SMJ- Sohag Medical Journal, Vol. 29 No(1) 2025

Print ISSN1687-8353 Online ISSN2682-4159

Original Article

Prevalence and Molecular characterization of Cryptosporidium spp. in Humans at Sohag Governorate, Egypt

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Abstract

Background: Cryptosporidiosis is a global infection caused by *Cryptosporidium*, which infects various vertebrates and humans, and causes gastroenteritis with varying severity. Several *Cryptosporidium spp.* with different genotypes and subtypes are implicated in human cryptosporidiosis.

Objective: estimate the prevalence of *Cryptosporidium* species in Sohag, assess its occurrence among various patient groups, and analyze the relationship between *Cryptosporidium* infections and associated risk factors and assessed the genetic diversity of *Cryptosporidium* spp in Humans at Sohag Governorate, Egypt

Patients and Methods: A total of 245 human stool samples were collected from patients attending the outpatient clinics of Sohag hospitals. The collected samples were examined microscopically by direct smear and modified Ziehl-Neelsen stain (MZN). All positive *Cryptosporidium* samples detected by MZN were genotyped using nested polymerase chain reaction-restriction fragment length polymorphism (nPCR-RFLP) targeting cowp gene encoding *Cryptosporidium* oocyst wall protein (Cowp).

Results: *Cryptosporidium* was detected in 20% (49/245) of the examined samples using MZN. Only 2/45 (4%) of samples positive by microscopy were successfully amplified using cowp gene. All amplified samples were confirmed as *C. hominis* by nPCR-RFLP analysis of the cowp gene. Significant associations were found between cryptosporidiosis and age, residence and patient categories.

Conclusion: The identification of *C. hominis indicates* anthroponotic transmission. the relatively low prevalence of *Cryptosporidium* underscores the positive impact of public health measures implemented during and after COVID-19 pandemic on reducing parasitic infections.

Keywords: Cryptosporidium, C. hominis, Sohag, nested PCR-RFLP, sequencing

DOI : 10.21608/SMJ.2025.347066.1520 **Received:** December 11, 2024 **Accepted:** December 25, 2024

Published: January 01, 2025

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Citation: **Salwa Gamal Ahmed. et al.,** Prevalence and Molecular characterization of Cryptosporidium spp. in Humans at Sohag Governorate, Egypt SMJ,2025 Vol. 29 No (1) 2025: 41- 56

Introduction

Cryptosporidiosis is a global infection caused by the protozoan *Cryptosporidium*, which infects various vertebrates, including humans. It is primarily transmitted through the fecal-oral route by ingesting viable oocysts from contaminated food or water. While waterborne transmission is well-documented, the natural reservoir and exact infection route of *Cryptosporidia* remain unclear.(1)

Cryptosporidium causes acute gastroenteritis, abdominal pain, and diarrhea. The severity of symptoms varies with the host's immune status. Healthy individuals usually have self-limiting watery diarrhea, while young children in developing countries may face prolonged diarrhea and malnutrition. Immunocompromised individuals can experience severe, chronic, and potentially fatal cryptosporidiosis.(2)

Currently, there are 41 species of *Cryptosporidium*, with over 60 genotypes. Each species has distinct sources of infection, transmission routes, and levels of pathogenicity. Species identification is crucial for assessing local transmission risks and implementing control measures to reduce exposure to infectious oocysts. In humans, nearly 20 species have been identified, with *C. hominis* and *C. parvum* responsible for 90% of infections, leading to nearly a million deaths annually. Other species like *C. felis, C. meleagridis, C. canis, C. suis,* and *C. muris* can also infect humans. *C. hominis* is transmitted solely between humans (anthroponotic), while *C. parvum* causes zoonotic infections in both humans and animals.(3)

Cryptosporidium infection causes over 50,000 deaths annually and is more prevalent in developping countries. The epidemiological features of human cryptosporidiosis in developing countries differ from those in developed countries due to higher endemicity, lower hygiene levels, and less intensive animal farming.(4)

During the COVID-19 pandemic, increased hygiene practices resulted in a significant decrease in gastrointestinal illnesses, including cryptosporidiosis. This underscores the effectiveness of improved public health measures in controlling infectious diseases, especially in endemic areas.(5)

Microscopy with acid-fast staining is the primary global screening technique for diagnosing *Cryptosporidium*, especially in rural areas, due to its cost-effectiveness and high sensitivity. However, it cannot identify the specific species or subspecies. Advanced molecular genetics tools can identify and subtype *Cryptosporidium* species.(6)

The present study aimed to estimate the prevalence of *Cryptosporidium* species in Sohag, assess its occurrence among various patient groups, and analyze the relationship between *Cryptosporidium* infections and associated risk factors and assessed the genetic diversity of *Cryptosporidium spp* .

Material and Methods

A cross-sectional study was performed on fecal samples collected during the period between March 2022 to February 2024, Microscopic examinations carried out in the Medical Parasitology Research Laboratory, Faculty of Medicine, Sohag University, Egypt. Copro-nested (n) PCR-RFLP assay was performed in Molecular Medical Parasitology Lab., Department of Medical Parasitology, Faculty of Medicine, Cairo University, Egypt.

