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# Some Epidemiological Aspects of Cryptosporidium parvum Among Children Below Five Years in Kirkuk Province

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## ABSTRACT

**Background:** Intestinal protozoan parasite infection impacted malnutrition, public health, and young children. The zoonotic pathogen Cryptosporidium spp. generates diarrhea in immunocompetent and immunocompromised hosts. Its global burden, epidemiology, diagnosis, and management are poorly understood.

**Setting:** A cross-sectional study of 330 under-5 years with gastroenteritis (177 men and 153 females).

**Methodology:** After each patient completed a questionnaire, stool samples were obtained for Modified Ziehl-Neelsen, direct microscopy, concentration, the flotation technique), and fluorescent stains.

**Results:** 204 stool samples had 61.68 percent Cryptosporidium. This rate involved: 65.35 percent in females against 58.75 percent in males ($p > 0.05$), although the association between male age and cryptosporidium frequency was significant (p 0.05). Samples from children under one year and one to two years had high rates of 32.35 percent and 26.24 percent, respectively, compared to older children. $P<0.05$. Artificially fed children had 43.07% Cryptosporidium compared to 16.93% breastfed; ($P <0.05$). Children drinking mineral sterile water and tank water had stool rates of 59.63 % and 35.79 %, respectively, compared to 4.58 % for municipal water; $P<0.05$. Alkaline stool samples had 26.31 percent cryptosporidiosis, $p<0.05$. Patient residency did not affect Cryptosporidium dispersion. Diarrhea-associated Cryptosporidium positivity was 62.26 percent compared to 37.74 percent in non-diarrheic stools, especially in infants under one year. Entamoeba histolytica (19.74%), Blastocystis hominis (9.8%), and Giardia lamblia (6.37%) were other frequent intestinal parasites. Cryptosporidium was easily visible using modified Ziehl-Neelsen, Calcofluor, and Auramine methods.

**Conclusions:** In Kirkuk Province, children under five had the most gastroenteritis due to Cryptosporidium parvum infection. Age, feeding, and water intake are factors.

**Keywords:** *Cryptosporidium, gastroenteritis, Auramine, Calcofluor, Entamoeba*

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## INTRODUCTION

Cryptosporidium was first identified in 1976 and is one of the most common waterborne diseases found worldwide [1]. Cryptosporidium species infect epithelial surfaces in the digestive and respiratory systems of people and animals all over the world. There are several species of Cryptosporidium, and all of them are protozoan parasites that can only survive inside host cells.[2 ]. But only C. hominis and C. parvum are known to cause illness in humans. When compared to the latter, which is more common in underdeveloped countries or occurs as a zoonotic illness in wealthy countries, the former is more frequently isolated from developed countries. Hence, impoverished countries bear a disproportionate share of the cost of cryptosporidiosis because of inadequate sanitation systems [3]. The life cycles of these parasites are accomplished entirely within a single host (a monoxenous life cycle). Cryptosporidiosis can be fatal [4]. Oocysts, a primary cause of diarrhea, can be found in food and drink [5]. Cryptosporidium epidemics in drinking and recreational water are exacerbated by the infective oocyst's pervasiveness, resilience to environmental stresses, and low infection dosage[6]. This situation is worse than in developing nations, where Cryptosporidium diarrhea is caused by a lack of potable water and limited diagnosis and treatment [7]. Cryptosporidiosis causes both short- and long-term diarrhea in children. Those with healthy immune systems are just as susceptible to it as those without, but those with weakened immunity tend to suffer from a more chronic form of the disease [8]. Although cryptosporidiosis has been documented in animals, prior to the last two decades, little attention was paid to Cryptosporidium as a human and, more specifically, as a young child protozoan intestinal parasite .Othman[9] collected similar data on the frequency of Cryptosporidium in Kirkuk Province; this study is generally regarded as the most comprehensive overview of the topic to date. She found that 12.62 percent of infants were infected with cryptosporidium. [10] conducted a similar study in the same province using a modified Ziehl-Neelsen stain (hot technique), and they found that 4.36 percent of the children tested positive for cryptosporidiosis. Studies on Cryptosporidium in Kirkuk were conducted after 2010 by [11, 12 and 13]. The rates were 14.47%, 7.60%, and 6.45%, respectively. The current study was planned and done to estimate some epidemiological aspects of Cryptosporidiosis using some lab diagnostic techniques. This was done because an increase in gastroenteritis among children under five years old in Kirkuk Province in 2022 has been linked to the fact that basic infrastructure in the province has gotten worse as a result of what happened in Iraq in 2014.

