen complex. After excess enzyme conjugate was washed off, TMB chromogenic substrate was added. The intensity of resulting color reaction is directly proportional to the quantity of IgG specific antibody in serum samples. Optical density was read at 450 nm by a microwell reader.

Stool samples collection and examination

From each participants three successive stool samples were collected in wide-mouth plastic labelled containers with tight-fitting lids. Each obtained sample was separated into two sections: One was kept fresh and analysed by microscopy. The second half was maintained at (-20) for antigen testing using RIDA®QUICK Combi kit.

Direct parasite detection

Stool samples were examined macroscopically and microscopically. Samples were examined by the naked eye for determination of stool consistency, colour and odour and for the presence of blood or mucus or parasites. Microscopic examination for parasites detection was conducted through direct examination by unstained smear with saline and Lugol's iodine-stained smear [9]. In addition, concentration techniques such as formol-ether sedimentation [10] and zinc sulphate centrifugal floatation [11] were performed together with modified Ziehl-Neelsen permanent staining technique [10].

RIDA®QUICK Combi immunochromatographic test

This is a quick immunochromatographic test (ICT) for the qualitative determination of Cryptosporidium parvum and/or Giardia lamblia and / or Entamoeba histolytica/ dispar antigens in faecal samples using RIDA®QUICK Combi kit (R-Biopharm, Darmstadt, Germany). In this assay, specific antibodies against each parasite are bound to latex particles that are color-coded: green for *Entamoeba*, red for *Giardia*, or blue for *Cryptosporidium*. If the corresponding antigens are present in the sample, antibodies bind to them and coloured band appears. The test was performed according to manufacturer's guidelines [12].

Statistical analysis

IBM SPSS 23.0 for Windows, a database software tool, was used to analyze the gathered data (SPSS Inc., Chicago, IL, USA). Chi-Square [X2] test, fisher's exact test (f), Cohen's Kappa Test (κ), independent t-test were used.

RESULTS

There was no significant difference between the two groups in terms of demographics and socioeconomic status (P>0.05), **table (1)**. Regarding clinical manifestations, no significant difference was detected between the studied groups except for constipation, as (40%) of the patients in the autistic group had constipation in comparison to (12.5%) of the control group, **table (2)**.

Serum examination for detecting anti-*T. gondii* IgG antibodies revealed higher detection rate among autistic children compared to non-autistic group with a statistically significant difference (P< 0.05). 57.5% of the autistic group had positive IgG antibodies, while 35% of the control group had positive results as shown in **table (3)**.

Regarding, direct stool analysis, a significantly higher number of parasitic infections were detected among the autistic group than among the control group (P< 0.05). However, there was no significant difference between the studied groups in terms of the specific types of parasites identified in stool samples by microscopic examination (P>0.05), **(Table 4; Fig.1)**. Regarding results of ICT for detecting *C. parvum* and/or *G. lamblia* and / or *E. histolytica/ dispar* antigens, a statistically significant difference was observed between the studied groups. Positive results detected in 62.5% of the autistic group in comparison to 32.5% of the control group, **(Table 5; Fig.1s)**. Additionally, when comparing stool microscopy and ICT findings for these protozoa, a highly significant difference was detected between the two methods of diagnosis (P<0.001), **table (6)**.

Table 1: Socio-demographic data among the studied groups

	Autistic group (n=40)	Control group (n=40)	Test Used	χ^2/t test	P-value
Age (years) ***Mean ± SD***	9.46 ± 4.26	9.61 ± 4.25	Independent t-test	0.16	0.97
Sex ***(N. %)*** **Male** **Female**	19 (47.5%) 21 (52.5%)	15 (37.5%) 25 (62.5%)	Chi-square test	0.82	0.37
Residence ***(N. %)*** **Rural** **Urban**	28 (70%) 12 (30%)	31 (77.5%) 9 (22.5%)	Chi-square test	0.58	0.45
Socio-economic ***(N. %)*** **High** **Moderate** **Low**	4 (10%) 22 (55%) 14 (35%)	7 (17.5%) 22 (55%) 11 (27.5%)	Chi-square test	1.18	0.61
Dealing with pets ***(N. %)*** **Yes** **No**	22(55%) 18(45%)	23 (57.5%) 17 (42.5%)	Chi-square test	0.05	0.82
Type of drinking water **Tap water** **Domestic water filter** **Mineral water**	21 (52.5%) 12 (30%) 7 (17.5%)	22 (55%) 13 (32.5%) 5 (12.5%)	Chi-square test	0.39	0.82