Samples collection: A total of 245 human fecal samples were collected from patients attending the outpatient clinics of Sohag hospitals (Sohag University Hospitals- Endemic Diseases Hospital-Sohag Oncology Institute). The patients were of different ages: 124 Children (2 to 12 years old), 27 Adolescents (13 to 19 years old), 26 Young Adults (20 to 35 years old), 38 Middle age (36 to 55 years old), 30 old age (≥ 56 years old); of both sexes, and different immune status. A predesigned questionnaire containing demographic, clinical and environmental data such as the source of drinking water and animal contact was fulfilled for each patient.

Fecal samples: Fresh samples were collected in 100 ml clean, labeled containers. Each sample was divided into three portions; a small part for direct smear examination, another part was preserved in tight containers using 7% formalin for microscopic examination and staining, and the third portion was stored at -20°C in Eppendorf tubes for molecular studies.

Microscopic examination: All collected samples were examined microscopically using direct wet smear, formalin-ethyl acetate sedimentation concentration technique and MZN stain.(7)

Genomic DNA extraction from fecal samples: DNA extraction from the fresh frozen samples was performed using the QIAamp Stool Mini Kit

(Qiagen, Germany) according to the manufacturer's instructions. This kit is designed for rapid purification of total DNA from up to 220 mg stool and is suitable for both fresh and frozen samples. Briefly, extraction of DNA was done by lysis of samples in the fecal lysis buffer. One Inhibit EX tablet was added to each sample and vortexed immediately continuously for 1 min until the tablet was completely suspended. The suspension was then incubated for 5 min at room temperature to allow inhibitors to adsorb to the Inhibit EX matrix. Purification of extracted DNA was done on QIAamp spin columns. DNA was then eluted in 50 µl buffer (Qiagen) and stored at -20oC till use.

Amplification of extracted genomic DNA (Copro n-PCR): For the molecular characterization of *Cryptosporidium spp*., amplification of the extracted genomic DNA was performed using nPCR analysis of cowp gene. Amplification was done according to the manufacturer's instructions. Two sets of primers were used such that first set was used to produce an amplicon template for the nested reaction. The second set of primers was used to anneal internally within the previously obtained amplicon increasing the specificity of detection.[8]

The details of the target gene, primers sequences, and amplicon size are shown in table (1).

Table (1) Primers used in PCR for the target *COWP* gene and its sequence [8].

Primers	Sequence	Product size	Annealing temperature
Primary	1) Forward primer (COWP-F): 5' CCGCTTCTCAACAACCATCTTGTCCTC-3' 2) Reverse primer (COWP-R): 5'-CGCACCTGTTCCCACTCAATGTAAACCC-3'	769 bp	63oC
Secondary	1) Forward primer (Cry-15): 5'-GTAGATAATGGAAGAGATTGTG-3' 2) Reverse primer (Cry-9): 5'-GGACTGAAATACAGGCATTATCTTG-3	553 bp	54oC

Restriction fragment length polymorphism (RFLP): The amplified products of nPCR of positive samples were digested using restriction enzymes according to manufacture instructions. The DNA sample was digested by RsaI restriction enzyme (Thermo Scientific #FD1124) and the resulting restriction fragments were separated according to their lengths by gel electrophoresis. Restriction assays were performed in 30 µL volume with 2 units of restriction enzyme and 5 µL of PCR product per reaction. Mixtures were incubated in a heating block. Digestion products were visualized under UV light in 2% agarose gel electrophoresis after ethidium bromide staining. Digested PCR product with these enzymes resulted in a distinct band pattern for the human and bovine genotypes. The amplicon size of the digested products for *C. hominis* was distinct bands at: 106 bp, 125 bp, 285 bp.

DNA sequencing: PCR products of the cowp gene were purified using Illustra GFX PCR DNA and Gel band Purification Kit – GE Healthcare and cycle sequencing using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit and using Centri-Sep columns (Princeton separations) (cat no. CS-901) for dye terminator removal.

Statistical analysis: results were gathered, organized and tabulated in an Excel 2013 spreadsheet. Data were analyzed using IBM SPSS Statistics for Windows version 27.0. Quantitative data were expressed as mean ± standard deviation, median, and range. Qualitative data were expressed as number and percentage. The data were tested for normality using the Shapiro-Wilk test. The nonparametric kruskal-wallis test or Mann–Whitney test was used for data which was not normally distributed. Chi-square ($\chi 2$) test was used for comparison regarding qualitative variables as appropriate. A 5% level was chosen as a level of significance in all statistical tests used in the study.

Ethical approval: The research protocol was approved by the Medical Research Ethics Committee Faculty of Medicine - Sohag University (OHRP #: IRB00013006) under IRB registration number (Soh-Med-22-01-12) as it complies

with the 1964 Helsinki declaration. This study was registered at Clinicaltrials. Gov (ID: NCT05208970) [http://clinicaltrials.gov/ct2/show/record/NCT05208970]). Verbal consent from participants or from parents or guardians of children in the study was obtained after a clear explanation of the study objectives.

