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Article

Cryptosporidium parvum as a Causative Agent of Diarrhea in Children: A Study in Al-Ramadi and Falluja

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Abstract: This study investigated the prevalence of *Cryptosporidium parvum* in children experiencing diarrhea in Al-Ramadi and Falluja cities. Over a four-month period, 100 stool samples from diarrheic children and 30 control samples from children without gastrointestinal infections were analyzed using a combination of direct smear and modified Acid-Fast staining methods. The results indicated that 28% of the diarrheic children were infected with *Cryptosporidium parvum*, while no oocysts were detected in the control group. The research further explored the incidence of infection in relation to various factors, including gender, water source, place of residence, type of feeding, and stool pH. It was found that infection rates were highest in children under one year of age and that males were more significantly affected than females. Additionally, the study revealed that children consuming filtered water had a higher incidence of cryptosporidiosis compared to those using municipal water. Rural children were more likely to be infected than their urban counterparts, and those on artificial feeding were at greater risk than breastfed infants. The presence of *Cryptosporidium parvum* was also associated with acidic stool pH levels. The importance of this research lies in its identification of specific risk factors and demographics associated with *Cryptosporidium parvum* infection in children, which can inform targeted public health interventions and improve the management of diarrheal diseases in the study areas.

Keywords: Cryptosporidium parvum; Children; Diarrhea; Epidemiology; Public Health

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1. Introduction

Cryptosporidium is a type of protozoan pathogen that belongs to the Phylum Apicomplexa. It causes a diarrheal illness known as cryptosporidiosis, which affects the mammalian intestinal tract [1]. In particular, *C. parvum* infection is a cause of concern in patients with weakened immune systems. This is because the condition can result in severe diarrhea, with the volume reaching as high as 10-15 liters per day [2].

Some of the apicomplexan pathogens include *Plasmodium*, which causes malaria, and *Toxoplasma*, which causes Toxoplasmosis [3]. Unlike Plasmodium, *Cryptosporidium* doesn't require a mosquito vector to transmit and can complete its life cycle within a single host. It results in microbial cyst stages that are excreted in feces and can be transmitted to a new host [4, 5]. However, synanthropic filth flies may also be involved in the transmission of human and animal cryptosporidiosis according to studies [6].

Cryptosporidiosis is a disease caused by a parasite found all over the world. It is responsible for 50.8% of water-borne diseases caused by parasites according to Gideon (2009). In developing countries, *Cryptosporidium* is responsible for 8-19% of diarrheal diseases. Oocysts are excreted by 10% of the population in developing countries, while in developed countries the number is lower at 1-3%. Children aged 1 to 9 years old are the most affected by this disease [7].

Infection can occur when a person or animal comes into contact with contaminated material such as soil, water, or uncooked or cross-contaminated food that has been in contact with the feces of an infected individual or animal [8]. The infected material must then be transferred to the mouth and swallowed. This type of infection is particularly common among those who regularly come into contact with bodies of fresh water, including swimming pools [9]. Other potential sources of infection include untreated water supplies, contaminated food, or exposure to feces [10].

C. parvum is considered to be the most important waterborne pathogen in developed countries. It is resistant to all practical levels of chlorination, surviving for 24 hours at 1000 mg/L of free chlorine. Some outbreaks have happened in daycare related to diaper changes [11, 12].