## MATERIALS AND METHODS

### *Period and location*

This investigation was conducted between ion was conducted between July 15, 2022, and between July 15, 2022, and February 15, 2023, in the laboratories of the Kirkuk Medical College, the Kirkuk pediatric general hospital, and the Ibn-Nafees private medical laboratory.

### *Methodology and subjects*

A cross-sectional study was conducted on 330 children of both sexes, ranging in age from two months to five years. There were 177 boys and 153 girls. They primarily complain of gastroenteritis.

### *Exclusion criteria*

Children with additional symptoms and those older than five years were excluded from the trial and not included.

### *Ethical approval*

According to the functions of the scientific committee in the Kirkuk Health Directorate, permission to conduct the study was granted on October 2, 2022, in accordance with administrative issue 591. For each child, an informal questionnaire was completed with the child's name, age, address, type of feeding, water quality, and typical symptoms, as well as the patient's parents' signatures and consent to participate in the study.

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***Sample collections***

Each patient provided two stool samples, which were collected in airtight containers. Around 5 ml of a 2.5% potassium dichromate solution was added to the first container, which was then thoroughly mixed and placed in a cool box. A little amount of 0.85% normal saline was added to the second container, which was then thoroughly mixed[14]. Each child had one milliliter of venous blood collected and added to the EDTA tube mixed thoroughly for ABO blood grouping[15]

***Stool sample examination***

Each stool sample first was examined macroscopically for color, and consistency as well as for any foreign bodies in the specimen. Measurement of stool pH was done using gradient pH filter paper (Germany), while for microscopy briefly the following procedures were applied for each stool sample:

Each stool sample was tested for the presence of Cryptosporidium oocysts and other intestinal parasites using a direct double wet preparation of 0.85% NaCl (focusing the motility) and 1% Lugol's iodine (to study color internal contents) upon its arrival at the laboratory [16]. Each negative slide for Cryptosporidium and other intestinal parasites was examined using the flotation method (33% ZnSO4 solution) as outlined in the instructions [17]. According to Othman [9], a modified Ziehl-Neelsen technique was applied to all fecal smears. Briefly, a fecal smear slide was inundated with a strong carbol fuschin stain, and the slide's underside was heated until fine steam appeared on its surface. The slide was allowed to sit for 5 minutes before being discarded and washed with distilled water till a purple hue appeared. A 5% H2SO4 decolorizing solution was applied to the slide and agitated for one minute; then, an excess of acid was removed by rinsing with D.W. After one minute, the counterstain "methylene blue" was applied to the smear. Finally, the stain was removed, rinsed by D.W., air-dried, and observed under a microscope at a magnification of 100x, whereby the Cryptosporidium oocysts displayed as pinkish spheres against the blue backdrop of the slide [18].