Results

Out of the 245 study participants, the age of the study individuals ranged from 2.5 to 90 years with a mean and standard deviation of 23.6 ± 20.9

Microscopic examination: Using MZN-stained fecal smear, 49/245 samples (20%), were positive for *Cryptosporidium* oocysts. Figure (1)

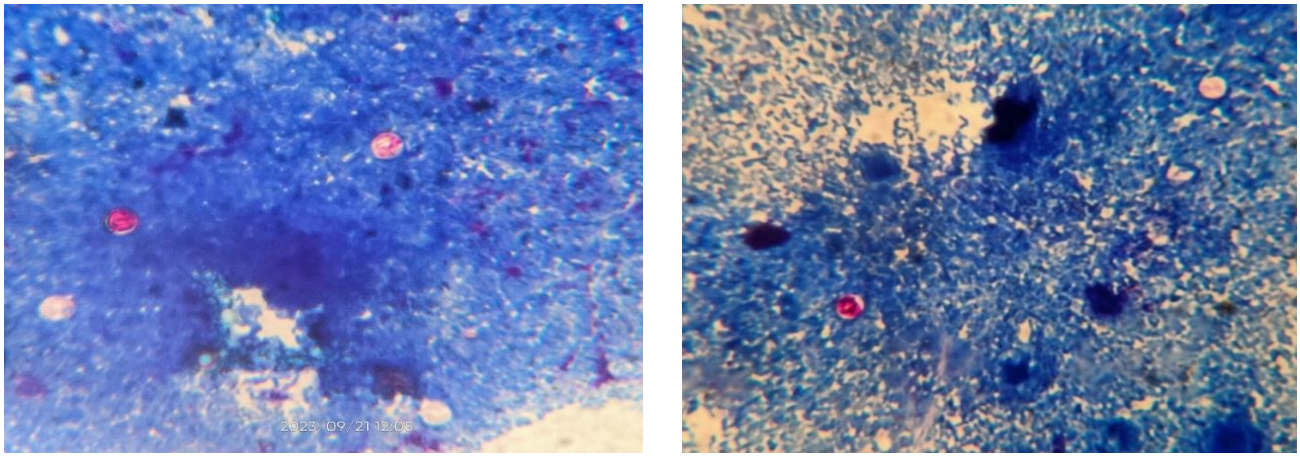

Figure (1) *Cryptosporidium* oocysts stained by MZN stain (1000X).

Prevalence of *Cryptosporidium* infections among different Sohag Governorate Districts: The analysis of *Cryptosporidium* prevalence across the districts of Sohag Governorate reveals variability in infection rates but there was no statistical significance (p-value= 0.09). the data highlighted Akhmim, Sohag and El Maragha as districts of particular concern, exhibiting the highest prevalence at 18 (7.3%), 9 (3.7%) and 7 (2.9%) respectively. Table (2)

Table (2) Prevalence of *Cryptosporidium* infections in Sohag Governorate Districts

Sohag Governorate Districts	*Cryptosporidium* diagnosed by MZN			p-value
	Positive	Negative	Total	
Akhmim	18 (7.3%)	40 (16.3%)	58	
Sohag	9 (3.7%)	66 (26.9%)	75	
El Maragha	7 (2.9%)	8 (3.3%)	15	
Girga	4 (1.6%)	18 (7.3%)	22	
Tahta	2 (0.8%)	13 (5.3%)	15	
Saqultah	2 (0.8%)	11 (4.5%)	13	
El Munsha	2 (0.8%)	15 (6.1%)	17	
El Balyana	2 (0.8%)	6 (2.4%)	8	
Juhaynah	1 (0.4%)	5 (2%)	6	
Dar al-Salam	1 (0.4%)	10 (4.1%)	11	
Tima	1 (0.4%)	4 (1.6%)	5	
Total	49 (20%)	196 (80%)	245	0.09*

Data presented as n and percentage with (%). kruskal-wallis test: *P* value ≤ 0.05 is significant*.

***Cryptosporidium* Infection in Various Patient Categories:** The study found significant differences in *Cryptosporidium* infection rates among patient categories, with a p-value of 0.002 indicating statistical significance. Asymptomatic immunocompetent individuals had a 6.9% infection rate, while symptomatic immunocompetent patients experienced a higher rate of 15.6%. Patients with chronic diseases exhibited the highest infection rate at 37.8%, particularly those with diabetes mellitus (55.6%) and chronic kidney disease

(43.8%). Among immunocompromised patients, including those with hematological malignancies and cancers undergoing chemotherapy, the infection rate was 31.3%. Table (3,4,5)

Table (3) Prevalence of *Cryptosporidium* Infection in Various Patient Categories

patient categories		***Cryptosporidium* diagnosed by MZN**		**Total**	**p-value**
		Negative	Positive		
Asymptomatic immunocompetent	Count	27	2	29	
	% within patient_category	93.1%	6.9%	100.0%	
	% of Total	11%	0.8%	11.8%	
Symptomatic immunocompetent	Count	124	23	147	
	% within patient_category	84.4%	15.6%	100.0%	
	% of Total	50.6%	9.4%	60 %	
Patients with Chronic disease	Count	23	14	37	
	% within patient_category	62.2%	37.8%	100.0%	
	% of Total	9.4%	5.7%	15.1%	
Immunocompromised patients	Count	22	10	32	
	% within patient_category	68.8%	31.3%	100.0%	
	% of Total	9 %	4.1%	13.1%	
Total	Count	196 (80%)	49 (20%)	245 (100%)	0.002*

Data presented as n and percentage with (%). kruskal-wallis test: P value ≤0.05 is significant*.

