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Cryptosporidiosis among pre-school children in Duhok city, Kurdistan Region, Iraq

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Abstract

Background and objective: The most prominent intestinal parasite causing watery diarrhea globally is cryptosporidiosis. The objectives of the study were directed to examine the frequency of *Cryptosporidium* spp. among pre-schoolchildren of age groups (≤5) years presenting with and without diarrhea, and determine associated risk factors related to this infection in children attending different daycare centers and kindergartens.
Methods: This cross-sectional study was conducted to find out the prevalence of cryptosporidiosis among preschool children. Participants examined from October 2022 to ending of February 2023 in the city of Duhok. Overall, (160) stool specimens were collected from children who attended different daycare centers and kindergartens in Duhok, all stool samples were examined microscopically by direct wet mount and modified Ziehl-Neelsen stain methods to detect the oocyst of *Cryptosporidium* spp.
Results: The infection rate of cryptosporidiosis among the total infected stool children was (11.25%). The parasite infection showed a significant difference between boys and girls at P <0.05, the highest infection rate was found in age group of (3-4) years old (25%), followed by age group (5) years old (10.64%). The Modified Zihel-Neelsen stain test (75%), performed more accurate than the direct wet mount method (25%).
Conclusion: The study showed Cryptosporidiosis were common health problems in various day care centers of Duhok city and active prevention programs must be considered for the improvement of cleanliness and health education.
Keywords: *Cryptosporidium* spp.; Children; Zihel-Neelsen Stain; Duhok; Iraq.

Introduction

The coccidian parasite *Cryptosporidium*, which infects many vertebrate species, including humans, is the cause of the global illness known as cryptosporidiosis. This prevalent coccidian parasite forms thick-walled oocysts that enter the host's intestinal tract through contaminated food or water.(1) Cryptosporidiosis is the parasite responsible for the majority of watery diarrhea cases worldwide and the sixth most significant food-borne parasite.(2) It is the second most common cause of diarrhea and pediatric accidental deaths, after rotaviruse.(3) According to epidemiologic studies, the overwhelming majority of human diseases caused by members of this genus are caused by the species *C. hominis* and *C. parvum*.(4) Such studies make the assumption that *C. hominis* can only spread between humans, but domestic livestock, particularly cattle, are the main reservoirs for *C. parvum*. Humans may acquire the parasite directly from infected cattle or indirectly by drinking contaminated water.(5) In most healthy individuals, symptoms resolve on their own. Dehydration and diarrhea can be more serious and even fatal in people with compromised immune systems.(6)

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Cryptosporidiosis typically identified by microscopic examination of stool samples for the presence of parasite oocysts, oocyst antigens, or oocyst DNA. When using acid-fast staining techniques on unconcentrated fecal smears, it is simple to see the *Cryptosporidium* oocysts.[7] Studies on cryptosporidiosis have generally reported varying rates of infection with regard to the disease's prevalence in Iraq and the Kurdistan Region.14% in Erbil,[8] 47.3% among children in Baghdad,[9] and 58% among diarrheal patients in Al-Najaf. [10] However, there is little data on the frequency of cryptosporidiosis in children in Duhok city, Kurdistan Region, particularly pre-school children. The study's objective was to determine the prevalence of *Cryptosporidium* spp. in preschoolers of both sexes and in age groups (≤5) years presenting with and without diarrhea, and determine associated risk factors related to these parasitic infections in children attending different daycare centers and kindergartens using a modified Ziehl-neelsen staining technique.

Methods

The Study Design and the Period of Study:

A cross-sectional study was conducted on 160 fresh fecal samples taken from the pre-school children of different governmental kindergartens and daycare center in the urban area of Duhok city, from October 2022 to the end of February 2023. Duhok city lies in the western part of Kurdistan Region, about 430-450 m above the sea level. The city's climate, with hot, dry summers and moderate, wet winters, is the same as that of the surrounding area. The study included all children for whom a consultation was obtained. Those who complied with the inclusion criteria and gave their consent to the study were immediately enrolled and chosen using a successive sampling process.

Data Collection

A questionnaires sheet was directed for the study in order to gather sociodemographic variables that consisted of: gender, age, residency, sources of drinking water, and other information. The stool specimens that were gathered were sent regularly to the Medical Laboratory Sciences Department's Parasitology Laboratory at Duhok University for microscopic and macroscopic analysis.

