

Investigation of *Cryptosporidium* spp. infection among patients with diarrhea

BASSAD A. AL-ABOODY¹, NOOR NIHAD BAQER² and AHMED SALIH MOHAMMED³

¹Department of Biology, College of Science, University of Thi-Qar, Nasiriyah 64001, Thi Qar Governorate, Iraq;
²Scientific Research Commission, Research and Technology Center for Environment, Water, and Renewable Energy, Baghdad 10001, Iraq; ³Bartellah Hospital, Ministry of Health, Mosul 410019, Iraq

Received March 24, 2025; Accepted September 1, 2025

DOI: 10.3892/wasj.2025.394

Abstract. The protozoan parasite, *Cryptosporidium* spp., causes an intestinal illness known as cryptosporidiosis in humans. Watery diarrhea, dehydration and weight loss are common symptoms of cryptosporidiosis, which can last for up to 2 weeks. The present study aimed to investigate *Cryptosporidium* spp. infection in humans, using the modified acid-fast staining technique. From November, 2023 to the end of January, 2024, a total of 200 fecal samples were collected from patients of different ages and both sexes. The analysis revealed an infection rate of 60/200 (30%) for *Cryptosporidium* spp. According to the findings, males had a higher infection rate (32.14%; 36/112) than females (27.27%; 24/88), although the difference was not statistically significant. The study also found a significant association between age and cryptosporidiosis; specifically, patients ≤ 5 year of age exhibited the highest infection rate at 52.94%, while the lowest rate was 20.83% for the age group of 36-50 years. As regards residency, the infection rate was higher among rural residents (30.90%) than urban residents (28.88%), with no significant differences between the groups. On the whole, the present study indicates that *Cryptosporidium* spp. is a critical factor in the causation of diarrhea, particularly in younger patients.

Introduction

Cryptosporidiosis, caused by the parasite, *Cryptosporidium* spp., is one of the most common intestinal illnesses. It is a widespread opportunistic disease that can affect both humans and animals. Cryptosporidiosis ranks fourth among the causes of diarrhea worldwide, particularly in immunocompromised

individuals who are more vulnerable to its potentially fatal chronic form (1,2). It leads to illness in newborns and young children, resulting in severe diarrhea and mortality (3), rendering it the second leading cause of mortality among children with diarrhea who are <5 years of age (4,5).

Cryptosporidium is a single-celled parasite belonging to the phylum Apicomplexa, with a complex life cycle that includes both sexual and asexual reproduction within a single host and does not require an intermediate host (6). Based on molecular and morphological data, nearly 46 species and >100 genotypes of *Cryptosporidium* have been identified from various hosts (7). The transmission of *Cryptosporidium* mainly occurs via two mechanisms: First, through the consumption of food and water contaminated with the parasites, and second, through direct contact with infected individuals or animals carrying the parasites (2). A previous study performed in Iraq demonstrated the prevalence of *Cryptosporidium* in water due to pollution with oocysts, particularly in agricultural regions contaminated with cow feces (8).

One of the traits of *Cryptosporidium* is its resistance to chemical disinfectants, environmental stress and chlorine (9). *Cryptosporidium* became significant when it was identified that this parasite had high resistance to the majority of drugs and disinfectants, and could cause infections of the stomach and intestines in humans and animals (10). Therefore, the present study aimed to survey *Cryptosporidium* spp. among patients with diarrhea in the Thi-Qar Province of Iraq, and to examine some of the factors affecting the prevalence of *Cryptosporidium* infection.

Patients and methods

The present study included 200 fecal samples collected from patients of an age range (≤ 5 - ≥ 51) years and a median age of 30.5 years for both sexes suffering from diarrhea, who attended the Nasiriyah General Hospital and Bint Al-Huda Teaching Hospital between November 1, 2023 and January 30, 2024. These patients resided in Nasiriyah Province near agricultural regions. Sterile containers were used to collect the fecal samples, and they were labeled with all necessary patient data. Of note, two methods were used to diagnose the parasite: Modified acid-fast staining and direct microscopy. The direct wet mount technique was used for detection. One drop of normal saline

Correspondence to: Dr Noor Nihad Baqer, Scientific Research Commission, Research and Technology Center for Environment, Water, and Renewable Energy, 923 Aljamia Street, Al-Jadiryah, Baghdad 10001, Iraq
E-mail: noornihadbaqer@gmail.com

Key words: acid-fast staining, cryptosporidiosis, *Cryptosporidium* spp., diarrhea

solution was added to a slide, and 1 mg of stool was mixed with a stick. The slide was covered with a coverslip, and examinations were conducted at 40X and 100X magnification.