Table (4) Prevalence of *Cryptosporidium* among Patients with Chronic Diseases

Patients with Chronic Diseases	Positive No. (%)	Negative No. (%)	Total No. (%)
CKD	7 (43.8%)	9 (56.3%)	16 (100%)
DM	5 (55.6%)	4 (44.4%)	9 (100%)
Hepatic diseases	0 (0.00%)	3 (100%)	3 (100%)
Chronic Pulmonary diseases	1 (50%)	1 (50%)	2 (100%)
Cardiac diseases	1 (33.3%)	2 (75%)	3 (100%)
Rheumatic diseases	0 (0.00%)	4 (100%)	4 (100%)
Total	14 (37.8%)	23 (62.2%)	37 (100%)

Data presented as n and percentage with (%).

Table (5) Prevalence of *Cryptosporidium* among immunocompromised patients

Immunocompromised patients	Positive No. (%)	Negative No. (%)	Total No. (%)
Haematological malignancies	7 (33.3%)	14 (66.7%)	21 (100%)
colorectal cancer	2 (25%)	6 (75%)	8 (100%)
Hepatocellular carcinoma (HCC)	1 (33.3%)	2 (66.7%)	3 (100%)
Total	10 (31.3%)	22 (68.7%)	32 (100%)

Data presented as n and percentage with (%).

Relationship between *Cryptosporidium* infection and different risk factors: The analysis revealed statistically significant associations between *Cryptosporidium* infections and various factors, with a p-value of 0.05 for age, indicating a significant relationship; children aged 2 to 12 years (8.2%) tested positive, while adolescents (2%), young adults (1.6%), middle-aged individuals (3.3%), and older adults (4.9%) were infected. The p-value for residence was 0.018, suggesting that living in rural areas correlates with higher infection rates. Gender did not show a statistically significant relationship. Although a higher prevalence was observed among individuals using tap water 13.5%, statistical analysis did not reveal a significant relationship between water source and infection rates, nor did contact with animals show any significant association. Table (6)

Table (6) analysis of different risk factors for *Cryptosporidium* infections among study individuals.

Variables	Categories	*Cryptosporidium* diagnosed by MZN		p-value
		Present	Absent	
Age	Children: 2 to 12	20 (8.2%)	104 (42.4%)	0.05*
	Adolescence: 13 to 19	5 (2%)	22 (9 %)	
	Young Adults: 20 to 35	4 (1.6%)	22 (9%)	
	Middle age: 36 to 55	8 (3.3%)	30 (12.2%)	
	Old age: ≥ 56	12 (4.9%)	18 (7.3%)	
Gender	Male	26 (10.6%)	93 (38%)	0.482
	Female	23 (9.4%)	103 (42%)	
Residence	Rural	34 (13.9%)	99 (40.4%)	0.018*
	Urban	15 (6.1%)	97 (39.6%)	
Animal contact	Yes	17 (7.6%)	81 (36.3%)	0.227
	No	30 (13.5%)	95 (42.6%)	
Drinking water	Filter	15 (6.7%)	56 (25.1%)	0.753
	Tap	**30 (13.5%)**	116 (52%)	
	Underground	2 (0.9%)	4 (1.8%)	

Data presented as n and percentage with (%). Chi-square ($\chi 2$) test: *P* value ≤0.05 is significant*.

Nested-PCR amplification of cowp gene: DNA was extracted from 49 stool samples that were all positive for *Cryptosporidium* by microscopic examination, using cowp gene, 2/49 stool (4%), samples were successfully amplified at 553 bp.

Molecular characterization of *Cryptosporidium* by n-PCR-RFLP: Results proved that *Cryptosporidium hominis genotype I.* was the only detected species. No other *Cryptosporidium spp.* was detected. Figure (2)