For performing the fluorescent staining the following procedure was applied for phenol-auramine "A phenolic auramine solution was spilled over each fecal smear for 15 minutes after they were air dried and cured with moderate flames. The slide was then wiped clean with distilled water. Decolorizer (Acid Alcohol Solution) was applied to the slide for two to three minutes. The decolorizing process was repeated on any slides that still showed pink after the first. The slide was given a thorough rinsing with distilled water, and the excess was shaken off. The counterstain (Potassium Permanganate Solution) was applied to the slide and left there for 3–4 minutes. cleaned with distilled water, then hung or blotted dry. confirmed under oil immersion lenses and examined at low and high magnifications 400X with a fluorescent microscope (UV light source)[19] Whereas the Calcofluor staining procedure involves the following "Calcofluor white stain is made of Calcofluor White M2R 1 g/l and Evans blue 0.5 g/l. Each fecal smear was covered with one drop of Calcofluor White stain, and then one drop of 10% potassium hydroxide was put on top. The sample was covered with a clean cover slip and left for 1 minute. Examined at 100x to 400x magnification under UV light [20]. Furthermore, for modified gram-stained chromotrope R2 briefly was applied as follow as "After fixation, each fecal smear was gram-stained except for the addition of Safranin, which was substituted by a hotly prepared chromotrope R2 stain: The slide was in warmed (50–55°C) chromotrope stain for at least 1 minute. Rinsed for 1–3 seconds in 90% acid-alcohol. This step requires special effort to stain oocysts correctly. 30-second ethanol rinse. Two 30-second rinses in 100% ethanol (two containers) were done. After drying, the slides were mounted with Canadian balsam and a clean coverslip and viewed microscopically" [15] Using a particular piece of equipment (a cassette) purchased from the Biozek firm in the Netherlands, a direct reaction was carried out between Cryptosporidium as an antigen (in a stool sample) and specific monoclonal antibodies bound on chromatography paper[21].

***Statistical analysis***

Using a Microsoft Excel file, all of the collected data were put into the right tables for statistical analysis. The manual statistical equations chi-square and t-student test for imposing variances and significance at the levels P 0.05 and P 0.01 were used to look at differences in the epidemiological characteristics of Cryptosporidium's spread.

## RESULTS

the overall positive rate for Cryptosporidium parvum was 61.81 % versus 38.19 % for negative, table 1. Also, the same table displays the distribution of Cryptosporidium-positive males and females among children under the age of five. The following proportions were documented: 58.75% and 65.35 % for males and females, respectively, as opposed to negative rates of 41.25 % and 34.65 % for males and females. There was no significant correlation between the distribution of positive infections and the gender of the patient.

**TABLE 1:** Positive and negative percentages of Cryptosporidium parvum among children below 5 years.

| **Percentages** | **Total** | **Cryptosporidium +ve** | **Cryptosporidium -ve** |
|---|---|---|---|
| Gender | No. Exam % | No. Exam % | No. Exam % |
| Males | 177 53.64 | 104 58.75 | 73 41.25 |
| Female | 153 46.36 | 100 65.35 | 53 34.65 |
| Total | 330 100 | 204 61.81 | 126 38.19 |

As for the spread of pathogens like the Cryptosporidium parasite and other intestinal parasites, the results showed that the infection was common in children younger than one year old. The fact that 32.25% and 31.57% of all participants were children under the age of one demonstrated this. With regard to the percentages that are the lowest, they are 12.74% and 9.21%, respectively recorded among children aged from 2 to 3 years, for each of the other intestinal parasites and the Cryptosporidium parasite; (P< 0.05). Table -2.

From the analysis of the questionnaires of the patients who participated in the current study and linking the positive results of cryptosporidium with the type of feeding used, Table 3 shows that children who are dependent on artificial feeding are more infected with cryptosporidium parasites, 43.07%, compared to 16.93% for children who are breastfed, respectively. This difference is because children who are breastfed have a higher immune system and are better able to fight off infections. P<0.05. Sterilized mineral, government-run, and tank water have the same morality (water from tanks equipped by tank trucks). Cryptosporidium prevalence was 59.63%, 35.77%, and 4.58% among sterilized mineral water, tub water, and government liquid water consumers, respectively, (P < 0.05).

**TABLE 2:** Positive percentages of infectious agents distribution according to ages.

| Infectious agents | Total examined | Cryptosporidium Positive | Other intestinal parasites |
|---|---|---|---|
| Percentages | No. % | No. % | No. % |
| Age group/years | | | |
| 0-1 | 157 47.57 | 66 32.35 | 24 31.57 |
| 1-2 | 78 23.63 | 54 26.47 | 19 25.00 |
| 2-3 | 27 8.18 | 26 12.74 | 7 9.21 |
| 3-4 | 30 9.09 | 28 13.75 | 14 18.42 |
| 4-5 | 38 11.51 | 30 14.70 | 12 15.78 |
| Total | 330 | 204 61.81 | 76 23.03 |