Consent was obtained from the participants in the present study for the collection of samples. For participants who were underage, consent was obtained from the parents. Ethical approval to collect samples from hospitals was obtained from the Ethics Committee of the Ministry of Health, Iraq (Reference no. 307/2022, 28-9-2022).

Detection of *Cryptosporidium parvum* by modified acid-fast staining. The staining procedure was performed as previously described (11) as follows: An appropriate amount of stool was obtained, and using a stick to mix it with a few drops of water, it was placed on the slide, submerged in carbolfuchsin dye (MilliporeSigma) for 5 min, and then heated at an intermittent temperature (60-70°C), while until the carbolfuchsin evaporated. To remove the remaining dye, the slide was washed with water. Rubbing alcohol was applied to the slide for 1 to 3 min. After rinsing the slide with water once more to remove the alcohol, it was submerged in methylene blue (MilliporeSigma) for 1 min at temperature room and then rinsed with water again. The slide was air-dried, and parasite oocysts appeared as dark pink or purple structures. The slide was examined first at x40 and x100 magnification. It was also necessary to apply specific oil when at x100 magnification to distinguish the oocysts.

Statistical analysis. The statistical analysis of the data was conducted using SPSS version 26 (IBM Corp.). Data are presented as numbers and percentages and were analyzed using the non-parametric and descriptive Chi-squared test.

Results

Direct wet mount examination. Only 60 sample out of the 200 sample were found as positive results following direct smear examination. Oocysts of *Cryptosporidium* were colorless and were shaped as spheres or had an oval-like shape.

Modified acid-fast staining results. Following staining with an acid-fast stain, the microscopical analysis yielded positive samples containing *Cryptosporidium* spp. oocysts, revealing that the oocysts were spherical in shape and reddish purple (Fig. 1).

Total infection rate with *Cryptosporidium* spp. The present study included 200 stool samples collected from patients with diarrhea in the Thi-Qar Province of Iraq. The present study revealed that 60 (30%) patients were infected with *Cryptosporidium* spp. while 140 (70%) were non-infected. The results revealed a significant difference between them ($P < 0.05$), as shown in Fig. 2.

Total infection rate of *Cryptosporidium* spp. according to sex. The findings of the present study demonstrated that males had a greater rate of *Cryptosporidium* infection than females, where the infection rates were 32.14% (36/112) and 27.27% (24/88), respectively. However, there were no significant differences between the sexes ($P > 0.05$; Table I).

Infection rate of *Cryptosporidium* spp. according to age groups. The data presented in Table II indicated that the prevalence of *Cryptosporidium* spp. infection varied according to age group. In particular, the age group ≤ 5 years had the highest infection rate, at 52.94% (18/34), while the age group aged 36 to 50 years had the lowest infection rate, at 20.83% (10/48). Significant differences ($P < 0.05$) were found between the groups. The mean ages of the patients in the different age groups were as follows: ≤ 5 years: Mean, 3.24 years; median, 3 years; 6-20 years: Mean, 12.3 years; median, 10 years; 21-35 years: Mean, 27.9 years; median, 26.5 years; 36-50 years: Mean, 42.7 years; median, 42.5 years; ≥ 51 years: Mean 56.6 years; median, 57 years.

Infection rate of *Cryptosporidium* spp. according to residency. According to the findings of the present study, the prevalence of *Cryptosporidium* spp. infection rates were higher in rural areas than in urban areas, with respective rates of 30.90 and 28.88%. There were no significant differences between the groups ($P < 0.05$; Table III).

Discussion

The human intestinal illness, cryptosporidiosis, is caused by the protozoan parasite, *Cryptosporidium*. Watery diarrhea, dehydration and weight loss are common symptoms of cryptosporidiosis, which can last for up to 2 weeks (12). In the present study, the parasite *Cryptosporidium* spp. in fecal samples of patients with diarrhea in Thi-Qar Province in Iraq was investigated microscopically by using the modified acid-fast staining technique.

Only four of the samples tested positive using the direct smear, indicating that *Cryptosporidium* was not detected in this manner, as the oocysts were small and required a specific detection method. Based on a modified acid-fast stain, which is simple, inexpensive and capable of clearly showing the interior structure of oocysts, the examination revealed the spherical shape of the oocysts, which appeared reddish-purple, while the remaining stool appeared blue.