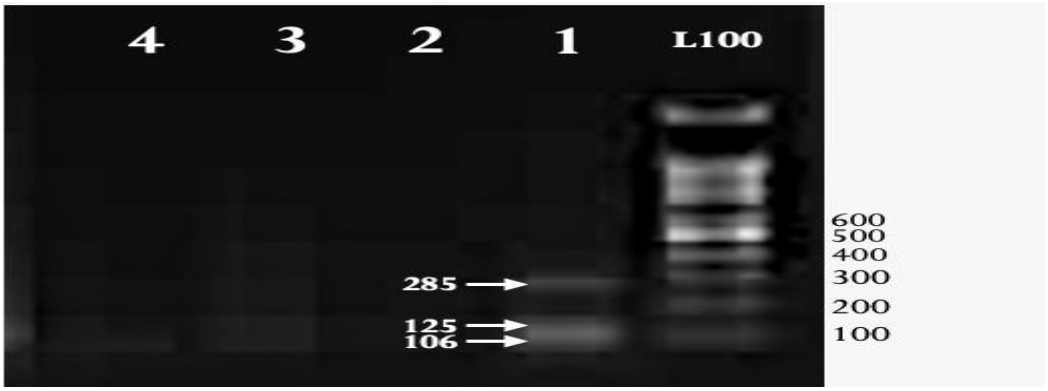

Figure (2) Agarose gel electrophoresis for COWP nPCR-RFLP products after digestion with RasI endonuclease. Lane L100: 100 bp DNA marker ladder. Lanes 1: *Cryptosporidium hominis genotype I* digestion products at 34, 106, 125 and 285 bp. Lanes 2-4: negative samples.

Sequencing Analysis: The sequencing of n-PCR products corresponding to the COWP protein gene confirmed the presence of *Cryptosporidium hominis* species. The sequence obtained was as follows:

(TTGCATTCACTATGCCTGAAAAATCATGTCCCCCAGGATTCGTCTTTTCTGGAAAACAATGTGT
TCAATCAGACACAGCTCCTCCTAATCCAGAATGTCCTCCAGGTACTATACTGGAAAATGGCAC
ATGTAAATTAATTCAACAAATTGATACCGTTTGTCCTTCTGGTTTTGTTGAAGAAGGAAATAGA
TGTGTTCAATATCTCCCTGCAAACAAAATCTGTCCTCCTGGATTCAATTTGTCAGGACAACAAT
GTATGGCACCAGAATCAGCTGAATTAGAATCGACATGCCCACCTAATTCAATATTTGAAAATG
GAAAATGTAAAGTGATTAAAAATATTGATATGGTCTGCCCACCAGGATATACAGATTCAGGAG
ATGATTGTGTACTATATGTAGCTCCTGCAAAGGAATGCCCACCAAATTTCATTTTACAAGGCCT
CCAGTGTATTCAAACTAGTTCTGCTCCAACTCAACCTGTCTGCCCTCCAGGTACAGTATTACAA
GATAATGCCTGTATTTCAGTCCAAGCAATC)

Discussion

SMJ- Sohag Medical Journal, Vol. 29 No(1) 2025

Print ISSN1687-8353 Online ISSN2682-4159

Original Article

The study determined the prevalence and species of *Cryptosporidium* in Sohag Governorate and identified associated risk factors, revealing that 20% of the total cases (49/245) were infected with *Cryptosporidium* using the MZN staining method. Among asymptomatic immunocompetent individuals, the prevalence was 6.9% (2/29), consistent with **El-Hady et al. (2017),**[9] who reported 10% in a similar group from Sohag.

Elsawey et al. (2020)[10] found a 4% prevalence among asymptomatic immunocompetent individuals in Mansoura, while **Abd Ellah et al. (2024)**[11] reported a lower rate of 3% in Sohag. In contrast, **Abdel-Hafeez et al. (2012)**[12] found a 42.2% prevalence among immunocompetent children in Minia, and **Ibrahim et al. (2016)**[13] reported 15.7% in asymptomatic immunocompetent patients from Beni-Suef.

The prevalence of *Cryptosporidium spp.* among symptomatic immunocompetent individuals with diarrhea, abdominal pain, low weight, or fever was 15.6% (23/147). This aligned with **Mohammad et al. (2021),**[14] who reported a 27.8% infection rate among children with diarrhea in Sharkyia Governorate, while **Yousof et al. (2023)**[15] found 10.5% in preschool children with diarrhea in Cairo, and **Mohamed & Masoud (2023)**[16] reported 17.1% in symptomatic patients. **Abd Ellah et al. (2024)**[11] noted a 20% prevalence in symptomatic patients from Sohag Governorate.

In contrast, **Helmy et al. (2013)**[17] reported a prevalence of 49.1% among children with diarrhea in Ismailia, and **Shalaby & Shalaby (2015)**[18] found 30.8% in school children with diarrhea in Mansoura. Additionally, **El-Nadi et al. (2017)** [19] reported a 31.5% prevalence among elementary school children aged 6 to 12 years in Sohag, while **Dyab et al. (2018)**[20] found 40% among diarrheic children in the same region.

The study found a 37.8% (14/37) prevalence of *Cryptosporidium spp.* among patients with chronic diseases, indicating a significant association between chronic health conditions and this parasitic infection, with the highest prevalence observed in diabetes mellitus patients at 55.6% (5/9) and chronic kidney disease patients at 43.8% (7/16). This increased susceptibility can be attributed to acquired immunodeficiency from uremia impairing immune function,[21] as well as alterations in immune cell function due to diabetes that compromise defense mechanisms against pathogens. [22] Chronic inflammation associated with these diseases can lead to immune dysregulation and increased levels of pro-inflammatory cytokines like IL-6 and TNF-α, linked to both chronic diseases and heightened infection risk. [23]

These findings aligned with previous research highlighting the association between chronic illnesses and increased susceptibility to *Cryptosporidium spp.* infections; for instance, **El-Kady et al. (2018)**[24] reported a 40% prevalence among CKD patients receiving hemodialysis in Qena Governorate, while **Ahmed et al. (2022)**[25] found a 35% prevalence in CKD patients from Sohag. **Morsy et al. (2024)**[26] reported a 29.5% prevalence in diabetic individuals from Kafrelsheikh Governorate, and **Mohamed et al. (2024)**[27] noted a 36% prevalence in diabetic children from Minia Governorate.