Table 2: Clinical manifestation among the studied groups

	Autistic group (n=40)	Control group (n=40)	Test Used	P-value
No Symptoms	14 (35%)	13 (32.5%)	Chi-square test (test value 0.056)	0.81
Symptoms	26 (65%)	27 (67.5%)		
Constipation	16 (40%)	5 (12.5%)	Fisher's exact test	**0.01***
Fever	4 (10%)	2 (5%)	Fisher's exact test	0.68
Diarrhea	10 (25%)	8 (20%)	Chi-square test (test value 0.29)	0.59
Nausea	5 (12.5%)	5 (12.5%)	Fisher's exact test	1.00
Vomiting	2 (5%)	3 (7.5%)	Fisher's exact test	1.00
Abdominal cramps	6 (15%)	9 (22.5%)	Fisher's exact test	0.57

***Significant difference (P< 0 .05)

Table 3: Seroprevalence of anti-*T. gondii* IgG antibodies

	Autistic group (n=40)	Control group (n=40)	Chi-square test (χ^2)	P value
Positive	23 (57.5%)	14 (35%)	4.1	**0.04***
Negative	17 (42.5%)	26 (65%)		

***Significant difference (P< 0 .05)

Table 4: Detected parasites in stool samples by microscopic examination in studied groups

	Autistic group (n=40)	Control group (n=40)	Test Used	P-value
Negative	11 (27.5%)	21 (52.5%)	Chi-square test (test value 5.21)	**0.02***
Positive	29 (72.5%)	19 (47.5%)		
E. coli	3 (7.5%)	2 (5%)	Fisher's exact test	1.00
E. histolytica/dispar	5 (12.5%)	3 (7.5%)	Fisher's exact test	0.71
C. parvum	10 (25%)	4 (10%)	Fisher's exact test	0.14
G. lamblia	4 (10%)	2 (5%)	Fisher's exact test	0.68
Blastocystis hominis	5 (12.5%)	3 (7.5%)	Fisher's exact test	0.71
A. lumbricoides	1 (2.5%)	2 (5%)	Fisher's exact test	1.00
Taenia spp.	1 (2.5%)	1 (2.5%)	Fisher's exact test	1.00
E. vermicularis	0 (0%)	1 (2.5%)	Fisher's exact test	1.00
H. nana	0 (0%)	1 (2.5%)	Fisher's exact test	1.00

*Significant difference (P< 0 .05)

Table 5: ICT results for antigen detection of *G. lamblia*, *E. histolytica/ dispar*, and *C. parvum* in stool samples

	Autistic group (n=40)	Control group (n=40)	Test Used	P-value
Negative	15 (37.5%)	27 (67.5%)	Chi-square test (test value 7.22)	**0.007***
Positive	25 (62.5%)	13 (32.5%)		
G. lamblia	7 (17.5%)	5 (12.5%)	Fisher's exact test	0.76
C. parvum	12 (30%)	5 (12.5%)	Fisher's exact test	0.09
E. histolytica/ dispar	6 (15%)	3 (7.5%)	Fisher's exact test	0.48

***Significant difference (P < 0 .05)

Table 6: Comparison between microscopic stool examination and ICT results among the studied groups

	Microscopic		ICT		Kappa test	P-value
	Autistic group (n= 40)	Control group (n= 40)	Autistic group (n= 40)	Control group (n= 40)		
G. lamblia	4 (10%)	2 (5%)	7 (17.5%)	5 (12.5%)	0.528	**<0.001****
C. parvum	10 (25%)	4 (10%)	12 (30%)	5 (12.5%)		
E. histolytica/dispar	5 (12.5%)	3 (7.5%)	6 (15%)	3 (7.5%)		
Positive	19 (47.5%)	9 (22.5%)	25 (62.5%)	13 (32.5%)		
Negative	21 (52.5%)	31 (77.5%)	15 (37.5%)	27 (67.5%)		