The fecal smear, which is applied using a modified acid-fast stain, is considered the standard method for detecting *Cryptosporidium* oocysts in stool. This technique is commonly used in microbiological laboratories to help identify *Cryptosporidium* oocysts. Although the staining process requires time and an experienced microscopist to examine the slides, it is inexpensive and permits the detection of other parasite species, such as *Cyclospora* and *Isospora* (13).

In the present study, the outcome, based on acid-fast stain, revealed the infection rate with *Cryptosporidium* in humans, which was similar to previous studies conducted in Iraq. In the study by Alasady and Al-Hasnawy (14), in the Babylon Province, they recorded the rate of infection with *Cryptosporidium* spp. as 25%, by using microscopic examination. Moreover, the study by ALYasary and Faraj (15) in Karbala Province, found a positive result, with rate of infection being 26%.

Furthermore, another study revealed the prevalence of *Cryptosporidium* in the domestic pigeon in Baghdad (16). However, there are limited reports available on the genetic characteristics of several species of *Cryptosporidium* in

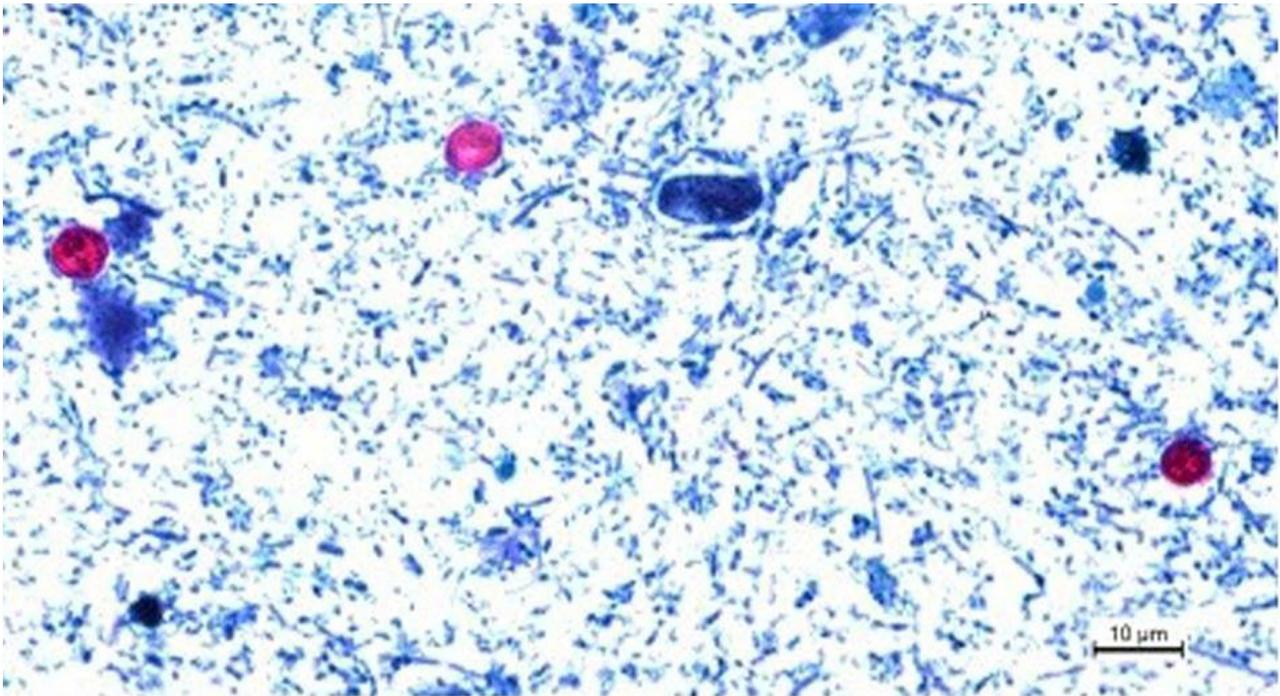


Figure 1. Oocysts of *Cryptosporidium* spp., isolated from human feces stained with acid-fast stain (100X magnification).