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**TABLE 3:** The impacts of feeding modes on the distribution of Cryptosporidium and intestinal-viruses.

| Feeding modes | Cryptosporidium Positive | Type of water | Cryptosporidium Positive |
|---|---|---|---|
| | No % | | No % |
| Artificial | 28 43.07 | Mineral | 65 59.63 |
| Breast feeding | 11 16.93 | Municipal | 5 4.58 |
| Without feeding | 26 40.00 | Tank | 39 35.77 |
| Total | 65 100 | Total | 109 100 |

The results showed that there is a strong link between where children's families live and how many of them have cryptosporidium. The infection was found at a rate of 34.84% in rural areas and 26.96% in urban areas. 0.05 or more Table No. 4. The similar relationship regarding other intestinal parasites occurrence revealed 12.12% in a rural area versus 10.91 % in an urban area.

**TABLE 4:** Patients' residency, and gender considering the frequency of Infectious agents.

| Rural area | Crypto +ve | Parasite | Crypto -ve |
|---|---|---|---|
| Males | 56 48.34 | 23 57.50 | 42 58.33 |
| Females | 59 51.66 | 17 42.50 | 30 41.67 |
| Total | 115 34.84 | 40 12.12 | 72 21.81 |
| Urban area | | | |
| Males | 48 53.93 | 27 75.00 | 31 57.40 |
| Females | 41 46.07 | 9 25.00 | 23 42.60 |
| Total | 89 26.96 | 36 10.91 | 54 16.37 |
| All total | 204 61.81 | 76 23.03 | 126 38.19 |

The connection between diarrhea and the appearance of instances of the cryptosporidium parasite was substantial, as this parasite recorded 62.62% of cases with diarrhea and 37.74% without ($P<0.05$). Although the percentages varied according to the age of the patients, the presence of diarrhea in cases positive for the Cryptosporidium parasite was significant for the age group less than one year, with the following percentages recorded: 38.58% for cases of diarrhea and 25.97% for cases of non-diarrhea. Table-5.

**TABLE 5:** Frequency of Cryptosporidium parvum according to diarrhea and non-diarrhea existence among Children concerning ages.

| Intestinal disorders | None diarrheic Cryptosporidium Positive | Diarrhea Cryptosporidium Positive | Total positive |
|---|---|---|---|
| Ages/years | No. % | No. % | |
| Below 1 year | 20 25.97 | 49 38.58 | 69 33.82 % |
| 1-2 | 24 31.16 | 30 23.62 | 54 26.47 |
| | | | |

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| | | | |
|---|---|---|---|
| 2-3 | 10 12.98 | 14 11.02 | 24 11.76 |
| 3-4 | 12 15.58 | 15 11.81 | 27 13.23 |
| 4-5 | 11 14.28 | 19 14.96 | 30 14.70 |
| Total | 77 37.74 | 127 62.26 | 204 100 |

Total stool samples positive for Cryptosporidium =204.

Those with a positive Cryptosporidium parasite test most often had a fever, which happened in 72.05 percent of cases. In females, the number was even higher, at 81.1 percent. Other common complaints included abdominal pain (62.25%), vomiting (13.23%), and lactose intolerance (the least common reaction to lactose was 5.39%). intestinal allergy by 3.43 percent. A total of 16 parasite-positive cases (15.38%) were found to exhibit none of the aforementioned symptoms. table No.6.

**TABLE 6:** Common symptoms among children below five years positive for Cryptosporidium.

| **Genders** | **Males** | **Females** | **Total** |
|---|---|---|---|
| Symptoms | No. % | No. % | No. % |
| Allergy | 3 2.88 | 4 4 | 7 3.43 |
| Lactose intolerance | 6 5.76 | 5 5 | 11 5.39 |
| Fever | 66 63.46 | 81 81 | 147 72.05 |
| Vomiting | 12 11.53 | 15 15 | 27 13.23 |
| Abdominal pain | 67 64.42 | 60 60 | 127 62.25 |
| Asymptomatic | 16 15.38 | 12 12 | 28 13.72 |

Cryptosporidium positive; males 104 and females100.