** Highly significant (P < 0 .001)

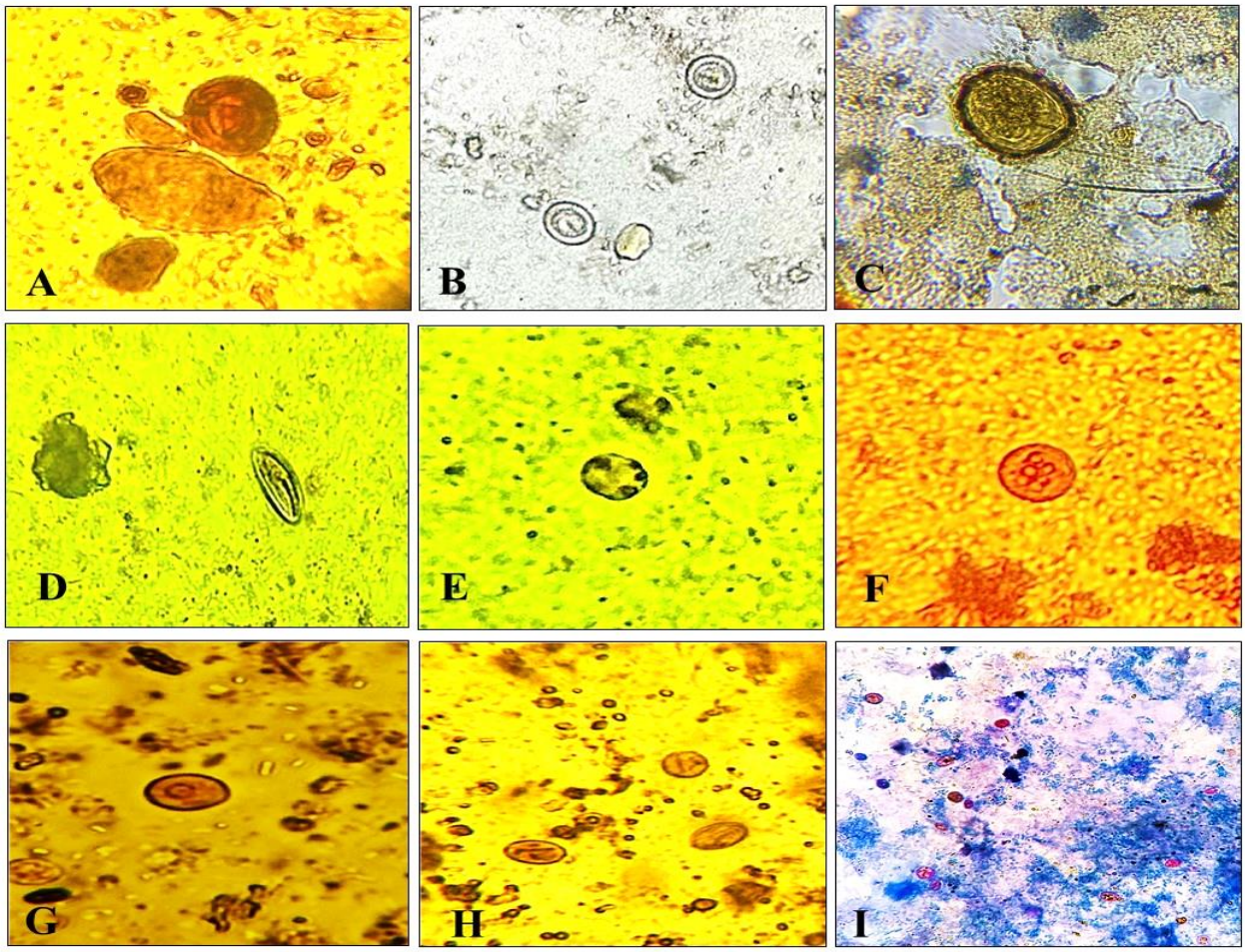

Fig. 1: Stool smears examination showing various detected parasites **A)** *Taenai spp.* egg (iodine stained x400); **B)** *H. nana* egg (wet mount unstained); **C)** *A. lumbricoides* egg (iodine stained x400); **D)** *E. vermicularis* egg (iodine stained x400); **E)** *Blastocystis hominis* vacuolar form (iodine stained x400); **F)** *E. coli* cyst (iodine stained x400); **G)** *E. histolytica/dispar* cyst (iodine stained x400); **H)** *G. lamblia* cysts (iodine stained x400) and **I)** *Cryptosporidium* oocysts (MZN stained x1000).