Stool Specimen Collection

Parents/guardians of children were given labeled stool containers to collect fresh stool sample. All of the fresh feces' samples were taken and stored frozen on a completely labeled cold pack at the lab in sterile, screw-capped sampling containers with clear labels. Parasitology laboratory for examinations at the end of each working day. The labels of the containers were also checked and matched to their corresponding questionnaires with child's data after taking ethical consideration permission from daycare centers administrators and verbal permission from parents of children.

Sample Analysis

In the laboratory, all fresh fecal samples were analyzed macroscopically by naked eye to determine stool consistency (formed, semi-formed, liquid, and watery), odor, and the presence of blood, or mucus. In addition to the microscopic examination was done by direct wet mount method and the gold standard Modified Acid-Fast stain used for detecting of oocyst of *Cryptosporidium* spp. parasite.

Modified Ziehl-nelseen Staining Method

In the child's fecal sample, the staining procedure was suitable for identifying the intestinal coccidian *Cryptosporidium* spp. Before fecal smears were made on microscope slides using wooden stick applicators, stool samples were removed from the lab refrigerator and allowed to come to room temperature. The smears were then allowed to air dry on racks. Slides were placed in staining racks and fixed for three minutes in absolute methanol, then for five minutes in a strong carbolfuchsin stain. After that, tap water was used to rinse the slides. After adding

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1% hydrochloric acid alcohol for 15 seconds and rinsing with tap water, decolonization was achieved. After one minute, a 1% methylene blue counterstain was added, thoroughly rinsed, and allowed to air dry. The stained slides were examined microscopically using 100x objectives with oil immersion and the presence or absence of oocysts was reported. In the current method, the *Cryptosporidium* spp. oocysts were visualized as bright red round bodies against a blue background.[11] Cryptosporidiosis infection was recorded either positive or negative based on the presence or absence of the oocysts in each stool sample's examined microscopic fields.

Statistical Analysis

Analysis of data was carried out using Package for Social Science (SPSS) software version 23.Chi square association test used for categorical variable. *P* value ≤ 0.05 was considered significant for all statistical analyses.

Ethics Approval

Scientific and ethical approval for the study was approved by the Scientific Committee of the College of Health Sciences/Duhok University. Also, clearance from the General Directorate of Education in Duhok (approval no. 531 on November 3rd, 2022) was also attained before the collection of samples from selected several kindergartens and daycare centers by receiving a formal request from the College of Health Sciences, the children's parents and/or guardians were contacted to outline the aim the study to them.

Results

In this study, a total of 160 stool specimens were collected and examined from age groups (≤5) years of both genders. A percentage of (11.25%) specimens were detected with the infected stool, and (88.75%) specimen was recognized with non-infected stool. Overall, the frequency of infection among the total examined specimens of the children's stool showed in Figure 1.

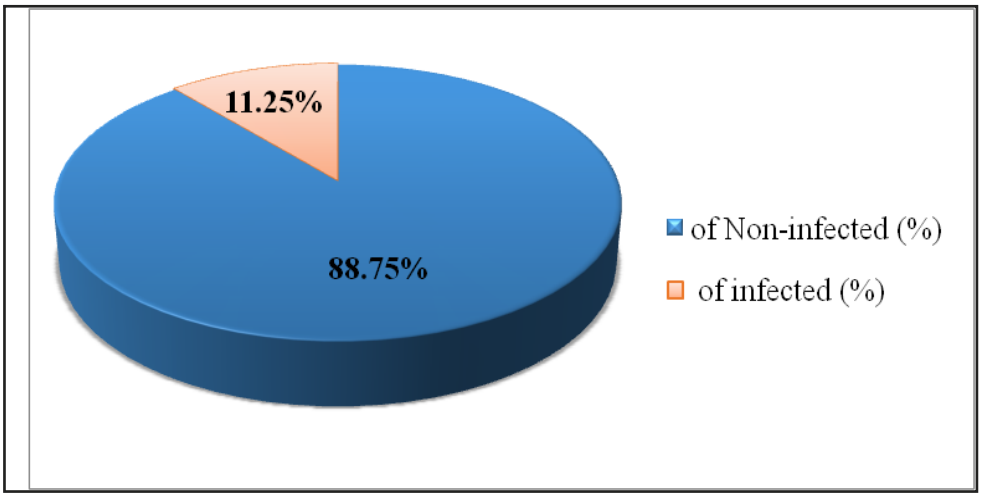

Figure 1 Overall infection of Cryptosporidiosis among the examined stool speciemens