Cryptosporidium parvum has a complex life cycle consisting of asexual and sexual stages. After ingestion, oocysts release sporozoites that attach to the small intestine's epithelial cells. Trophozoites reproduce asexually, forming Type 1 meronts with 8 daughter cells [13]. Some daughter cells cause autoinfection, while others develop into Type II meronts with 4 merozoites. Merozoites attach to epithelial cells and become macrogamonts or microgamonts, the female and male sexual forms, respectively [14]. Zygotes form from the penetration of microgametes into macrogamonts, developing into two types of oocysts. Thin-walled oocysts can reinfect the host, while thick-walled oocysts are excreted into the environment and remain infective for months [15]. In immunocompromised individuals, such as AIDS patients, cryptosporidiosis resolves slowly or not at all and frequently causes a particularly severe and permanent form of watery diarrhea coupled with a greatly decreased ability to absorb key nutrients through the intestinal tract [14, 16]. The result is progressively severe dehydration, electrolyte imbalances, malnutrition, wasting, and eventual death [17]. Spiramycin can help treat diarrhea in patients who are in the early stages of AIDS[18]. The mortality rate for infected AIDS patients is generally based on CD4+ marker counts; patients with CD4+ counts over 180 cells/mm^3 generally recover with supportive hospital care and medication, but in patients with CD4+ counts below 50 cells/mm^3, the effects are usually fatal within three to six months [19, 20]. This research sought to determine the occurrence of Cryptosporidium parvum in children experiencing diarrhea in Ramadi and Fallujah city. Additionally, the study underscores the significance of parasitological examination for efficient disease management and control, particularly in relation to its connection with various other factors.

2. Materials and Methods

Study Design:

This research was conducted on a group of 100 patients suffering from diarrhea, with stool samples collected from each patient over a period from 15 November 2023 to 20 February 2024. The patients ranged in age from one month to six years and were seen at the Maternity and children teaching hospitals in Al-Ramadi and Fallujah cities. Additionally, stool samples were obtained from 30 healthy children without gastrointestinal infections to serve as a control group for this study.

To gather medical information, an interview was conducted with each patient's parent, and a questionnaire was completed. The data collected included the patient's age, gender, type of water source used, place of residence, type of feeding, and the pH level of the stool sample.

General Stool Examination:

Each stool sample underwent a visual examination, with the following characteristics recorded: its consistency (formed, unformed, or liquid) and its color (white, yellow, brown, or black). The presence of any abnormal components, such as mucus or blood, was also noted.

The examination procedure was as follows: A small piece of the stool specimen was placed in a drop of normal saline on a clean slide and thoroughly mixed. A coverslip was then placed on the saline-specimen mixture, taking care to avoid the formation of air bubbles. After waiting for 2 to 3 minutes, the slide was examined under a microscope using the high-power objective (40x magnification). The presence of pus cells, red blood cells (RBCs), and parasites was recorded according to the method described by [21].

Modified acid-fast stain:

Reagent Preparation: As per the method described by [22], with certain modifications, the Carbolfuchsin reagent was prepared as follows:

CARBOLFUCHSIN:

Basic Fuchsin: 4 g; Phenol: 8 ml; Alcohol (95%): 20 ml; Distilled Water: 100 ml

The basic fuchsin was first dissolved in alcohol, and then distilled water was gradually added while the solution was shaken to ensure thorough mixing. Phenol was melted in a water bath set to 56°C, and 8 ml of the melted phenol was carefully added to the stain using a pipette equipped with a rubber bulb.

DECOLORIZER:

Slowly add 3 ml of concentrated HCL to 97 ml of ethanol (95%) while working under a chemical fume hood.

COUNTER STAIN (Option 1):

Dissolve 0.3 g of methylene blue in 100 ml of distilled water.

COUNTER STAIN (Option 2):

1. Dissolve 0.4 g of malachite green in 100 ml of deionized water.
2. Mix the solution on a magnetic stirrer.
3. Filter the solution through Whatman No.1 filter paper into a stock reagent bottle [23].

i. The smear was fixed by heating at 70 C for 10 minutes.
ii. The fixed smear was stained for 3 - 5 minutes (no heat necessary).
iii. Wash in distilled water and shake off excess water.
iv. Float with decolorizer for approximately 1 minute. Checked to see that no redder color runs when the slide is tipped. Add a bit more decolorizer for very thick slides or those that continue to bleed red dye.
v. Wash thoroughly with filtered water as above and shake off excess.
vi. Float with counter stain for approximately 1 minute.
vii. Wash with distilled water and drain by standing slides upright. Do not blot dry [22].