As for the pH of stool samples and the distribution of pathogens, it is evident from Table No. 7 that a pH of 7 to 8 was associated with Cryptosporidium parasites, whereas the lowest rates were recorded in the pH range of 6 to 7, which was 18.18% with wave models for other intestinal parasites ($P< 0.05$). The lowest positive Cryptosporidium percentage, 17.27%, was found in stool samples with a pH between 5 and 6.

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**TABLE 7:** Relationship between stool pH and microorganisms' distributions.

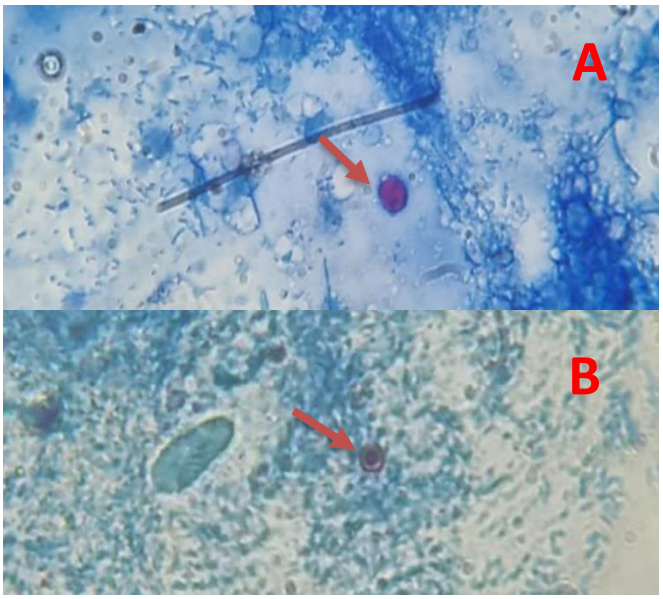
| Organisms | Cryptosporidium positive | Other intestinal Parasites |
|---|---|---|
| pH | No. % | No. % |
| 5-6 | 57 17.27 | 15 4.54 |
| 6-7 | 60 18.18 | 12 3.63 |
| 7-8 | 87 26.36 | 49 14.84 |
| Total | 204 61.81 | 76 23.03 |

The study also looked at how well different techniques and fluorescent stains work compared to the modified Ziehl-Neelsen technique to find Cryptosporidium-positive cases. With Calcofluor white, Phenol auramine, and Modified Gram-Chromotrop-2), the following percentages were found: 83.33%, 77.45%, and 61.67%. When compared to the modified Ziehl-Neelsen technique, the flotation technique (ZnSO2) and the double wet swab preparation technique (normal saline 0.9% and Lugol's iodine 5%) had the lowest positive percentages at 45.09% and 30.39%, respectively. Table 8.

**TABLE 8:** Efficacy of different laboratory techniques in Cryptosporidium detection microscopically.

| Genders | Males | Females | Total |
|---|---|---|---|
| Percentages | No. % | No. % | No. |
| Types of techniques | | | |
| Modified Ziehl-Neelsen | 104 50.98 | 100 49.02 | 204 100 |
| Calcofluor | 92 45.09 | 78 38.23 | 170 83.33 |
| Phenol-auramine | 87 42.64 | 71 34.80 | 158 77.45 |
| Chromotrop-2 Modified gram | 80 39.28 | 46 22.54 | 126 61.76 |
| Flotation 33% ZnSO4 | 52 25.49 | 40 19.60 | 92 45.09 |
| Direct wet double Preparations | 37 18.13 | 25 12.25 | 62 30.39 |
| Cassette | 27 13.23 | 12 5.88 | 39 19.11 |

The total number of stool samples=330.




Cryptosporidium oocysts: picture A by using the modified Ziehl-Neelsen technique. Picture B using modified gram stain-Chromotrope R2 technique.