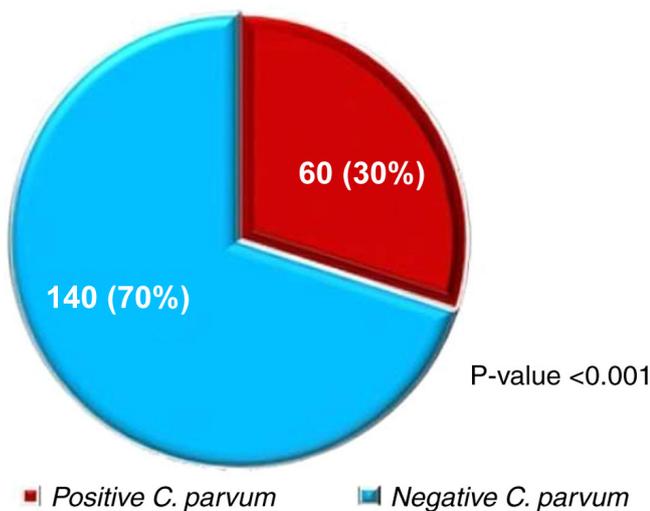


Figure 2. Infection rate with *Cryptosporidium* spp.

Iraq; nevertheless, both *Cryptosporidium hominis* and *Cryptosporidium parvum* have been identified in humans, with the latter being more abundant in isolates from cattle, sheep, goats and birds. A national investigation is necessary to obtain a sufficient number of samples from various hosts and environmental matrices, utilizing modern diagnostic methods to accurately assess the epidemiological status of cryptosporidiosis in Iraq (17).

Additionally, molecular genotyping research needs to be undertaken in Iraq to delineate the species and subtypes of *Cryptosporidium* infecting humans and animals, particularly during outbreaks. Consequently, the *Cryptosporidium* parasite needs to be incorporated into the standard diagnostic and surveillance framework for infectious diseases in Iraq and

should be acknowledged as a significant public health issue of concern.

The findings of the present study concur with a study conducted in Pakistan by Khan *et al* (18), which found, by using the MZN stain, that 127 of the 425 samples collected were found to be positive for *Cryptosporidium* with a prevalence of 29.88% (127/425). On the other hand, the findings of the present study are in contrast to those of the study by Sayal (19) in Al-Najaf AL-Ashraf in Iraq. He recorded the total percentage of infection with *Cryptosporidium* to be higher than that in the present study. The positive result was 58%. The findings of the present study are also in contrast to those of the study by Sharbatkhori *et al* (20) from Iran, in which they recorded an infection rate lower than that in the present study. They found that, out of 547 collected samples, 27 (4.94%) were positive for *Cryptosporidium*. Numerous factors, including variations in the study population, personal hygiene, sewage water management, ingestion of contaminated food, untreated water and meteorological conditions, were linked to the fluctuations in the prevalence of *Cryptosporidium* infection. The high prevalence of cryptosporidiosis that currently exists is partly due to the season and low economic standing (18,21).

According to the findings of the present study, males had a greater infection rate than females, although there was no statistically significant difference between the sexes ($P>0.05$). This finding is in agreement with the findings of other studies from Iraq, from the Basra Province, Duhok Province and Babylon Province (14,22,23). They had recorded higher infection rates for males than females, but without a significant difference.

Conversely, the findings of the study were not in agreement with the findings of the study by Saneian *et al* (24), which demonstrated that the infection rate in Iranian females was 2.6% and that in Iranian males was 2.0%. Mohammad (25)

Table I. Infection rate of *Cryptosporidium* spp. according to sex.

Sex (age range) years	Examined Sample		Positive samples		Negative samples	
	No. of patients	%	No. of patients	%	No. of patients	%
Male (≤ 5 - ≥ 51) years	112	56	36	32.14	76	67.85
Female (≤ 5 -51) years	88	44	24	27.27	64	72.72
Total	200	100	60	30	140	70
P-value 0.456						

Table II. Infection rate of *Cryptosporidium* spp. according to age groups.

Age groups (years), mean and median	No. of examined samples		Positive samples		Negative samples	
	No. of patients	%	No. of patients	%	No. of patients	%
≤ 5 (3.24 and 3)	34	17	18	52.94	16	47.05
6-20 (12.3 and 10)	56	28	14	25	42	75
21-35 (27.9 and 26.5)	36	18	10	27.77	26	72.22
36-50 (42.7 and 42.5)	48	24	10	20.83	38	79.16
≥ 51 (56.6 and 57)	26	13	8	30.76	18	69.23
Total	200	100	60	30	140	70
P-value 0.024						

Table III. Infection rate of *Cryptosporidium* spp. according to residency.