Statistical analysis:

All data were analyzed using Chi-square (Cross tabulation) test by SPSS statistical program version 18 [24]. All the study graphics (bar chart, bi chart) were done by using Microsoft Office Excel 2007.

3. Results

During the study period,100 children with diarrhea had been involved in this study, those age range from (1 month - 6years), the Oocysts stain bright red against a background of blue or green fecal debris and yeasts depend on type of counter stain as shown in (figure 1 A, B).

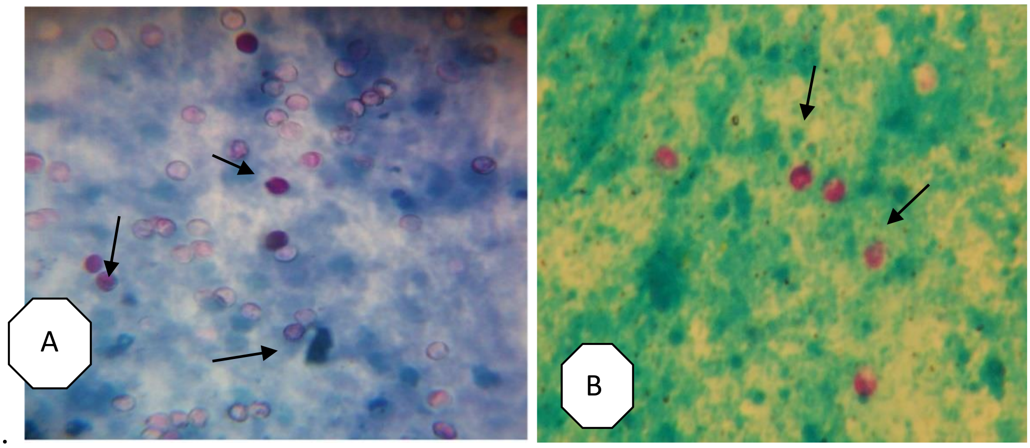

Figure 1. *Cryptosporidium parvum* (counter stain: A: methylene blue, B: malachite green 100x)

Incidence of Cryptosporidiosis concerning age:

The total children involved in this study were divided into six age groups (Figure 3.2). Cryptosporidiosis was detected in (28) patients, among children < 1 year of age group the patients were (n=19,67.8% from infected patients), in the second group was (n=6, 21.4 %), and in the third group (n=3,10.7%) from infected patients. then it began to decrease to (0.00%) in other groups, while in control groups there was no infection recorded in all age groups, this result was significant (P. value is 0.001, Chi ^{2}=54.1), as shown in (Figure 2).

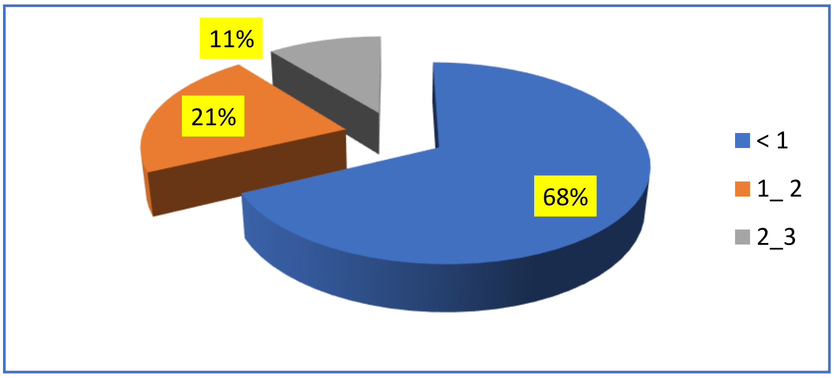

Figure 2. Distribution of Cryptosporidiosis among age groups

Frequency of Cryptosporidiosis according to gender:

Males showed a higher infection rate 53.6 % than females 46.4% in patient groups, this result was significant (P. value< 0.05 is 5.99, Chi ^{2}=0.884), as shown in (Table 1).