## DISCUSSION

In children younger than five years old, diarrhea is the main cause of both morbidity and mortality. Intestinal parasites, such as Entamoeba histolytica, Giardia lamblia, and Cryptosporidium spp., are well-known etiological agents causing diarrhea in children, despite the fact that bacteria and viruses are the most common agents responsible for this condition. The rate of Cryptosporidium 61.81 % was very high compare to other studies carried on in the same province. This finding contradicted the findings of Karyaghdi [22,23,12, 14 and 24] in the province, which found rates of 10.8%, 20.6%, 6.45%, 16.2%, and 1.43%, respectively. In addition, compared to the current study, the rate in Basrah-Iraq was 16.76%, while the rates in Al-Najaf and Babylon governorates were 72.9% [25] and 87.57% [26]. Our findings are lower than those reported in Bangladesh and India, where 90% of cryptosporidiosis was recorded collectively among children under the age of two [27 and 28] and are also not agreed upon [29] in rural Western Kenya among children under the age of five. It is unclear how much of these disparities may be explained by

changes in how the study was designed, where it was conducted, the population studied, the lab methods used, or the stage of the disease. While Cryptosporidium is a monoxenic parasite, meaning that its life cycle is completed in a single host, this high infection rate might also have something to do with the way the parasite completes its life cycle. Also, the end product of schizogony in this protozoan gives rise to different kinds of infective stages, such as rough and smooth oocysts; the latter had a part in raising the rate of autoinfection in addition to giving rise to rough-type oocysts. According to our findings, children aged one year or younger to two years old had a much higher risk of being infected with Cryptosporidium than children aged three to five years old. Infants and children in the city of Xuzhou, China, were examined, and identical findings were discovered [30]. This could be due to the increased susceptibility of younger children as compared to the somewhat improved awareness of the importance of personal cleanliness among older children. Because the majority of their mothers are officers, the children in our study who were between the ages of one and two were still attending infant day care. The number of children in this age range who participated in the study was 235 out of a total of 330. In addition, it's possible that they don't get as much teaching about proper hygiene as students in primary schools do. Also, younger children may be more likely to get opportunistic illnesses because they are more likely to be around sources of infection that are contaminated. In a number of studies conducted in various countries [31,32 and 33], the correlation between a lower age and an increased risk of infection with Cryptosporidium was shown to be quite strong. Considering patient sexes, finding the negative relation with Cryptosporidium positivity, this might be attributed to high rate of infection among young aged children as they contributed high participation in the current study. Therefore, they were not active as children above 5 years. This finding was compatible with that recorded in China by [34]. No statistical difference was found between male and female children in terms of Cryptosporidium infection in our study. This is in agreement with observations in similar studies [35].

The fact that infants who are breastfed have passive immunity despite the fact that children who depend on artificial feeding have a much higher rate of Cryptosporidium infection than children who depend on breast feeding reflects the fact that infants who depend on artificial feeding have a much higher rate of Cryptosporidium infection than children who depend on breast feeding. Additionally, the possibility of more contamination in milk preparation played a role in increasing the rate of infection. Mineral water and tank (vehicle) water users are more likely to be contaminated than those who use municipal or piped water. This could be because poor quality filters are employed, which allow oocysts to slip through and cause infections, and because most sterile or mineral water is not certified by a governmental lab. Tank water may be contaminated because local government directors fail to inspect cars and disregard daily assessments of tank contamination our result was not agree that recorded in Taiz-Yemen by [36] who found high rate of Cryptosporidiosis among children drinking tank water. Finally, the water directory oversees the daily control of the majority of municipal water in checking water and minimizing the incidence of contamination by infectious agents, along with recent adjustments to or maintenance of water supply pipes to dwellings in the study district. There may be a much lower risk of infection from using public water supplies for drinking because of these factors [37].

Those living in rural areas were more susceptible to contracting Cryptosporidiosis (34.84%) than those living in urban areas (26.08%), which was thought to be due to the higher concentration of animal excrement in the soil in rural areas. In addition to using water from lakes, ponds, or tanks for drinking. Although there was no significant difference between males and females in either residency, the high percentages are indicative of pollution in Kirkuk Province, especially during the October and September 2022 rains.