Among immunocompromised patients—including those with hematological malignancies, colorectal cancer, and hepatocellular carcinoma receiving chemotherapy—the prevalence of *Cryptosporidium spp.* was found to be 31.3% (10/32), with rates of 33.3% (7/21) for hematological malignancies, 25% (2/8) for colorectal cancer, and 33.3% (1/3) for hepatocellular carcinoma. Hematological malignancies can significantly impair the immune system due to the disease or chemotherapy, creating conditions favorable for opportunistic infections like *Cryptosporidium spp.*[28]

Epidemiological evidence also links *Cryptosporidium* infections to gastrointestinal cancers, particularly colorectal and hepatocellular carcinoma, where chronic infections may promote

tumor growth in immunocompromised individuals. [29]

These findings are consistent with studies indicating notable prevalence rates of *Cryptosporidium spp.* infections among patients with hematological malignancies and gastrointestinal cancers; **Mohammad et al. (2021)**[30] reported a 29.7% prevalence among children with hematological malignancies in Sharqiyah Governorate, while **Seleem et al. (2024)**[31] found a 14% prevalence in intestinal cancer patients from Sohag Governorate. **Zhang et al. (2020)**[32] reported a 13.3% infection rate among gastrointestinal cancer patients, including 17.24% for colorectal cancer and 14.29% for liver cancer.

In contrast, **Abdel-Hafeez et al. (2012)**[12] reported a higher prevalence of *Cryptosporidium* spp., at 60%, among immunosuppressed children in Minia Governorate; **El-Hady et al. (2017)**[9] found a prevalence of 45% among children undergoing chemotherapy in Sohag Governorate, while **Mohamed et al. (2020)**[33] also reported a 45% infection rate among immunocompromised patients in Sohag. This contrasts sharply with a study conducted in Saudi Arabia that revealed a *Cryptosporidium* infection rate of 70.3% among patients with various malignancies—including rates of 50% for both colon and liver cancer and an alarming 100% for lymphoma.[34]

The study revealed statistical significance between *Cryptosporidium* infection and patient categories with a p-value of 0.002.

Parasitic infections are a significant public health concern, leading to considerable morbidity and mortality. The COVID-19 pandemic may have contributed to a decline in the prevalence of *Cryptosporidium spp.* infections across various patient categories, as evidenced by studies examining prevalence changes before and after the pandemic. [35]

A study in Saudi Arabia found that quarantine measures led to a reduction in parasitic infections, with the prevalence of *Cryptosporidium* dropping from 2.5% pre-COVID to 1.3% during the pandemic. [36] Research in New Zealand indicated that *C. hominis* disappeared from early 2020 to early 2021 due to lockdown measures, while *C. parvum* maintained its typical transmission patterns. [37]

Similarly, a study in England and Wales reported a 97.5% reduction in *C. hominis* and a 49% decrease in *C. parvum* cases following public health measures that limited social interactions and travel.[38] In Scotland, there was a significant drop in *C. hominis* cases during the pandemic, with only five cases reported compared to previous years, although a small peak in *C. parvum* cases was noted in November 2020. [5]

The COVID-19 pandemic has led to significant changes in hygiene practices, which may have inadvertently reduced the prevalence of intestinal parasitic infections including cryptosporidiosis. Public health campaigns promoted handwashing, the use of hand sanitizers, and general cleanliness as measures against COVID-19. [39] Lockdowns minimized human interactions, reducing opportunities for the spread of intestinal parasites often transmitted through contaminated food or water. [40]

Additionally, improvements in sanitation and waste management were observed, with increased public awareness and investment in sanitation infrastructure leading to decreased contamination of water sources and food supplies.[41] Antiparasitic medications such as Metronidazole, Nitazoxanide, and Ivermectin were also incorporated into COVID-19 treatment protocols,[42-44] The sustained focus on hygiene and sanitation may yield long-term effects on the prevalence of intestinal parasites. [45]

This study was the first conducted in the Sohag Governorate to detect *Cryptosporidium* species which was *Cryptosporidium hominis genotype I;* using n-PCR\RFLP and sequencing techniques. *C. hominis* is one of the two most common species of *Cryptosporidium* that infect humans, responsible for more than 90% of cases. *C. hominis* is predominantly anthroponotic, meaning it is primarily transmitted from human to human. This genotype is rarely associated with zoonotic transmission. [46]