Table 1. Cryptosporidiosis according to gender and age groups in patients

Distribution of ages (Years)	Female No. (%)	Male No. (%)	Total No. (%)
< 1	9 (32.1%)	10 (35.8 %)	19 (67.9 %)
1 _ 2	3 (10.7 %)	3 (10.7 %)	6 (21.4%)
2 _ 3	1 (3.6 %)	2 (7.1 %)	3 (10.7%)
Total No. (%)	13 (46.4%)	15 (53.6%)	28 (100%)

Frequency of Cryptosporidiosis concerning water sources:

The infection was higher among patients who consumed filtration systems water (60.7 %) than those who consumed municipal tap water (39.3 %) this result was not significant (P. value >0.05, Chi ^{2}=0.90), (Table 2).

Table 2. Cryptosporidiosis in relation to water sources and age groups in patients

Distribution of ages (Years)	Municipal tap water No.	Filtration system No. (%)	Total No. (%)
< 1	8 (28.6 %)	11 (39.3 %)	19 (67.9%)
1 _ 2	2 (7.1 %)	4 (14.3%)	6 (21.4%)
2 _ 3	1 (3.6 %)	2 (7.1 %)	3 (10.7%)
Total No.	11 (39.3 %)	17 (60.7 %)	28 (100%)

Incidence of Cryptosporidiosis in relation to residence:

The higher infection rate was among those in rural (57.1%) than those who reside in urban areas (42.9%) of patients, this result was significant (P. value is 0.001, Chi ^{2}=0.28) as shown in (Table 3).

Table 3. Cryptosporidiosis in relation to residence in patients and control groups

Distribution of ages (Years)	Urban No. (%)	Rural No. (%)	Total No. (%)
< 1	9 (32.2%)	10 (35.7%)	19 (67.9 %)
1_ 2	3 (10.7 %)	3 (10.7 %)	6 (21.4%)
2 _ 3	0 (0.0 %)	3 (10.7 %)	3 (10.7%)
Total No. (%)	12 (42.9%)	16 (57.1%)	28 (100%)

The effect of feeding type on *Cryptosporidium* infection:

This study revealed that 25% of patients who were breastfed their stools were positive for *Cryptosporidium* Oocysts, while the positive results regarding stools of patients who were artificially fed were 75%, this result was significant (P. value is 0.001, Chi ^{2}=0.43) as shown in (Table 4).

Table 4. Cryptosporidiosis according to type of feeding in patients and control

Distribution of ages (Years)	Breastfeeding No. (%)	Artificial No. (%)	Total No. (%)
< 1	6 (21.5%)	13 (46.4%)	19 (67.9 %)
1 _ 2	1 (3.5 %)	5 (17.9%)	6 (21.4%)
2 _ 3	0 (0.0 %)	3 (10.7 %)	3 (10.7%)
Total No.(%)	7 (25 %)	21 (75 %)	28 (100%)

The effect of pH of stool on Cryptosporidiosis:

This study revealed that *Cryptosporidium* Oocysts mostly detected in patients whose stools pH were acidic i.e. below 6.1 but the parasite could not be detected in stools with pH higher than (6.5).

4. Discussion

Among 100 stool samples collected from studied patients, Oocysts were detected in (28%). This result was almost higher than the 2% reported by [25] in Al-Ramadi city, 14.3% reported by [26], in Baghdad recorded 14.6% [27]. [28] reported 4% in Salah Aldeen, and lower than 20.52% reported by [29] in children in Some Regions in Nineveh Governorate.