DISCUSSION

In the current study, the age of the participants was between 2 to 18 years old. Most of the participants were from rural areas (70% of the autistic group & 77.5% of the control group), with a moderate socioeconomic level (55% of both groups) with no significant difference between both groups regarding sociodemographic data. A study by Hamid et al. [13] found that higher socioeconomic status, was significantly related to autistic children compared to those of non-autistic children. This suggests that other factors could be affecting infection in autism rather than sociodemographic factors.

In the current study there is no significant difference between the studied groups as regards the clinical manifestations except for constipation. Children in the autistic group showed higher incidence of constipation than the non-autistic group. This could be attributed to autism itself rather than parasitic infections. This is supported by studies indicating that children with autism may experience constipation, most probably due to a combination of neurological and developmental impairments [14].

Ibrahim et al [15] found that compared to the control group, autistic patients experienced an increased incidence of eating disorders and constipation. Although there was no significant correlation found between autism case status and the overall incidence of gastrointestinal symptoms, this suggests that a neurobehavioral

etiology rather than a primary organic gastrointestinal etiology may be the cause of the higher incidence of these gastrointestinal symptoms in children with autism. Similarly, Ahmed et al. [16] and Gyamenah et al. [17] reported that children with autism have higher risks of constipation. Li et al. [18] also stated that compared to the control group, children with ASD had worse diets, with fewer fruits and vegetables, less diversity in food, a higher degree of inadequate or imbalanced dietary intake, and more severe constipation and overall gastrointestinal problems. Based on these findings, we suggest that the parasitic infections may produce similar symptoms in both autistic and non-autistic hosts when exposure risk is similar. Also, these findings support the notion that GIT symptoms in autistic children may not be caused solely by parasitic infections.

Regarding anti *Toxoplasma*-IgG results in the current study, there was a statistically significant difference between autistic and non-autistic group as regarding anti-*T. gondii* IgG antibodies, (with higher sero-positivity among the autistic group . Similar findings detected by Nayeri et al, [19], who discovered a link between latent *T. gondii* infection and the likelihood of ASD. But there was no connection between ASD and acute infection. Likewise, in accordance with Bazzaz and Jameel, [20], compared to the healthy control group, autistic children had considerably higher levels of anti-*Toxoplasma* IgG.

A recent Egyptian study by El-Sayed et al. [21], reported that 36% of children with ASD were IgG positive, compared to 10% of healthy controls with a statistically significant difference. The study revealed that the old but not the recent infection with *T. gondii* in children could be linked to ASD. *T. gondii* was the most common protozoan parasite among people with mental health issues, according to earlier cross-sectional investigations. Toxoplasmosis has been found to have a strong correlation with a variety of mental health conditions in recent decades. This could be explained by the parasite's intracellular neurotropic properties as well as the development of intracellular cysts in glial and neuronal cells [22, 23].

In contrast, Esnafoglu et al. [24] and Hassan et al. [25] found no significant difference in anti-*Toxoplasma* IgG seroprevalence between ASD and control groups. This discrepancy likely stems from different sample sizes, different environmental conditions, eating habits, variations in climatic conditions as well as regional variation in parasite endemicity [21].

Regarding results of microscopic stool examination of the present work, more parasitic infections were detected among autistic children compared to control ones with a statistically significant difference. Various parasites were found with no discernible difference between the studied groups as regarding each parasitic infection detected in the stool samples. This is close to the results of Heisel et al. [26] who found that the autistic group had a statistically significant greater prevalence of helminthic and protozoal illnesses than the control group. Compared to children without ASD, autistic children showed a higher rate of gastrointestinal infections.

In the current study, the most frequent parasite detected in autistic group was *C. parvum* (25%) followed by *E. histolytica/dispar* and *B. hominis* each (12.5%), then *G. lamblia* (10%), then *E. coli* (7.5%), then *A. lumbricoides* and *Taenia spp*. each (2.5%). In the control group, the most detected parasite in this study was also *C. parvum* (10%), then *E. histolytica/dispar* and *B. hominis* each one was (7.5%), then *G. lamblia, E. coli,* and *A. lumbricoides* were (5%) for every one of them, then *Taenia spp., E. vermicularis* and *H. nana* were (2.5%) for every one of them.