Residency	Examined samples		Positive samples		Negative samples	
	No. of patients	%	No. of patients	%	No. of patients	%
Urban	90	45	26	28.88	64	71.11
Rural	110	55	34	30.90	76	69.09
Total	200	100	60	30	140	70
P-value 0.756						

reported in his study, from the Al-Qadisiyah Province in Iraq, that the infection rates in women were higher than those in males at 56.6 and 43.3%, respectively. The increasing incidence observed in the present study among males may be attributed to several factors, including, the way of life that men lead and their ongoing interaction with the outside world, which is significant. Various factors contribute to the spread of oocysts, such as improper feeding practices, consuming exposed food in public areas and apathy towards maintaining personal cleanliness (26). According to the present study, age and cryptosporidiosis were significantly associated. Those aged ≤ 5 years had the highest infection rate of 52.94%, while the lowest rate was 20.83% for age group of 36-50 years. The results presented herein are consistent with the research conducted in Kirkuk City, Iraq, by Salman *et al* (27) and in Buner District, Pakistan, by Khan *et al* (18), which demonstrated greater infection rates among children < 6 years of age, at 25.45 and 41.0%, respectively. These findings contradict

those of other studies by Yang *et al* (28), in southwest China, Abdel Gawad *et al* (29) in Beni-suef, Egypt, and in Duhok Province/Iraq by Shahoi *et al* (23); all these studies found that the highest rates of cryptosporidiosis were found in the age groups of 41-50 years and 31-40 years, with rates of 17.3, 25 and 91.7%, respectively. Children are more likely to acquire infections than adults due to their immature immune systems. As a result, consuming a small number of oocysts can lead to cryptosporidiosis, and frequent low-dose infections can build up immunity to *Cryptosporidium*, which may protect children from developing comparatively more severe illnesses than adults (30,31). According to residency, the rate of infection with *Cryptosporidium* was higher among rural residents than urban residents, with no significant difference between them. The results of the present study are in agreement with those of the study by Khoshnaw *et al* (32) in Erbil City, Iraq, as they did not record any significant association between cryptosporidiosis and residency (rural, 21.1% vs. urban,

19.7%). These findings are also in agreement with those of the study by Alasady and Al-Hasnawy (14) in Babylon Province, who recorded non-significant differences ($P>0.05$) in the rate of infection in rural areas (36.36%), compared with urban areas (16.07%). However, these findings contradict those of Shahoi *et al* (23) in the Duhok area of Iraq, which found that suburban inhabitants had a greater infection rate (87.6%) than urban residents (75%), with a significant difference ($P<0.05$) between the two groups. These findings are also in contrast to those of the study by Abdel Gawad *et al* (29) in Egypt, who reported significantly higher rates of infection in rural residents compared to urban ones (59.5 vs. 40.5%). High rates of infection in rural areas may be caused by several factors that are considered to favor the spread of intestinal parasites, such as, the lack of clean drinking water and reliance on the river for water, the need to deal with contaminated soil from farms and gardens that contain cysts of parasite, animal breeding, contact with parasite reservoirs, the use of animal waste as organic fertilizer, and the poor health and cultural level of the rural population.

Of note, a limitation of the present study is that species-level diagnosis of *Cryptosporidium* was not conducted. The diagnosis was based on acid-fast staining, which is useful for detecting the presence of *Cryptosporidium* oocysts, but does not allow differentiation between the two main human-infecting species, *Cryptosporidium parvum* and *Cryptosporidium hominis*, due to their morphological similarity and sample loss for genetic diagnosis. Future studies employing molecular methods (e.g., PCR and sequencing) would be necessary to distinguish species and investigate their epidemiological distribution accurately.

In conclusion, the present study demonstrated the prevalence of *Cryptosporidium* in Thi-Qar Province, Iraq. Additionally, the prevalence was higher in males than in females. The infection rate also increased in rural areas compared to urban areas due to contact with animals. The incidence rates were higher in children; this may be due to the handling of animals and soil polluted with feces from animals by their parents; this contamination was then transmitted to their children by their food and drink through inadequate personal care.

Acknowledgements

The authors would like to thank all the staff of Nasiriyah General Hospital and Bint Al-Huda Teaching Hospital in Iraq for their assistance with sample collection.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

BAAA was involved in the conceptualization of the study, in data curation and investigation, in the study methodology, in project

administration, in the provision of resources (kits) and software (for statistical analysis), and in the writing of the original draft of the manuscript, and in the writing, review and editing of the manuscript. NNB was involved in data validation, and in the writing, review and editing of the manuscript. ASM was involved in data curation, in the study methodology, and in data investigation. All authors have read and approved the final manuscript. BAAA and NNB confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Consent was obtained from the participants in the present study for the collection of samples. For participants who were underage, consent was obtained from the parents. Ethical approval to collect samples from hospitals was obtained from the Ethics Committee of the Ministry of Health, Iraq (Reference no. 307/2022, 28-9-2022).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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