While in other countries such as Wales (UK) reported 2% [30], 2.8% in immunocompetent patients of Massachusetts (U.S.A) [31] and 5% in an urban community of Bristol (England) [32], and the reason for this high result may be attributed to the deterioration in the standards of general hygiene and the treatment of municipal water was not efficient and the efficacy of various water filtration systems was not good. The high infection rate of Cryptosporidiosis, caused by the parasite Cryptosporidium, can be attributed to several interrelated factors. Firstly, the parasite's robust oocysts are highly resistant to environmental stressors, allowing them to survive in water sources and contribute to widespread transmission [33]. Additionally, studies indicate that certain populations, particularly immunocompromised individuals, exhibit a significantly higher susceptibility to infection due to their weakened immune responses [34]. Moreover, the prevalence of Cryptosporidium in contaminated water supplies, often linked to agricultural runoff and inadequate sanitation, exacerbates the risk of outbreaks [35]. Research also highlights the role of zoonotic transmission, where animals serve as reservoirs, further complicating control measures [36]. Lastly, the lack of effective treatment options and the parasite's ability to rapidly reproduce in the host contribute to the persistence of high infection rates [37]. Collectively, these factors underscore the challenges in managing Cryptosporidiosis outbreaks.

The highest rate of Cryptosporidiosis was found among children < 1 year of age group the patients were (n=19,67.8% from infected patients), in the second group was (n=6, 21.4%), and in the third group (n=3,10.7%) from infected patients. then it began to decrease to (0.00%) in other groups This result was in agreement with many other studies. Cryptosporidiosis, caused by the Cryptosporidium parasite, predominantly affects children under three years due to several factors. Young children have immature immune systems, making them more susceptible to infections. The transmission of Cryptosporidium often occurs through contaminated water sources, which are frequently accessed by children during play or bathing, increasing their exposure risk [38]. Additionally, the parasite's resilience in harsh environments allows it to persist in water supplies, further contributing to outbreaks in pediatric populations [26]. Research indicates that malnutrition and co-infections with other pathogens can exacerbate the severity of Cryptosporidiosis in young children, leading to higher morbidity rates [39]. Research indicates that males may have a higher susceptibility due to differences in immune response, with some studies suggesting that hormonal variations, particularly testosterone, can influence immune function and increase vulnerability to infections like Cryptosporidiosis [40].

The higher incidence of Cryptosporidiosis among patients consuming water from filtration systems compared to those using municipal tap water can be attributed to several factors. Filtration systems, particularly in rural areas, may not effectively eliminate Cryptosporidium oocysts, leading to increased exposure among users. In contrast, municipal water treatment processes typically include multiple barriers, such as chlorination and filtration, which are more effective at removing pathogens, including Cryptosporidium [40, 41]. Additionally, rural areas often face challenges such as inadequate infrastructure and less stringent water quality regulations, which can exacerbate the risk of contamination in filtration systems [42].Urban areas, benefiting from more robust public health measures and regular monitoring, tend to have lower incidence rates [42]. Therefore, the combination of less effective filtration methods and environmental factors in rural settings contributes to the observed disparities in Cryptosporidiosis incidence rates between these populations.

The relatively higher positive results for Cryptosporidiosis among patients who were artificially fed (75%) compared to those who were breastfed (25%) and this result was in agreement with [43] the explanation of this may be due to the fact that artificially prepared food was more exposed to contamination and the pH of stools of those patients were almost lesser than those who were breastfed. Regarding the pH of stool samples, it was found that the majority of positive cases were in stool samples with pH < 6.1 which means that the parasite prefers the acidic pH This is probably due to some physiological and enzymatic characteristics in the parasite. However, this character can be studied in the future to find out its role in the control of infection. No literature is available to compare our results.

5. Conclusion

Study found a high rate of Cryptosporidiosis, with oocysts detected in 28% of stool samples, which is higher than rates reported in other studies from the same region and other countries. The high infection rate is attributed to factors such as the resilience of Cryptosporidium oocytes, inadequate water treatment, and the susceptibility of immunocompromised individuals. The highest infection rates were observed in children under one year of age, which aligns with findings that young children are more susceptible to Cryptosporidiosis due to their immature immune systems and increased exposure to contaminated water. Males may have a higher susceptibility to Cryptosporidiosis due to differences in immune response, potentially influenced by hormonal variations. Patients consuming water from filtration systems, especially in rural areas, had a higher incidence of Cryptosporidiosis compared to those using municipal tap water, likely due to less effective filtration methods and environmental challenges.

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