These results aligns with those of Mohammad *et al.* [27] who found that, in examination stool samples from children in Sharkyia governorate, Egypt, cryptosporidiosis was the most prevalent parasitic infection (27.8%f), followed by *G. lamblia* (13.4%), *H. nana* (9.3%), *E. histolytica /dispar* (7.2%), *E. vermicularis* (6.2%), *A. lumbricoides* (2.1%).

Prior research demonstrated a strong correlation between neurodevelopmental problems and protozoal infections. Additionally, individuals with mental illnesses

were at increased risk to contract cryptosporidiosis [22]. Zain Alabeden [28] found close results for *G. lamblia* infection in autistic children in Khartoum state with prevalence of 8%. However, for *E. histolytica* was only 4%, and for *E. coli* was 2%. On the other hand, Ahmed and Abu-Sheishaa [29] found that *E. histolytica* was the most common parasitic species among Egyptian schoolchildren, followed by *G. lamblia, H. nana,* and *A. lumbricoides.* Also, Azzam and Khaled [30], discovered that *Entamoeba spp*. were the most common parasite among preschool- and school-aged children, followed by *G. lamblia* and *E. vermicularis. A. duodenale*, *T. trichiura*, and *Fasciola spp.* were less frequent parasites. They stated that living in a rural area, ages less than ten years, low socioeconomic standing, improper washing hands, eating raw vegetables and maternal education level were all important risk factors.

In the present study, a statistically significant difference between the groups under investigation was found as regards the results of ICT of stool samples. There was a predominance of *C. parvum* in autistic group (30%), followed by *G. lambli*a (17.5%), then *E. histolytica/dispar* (15%). While in control group, the results were (12.5%), (12.5%), (7.5%) for the same parasites, respectively. The significant difference observed in ICT positivity between autistic and non-autistic children suggests that autistic children in our sample had a higher exposure to intestinal parasites, particularly protozoa such as *C. parvum, G. lamblia* and *E. histolytica,* which are commonly detected by this method. This result aligns with the hypothesis that behavioral risk factors in autistic children such as poor hygiene, pica, mouthing non-food objects, or less supervision increase their likelihood of ingesting infective parasitic forms in contaminated objects [26, 31].

In the current study, all cases of G. lamblia, *C. parvum,* and *E. histolytica/dispar* that were positive by microscopy were positive by ICT with more detection rate by ICT, with highly significant difference. This finding aligns with those of Atia et al. [32] and Kafa et al. [33], who found that the ICT detected a higher number of protozoal infections compared to microscopy and concluded that antigen-based assays are more reliable for epidemiological screening, particularly in children. This could be attributed to that microscopy relies on operator skill, sample quality, and parasite load, which can vary significantly, especially in cases with low parasite burden or intermittent shedding. Furthermore, microscopy can miss protozoan infections if samples are not processed immediately or improperly stained. In contrast, ICTs like RIDA-QUICK can identify infections even in the absence of visible cysts or trophozoites and don't require experienced personnel [34].

CONCLUSION

Our study concluded that children with Autistic spectrum disorder are at higher risk of parasitic infections than non-autistic children. The most detected intestinal parasites in autistic children were as follows: *C. parvum, E. histolytica/dispar, Blastocystis hominis, G. lamblia, A. lumbricoides, Taenia spp.* respectively. Furthermore, a notably higher detection rate of anti- *T. gondii* antibodies among autistic children was detected, suggesting a potential association.

Conflict of Interest: None

Financial Disclosures: None

Availability of data: All data are available from the corresponding author upon reasonable request.

Authors' contribution: All authors contributed to the conception and design of the study, data collection and analysis, and manuscript drafting. All authors have read and approved the final version for publication.

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Fig. 1s: ICT test cassette showing: A) Negative result (crimson band only); **B)** Positive for *E. histolytica/ dispar* (crimson and green bands); **C)** Positive for *C. parvum* (crimson and blue bands); **D)** Positive for *G. lamblia* (crimson and red bands).

Citation

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