A bad side effect of a Cryptosporidium infection is explosive, watery diarrhea, which affects 62.26 percent of people and 37.74 percent of people who don't have diarrhea. These results are better than what was found in Iran [38], where C. parvum was found in 25.6% of children with diarrhea and 3.7% of children without diarrhea) and Yemen [39], where C. parvum was found in 13.9% of children with diarrhea) . The use of multiple lab techniques in this study was what caused the differences.

The severity of the infection can be judged by the most common symptoms, which were fever (72.05% of cases), abdominal pain (62.25% of cases), and vomiting (13.23% of cases). Fever was the first sign of initiation of any inflammation, while abdominal pain is due to intestine irritation due to the diarrhea mechanism. Interestingly, in most cases, the stool samples were alkaline, suggesting that changes in the pH of the gastrointestinal tract could be the cause of the vomiting. This result was very similar to what was observed in Sweden by [40].The more common symptoms associated Cryptosporidium infections included fever 72.05%, abdominal pain 62.25% and vomiting 13.23 % refer to the severity of the infection , because fever was the first sign of initiation of the any inflammation, while abdominal pain is due to intestine irritation due to diarrhea mechanism.while vomiting might be due to changes in the pH of the gastrointestinal tract as most of the cases stool samples were alkaline. This finding was close to that recorded in Sweeden by [40].

Evaluation of 7 different laboratory techniques in demonstrating the oocysts of the Cryptosporidium parasite showing high sensitivity of staining techniques than flotation and double wet preparation in the current study had an additive value for parasitologist and benefaction to lab technicians. The employee of hot modified Ziehl-Neelsen technique mostly due to the affinity of strong carbol fuchsin binding to mycolic acid in the cell wall of the parasite. And disability of 5 % of H2 SO decolorizing solution for removing the formed red color gives rise high sensitivity to this technique. The significant advantages of calcofluor white were its great sensitivity, speed, and ease of use between four laboratory staining techniques and the stability of solutions. Calcofluor solution may be held in the laboratory for a long period with minimal fluorescence loss if the solution was kept in the dark. The third efficacy was for phenol-auramine stain use which exhibited 77.45% , this finding was lower than that recorded in India by [41] .The real reason for this high efficacy that rapid auramine uptake due to accelerated oocyst wall penetration caused by phenol Cryptosporidium oocysts stain clearly against a dark backdrop, making examination of smears straightforward using a 20X or 40X objective. Because of its reduced screening time (30 seconds vs. 7 minutes per smear) and the possibility of screening at low magnification (400), the Auramine-O stain is preferable to chromotrope R2 modified gram staining in the current study. The faintly red to brown color of the oocysts of Cryptosporidium using chromotrope R2 modified gram staining with the lowest rate among the other 3 used stains may be attributed to the lesser affinity of the oocyst to gram staining. The faintly red to brown color oocysts of Cryptosporidium using chromotrope R2 modified gram staining with the lowest rate among other 3 used stains may attribute to lesser affinity of the oocyst to gram staining [13]. Also, the lighter weight of the oocysts that float in this solution is due to the better stool concentration (flotation technique, 33% ZnSO4) on double wet preparations, even though the isolation process is long and hard[42].

## CONCLUSION

To summarize, Cryptosporidium spp. is a major disease among youngsters in Kirkuk, Iraq. Fresh water supply, education, dietary habits, and domestic animals such as cattle are the main causes of cryptosporidiosis transmission. Hence, parents should consider health education to safeguard their children from becoming infected with Cryptosporidium spp., particularly through contaminated water, as well as hand cleanliness after contact with domestic animals.

## CONFLICTS OF INTEREST

The authors of this paper have said that they do not have any competing interests with the work they are presenting.

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### *Author's Contribution*

Salman and Mohiemeed both contributed to the study's conception and planning. Asker obtained all 330 samples of blood and stool. Salman provided guidance and instruction for all laboratory processes, solutions, and stains. Asker conducted the laboratory analysis. Mohiemeed did the data analysis, and Salman and Asker wrote the paper. The manuscript was evaluated by Mohiemeed. The corresponding author has been notified that all authors who contributed to the study approve of the manuscript as submitted and any revisions that may be made in the future.

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