Studies in various Egyptian governorates show that *Cryptosporidium hominis* was the predominant species. In Ismailia, 60.5% of cases were *C. hominis*, with 38.2% *C. parvum*[17]. In Cairo, **El-Badry et al. (2015)**[47] reported 95.8% for *C. hominis* and 3.0% for *C. parvum*. **Ibrahim et al. (2016)**[13] confirmed *C. hominis* as the main species in Beni-Suef, highlighting both anthroponotic and zoonotic transmission. **Naguib et al. (2018)**[48] found a notable prevalence of *C. hominis* in El-Dakahlia, El-Gharbia, and Damietta. **Elsawey et al. (2020)**[10] also reported that *C. hominis* was the most prevalent species 52.5%

among cryptosporidiosis-positive children in Mansoura Governorate. In Cairo Governorate, **Wahed et al. (2021)**[49] found a prevalence of 19% for *C. hominis* in children with acute gastroenteritis. **Amin et al. (2021)**[50] reported that among elderly patients over 60, *C. hominis* made up 80% of cases. In Sharkyia Governorate, **Mohammad et al. (2021)**[14] showed *C. hominis* in 65.2% of diarrhea cases and 62.2% in children with cancer. **Ghallab et al. (2022)**[51] noted a higher prevalence of *C. hominis* in Kafr El-Sheikh among both immunocompetent (60%) and immunocompromised (82.6%) patients.

This finding contrasted with a study in Alexandria Governorate, which identified *C. parvum* in children with malignancies. [52] Additionally, research in Fayoum Governorate found a predominance of *C. parvum* at 72.7%, indicating a higher prevalence of zoonotic transmission, particularly in farming areas.[16] Similarly, a study in Baghdad Governorate, Iraq, reported a human infection rate of 47.33%, with 46% attributed to *C. parvum* and only 1.33% to *C. hominis*. [53]

The observed discrepancy in this study between staining and molecular diagnosis for *Cryptosporidium* may be attributed to either the presence of false negative results of PCR or false-positive results in the staining method.

The detection rate of *Cryptosporidium* oocysts by PCR can be significantly affected by various factors, leading to false negatives. A low count of oocysts, around 150 per sample, can result in insufficient DNA for PCR. [54] In a study, 8.2% of samples that were positive by microscopy tested negative by PCR due to low oocyst counts. [55] Additionally, substances like bilirubin and bile salts can inhibit PCR performance, causing false negatives even when oocysts are present.[56] Improper handling and processing can also lead to DNA degradation, especially in small samples. [57] Carbol fuchsin stains can target *Cryptosporidium* oocysts but may also stain other organisms, such as yeasts and bacteria, leading to potential misinterpretation during microscopic examination.[58] **Chen et al.(2023)** [59] reported a prevalence of 3.0% for *Cryptosporidium* using staining, compared to 2.0% with molecular methods, indicating that the staining technique may yield unreliable results.

While PCR can have a reported specificity of up to 100%, stool samples with oocyst-like bodies that do not produce specific PCR amplification may still be deemed negative, although they cannot be entirely excluded. [60] The challenges in achieving accurate diagnoses are further highlighted by the specificity and sensitivity of methods like ELISA and acid-fast staining,[61] underscoring the complexities in diagnostic methodologies.

In the present study, we found a statistically significant association between *Cryptosporidium* infection and age (p-value of 0.05), with an 8.2% prevalence in children aged 2 to 12 years (20/245 cases). This aligned with a study in the Cairo Governorate, which reported a 14.5% prevalence in children versus 5% in adults.[47] A systematic review by **Dong et al. (2020)**[62] confirmed that children are more susceptible, with the highest prevalence among school children.

Elsawey et al. (2020)[10] attributed this to children's immature intestinal immunity, unhygienic behaviors, and poor handwashing. Among elderly patients over 56 years old, we found a 4.9% prevalence of *Cryptosporidium* spp., which is consistent with **Amin et al. (2021),**[50] who reported a 3.7% prevalence in those over 60 years.

In the present study, we found statistical significance between *Cryptosporidium* infection and residence, with p-values of 0.018. The findings indicated that the prevalence of *Cryptosporidium* was higher among participants from rural areas (13.9%) compared to those from urban areas (6.1%). This may be due to poor hygiene, low socioeconomic conditions, possibly contaminated water sources, occupational risk, sanitary conditions, and higher population densities with a risk of person-to-person transmission as well. [63]

This finding agreed with a study by **Dyab et al. (2018)**[20] in Sohag Governorate found that infection rates were higher in rural areas compared to urban areas (60% vs. 20%), with a significant p-value of 0.001. Similarly, **Ghallab et al. (2022)**[51] reported in Kafr El-Sheikh Governorate a significant relationship between residence and infection rates, also indicating a higher prevalence in rural areas (p-value < 0.005).

In contrast, A study in El-Dakahlia, El-Gharbia, and Damietta found similar prevalence rates of *Cryptosporidium spp.* among children in rural (1.5%) and urban (1.2%) areas, with a p-value of 0.76 indicating no significant difference. [48] A study in Cairo Governorate on *Cryptosporidium*

spp. infections in elderly Egyptians found that 70% of positive samples were from rural areas and 30% from urban areas. However, this difference was not statistically significant, indicating no strong correlation between residence type and infection rates. [50]

Regarding drinking water sources, the present study found that 13.5% of positive cases consumed tap water, which is partially impure, compared to only 6.7% who reported drinking filtered water, this may be explained by the fact that the routes of infection in this locality may be waterborne; however, there was no statistically significant association between cryptosporidiosis and the water source, as indicated by a p-value of 0.753.

It was consistent with a study investigating the epidemiology of *Cryptosporidium spp.* in diarrheic immunocompetent patients in Beni-Suef Governorate, which found that although tap water was associated with higher infection rates compared to filtered water (22.7% and 10.7%, respectively), there was no statistically significant association (p-value = 0.1). [57]

Similarly, in a study investigating cryptosporidiosis among outpatients with diarrhea in El-Fayoum Governorate, the highest rate (90.6%) was detected among tap water users without statistically significant association (p-value=0.24).[16] In contrast, a study by **Dwi et al., (2023)**[64] found that one person was infected with *Cryptosporidium* despite most research subjects using unhealthy healthy water as a drinking water source.

Regarding contact with animals, in the present study, we observed no significant association between cryptosporidiosis and contact with animals, as indicated by (p-value=0.227). This finding underscores the importance of anthroponotic transmission in the spread of *Cryptosporidium* spp.

This finding aligned with **Xiao and Feng (2008),**[65] who noted that many infections from zoonotic species may actually stem from human-to-human transmission rather than animal-to-human. **Boughattas et al. (2019)**[66] found no significant link between cryptosporidiosis and animal contact (p-value=0.092), suggesting that the role of animals might be overestimated. **Krumkamp et al. (2020)**[67] observed that transmission among infected children primarily occurred between household members and neighbors, indicating a higher prevalence of human-to-human transmission. Similarly, **Mohammad et al. (2021)**[14] reported no correlation with animal contact (p-value=0.6), underscoring the need to investigate alternative transmission routes. Conversely, **El-Sherbini and Mohammad (2006)**[68] identified a statistically significant association between farmers and their farm animals infected with *C. parvum*, indicating a zoonotic potential for the infection (p-value < 0.01).

Furthermore, **Izadi et al. (2014)**[69] reported that cryptosporidiosis poses an occupational risk for individuals in contact with cattle, providing strong evidence of zoonotic transmission from calves to humans (p-value < 0.0001). Additionally, a study by **Mohamed and Masoud (2023)**[16] found a significant infection rate among patients having indoor animals (p-value=0.001).

The predominance of anthroponotic *Cryptosporidium* strain, coupled with the lack of association between *Cryptosporidium* prevalence and animal contact in the present study population, suggests that population dynamics significantly influence the transmission patterns of *Cryptosporidium* in Sohag. Notably, *C. hominis* presents considerable public health challenges, particularly in regions with limited access to clean water and sanitation facilities.

Regarding gender, the analysis of gender differences in the study revealed that the infection rate among males was slightly higher than that of females, with 26 cases (10.6%) in males compared to 23 cases (9.4%) in females. However, this difference was not statistically significant, as indicated by a p-value of 0.48.

This finding aligned with **Boughattas et al. (2019)**,[66] which showed no significant gender difference in *Cryptosporidium spp.* prevalence among immigrants in Qatar (males 4.4%, females 4.7%; p-value 0.83). Similarly, **Khan et al. (2019)**[70] reported a p-value of 0.38 when comparing infection rates by gender, indicating no significant difference. Additionally, **Mohammad et al. (2021)**[14] found no significant relationship between gender and infection rates among Egyptian children with cancer (p-value 0.7), despite a higher prevalence in males. In contrast, **Dyab et al. (2018)**[20] who found the rate of *Cryptosporidium* infection among male children was significantly higher than the rate among females (p-value = 0.001). Additionally, **Sinyangwe et al. (2020)**[71] investigated factors associated with *Cryptosporidium* infection among

HIV-positive patients in Zambia and found that males were 2.5 times more likely to be infected than females, with a statistically significant p-value of 0.02.

Limitations

The study on *Cryptosporidium* prevalence in the Sohag Governorate has some limitations: the first was the low sample size which may restrict the findings' generalizability as the study was self-funded, Fluctuations in the dollar exchange rate and the broader economic conditions of the country affect the cost of materials and methods used in the study and due to the limited facilities.

Conclusion

The prevalence of *Cryptosporidium* in Sohag Governorate was 20% with significant associations in children aged 2-12 years and in rural areas. significant association with patient categories, especially immunocompromised and chronic disease patients. The identification of *C. hominis* indicates anthroponotic transmission. the relatively low prevalence of *Cryptosporidium* underscores the positive impact of public health measures implemented during and after COVID-19 pandemic on reducing parasitic infections.

Recommendations

Future studies should involve larger sample sizes for better generalizability and detailed subgroup analyses, focusing on age groups and clinical statuses. The study recommend researching environmental factors like water quality and sanitation practices to assess their impact on *Cryptosporidium* transmission. Continued monitoring of *Cryptosporidium* prevalence post-COVID-19 is also essential to understand the long-term effects of pandemic-related public health measures on intestinal parasitic infections.

Acknowledgements: The authors would like to thank all the participating patients, and we hope that they get better soon.

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