https://doi.org/10.15218/zjms.2025.048

Socio-Demographic Variables

According to socio-demographic factors, the distributions of *Cryptosporidium* infection were detected; according to gender, age group, and residency the prevalence of infections were shown in Table 1. Males had a higher infection rate (16.05%) than females did (6.33%), between boys and girls, there was a significant difference at level ≤0.05. Children who attended east of the city centers had a higher rate of infection (12.26%) than those who attended west of the city centers (9.26%). The *P*-value was 0.6094, not significant at level ≤0.05. Children aged between (3-4) years had the highest infection rate (25%), followed by those aged (5) years (10.64%).

The rate of infection was higher in boys than in girls in age group (5) years old (13.58%). As shown in Table 2, different age-groups together with gender factors.

Table 1 Distribution of Cryptosporidiosis among the total examined pre-school children according to (Socio-demographic Variables) in Duhok city

Socio-demographic data		N = of examined stool	+ve No.(%) of Stool	Chi-square Test	*P*-value ≤0.05
Gender	Boys	81	13 (16.05)	3.78467	*P* = 0.0517
	Girls	79	5 (6.33)		
Age-groups	(< one year)	2	0	2.443	*P* = 0.1181
	(1-2) years	5	0		
	(3-4) years	12	3 (25)		
	(5) years	141	15 (10.64)		
Residency	East of City	106	13 (12.26)	0.261	*P* = 0.6094
	West of City	54	5 (9.26)		
	Total	160	18 (11.25)		

Table 2 The percentage of Cryptosporidiosis concerning (Age groups) and (Gender)

Age groups	No. of Examined Stool		No. (%) of Infected Sample	
	Boy	Girl	Boy	Girl
(< one year)	1	1	0	0
(1-2) Years	4	1	0	0
(3-4) Years	8	4	2 (2.47)	1 (1.27)
(5) Years	68	73	11 (13.58)	4 (5.06)
Total	81	79	13 (8.125)	5 (3.125)

https://doi.org/10.15218/zjms.2025.048

The infection distribution by water source was as follows: groups that used both bottled and tape water had the highest infection rate (14.49%), while groups that used tap water had the lowest infection rate (8.33%), followed by the group that used bottled water (10.53%).

Clinical Presentations Associated with Cryptosporidiosis

There was a highly significant difference among groups at *P* <0.01. The highest infection rate was detected from diarrheic children (29.41%), whereas the lowest rate of infection (9.09%) was detected from Non-diarrheic samples.

Diagnostic Methods

The diagnostic methods used to examine the infected stool and detect oocyst of parasite are shown in table (5). Once examined by wet mount method (3.75%) cases were infected and when analyzed by modified acid-fast stain method was (11.25%).

Table 3 Distribution of Cryptosporidiosis among the total examined Pre-School Children according to (Water Supply of drinking) in Duhok city

Water Supply of drinking	N = of Examined Stool	+ve No. (%) of Stool	Chi-square Test	*P*-value ≤0.05
Tap water	72	6 (8.33)	1.074	*P* = 0.3000
Bottled water	19	2 (10.53)		
Both type of water	69	10 (14.49)		
Total	160	18 (11.25)		

Table 4 Distribution of Cryptosporidiosis among the Total Examined Pre-School Children according to (Clinical Presentations) in Duhok city

Clinical Presentation	N= of Examined Stool	+ve No. (%) of Stool	Chi-square Test	*P*-value ≤0.05
Non-Diarrheic	143	(9.09) 13	6.28388	P=0.0121
Diarrheic	17	(29.41) 5		
Total	160	(11.25) 18		

Table 5 Distribution of Cryptosporidiosis among the total examined Pre-School children according to (Diagnostic Methods) in Duhok city (N:18):

Diagnostic Methods	No. of Infected Stool	(%) of Infected Stool
Wet-mount	6	3.75
Modified ZN Stain	18	11.25

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Discussion

The study was addressing the incidence of cryptosporidiosis oocysts employing modified Ziehl-neelsen stain and their association with certain risk variables in Duhok City's preschool children. The rate of infection in this study was (11.25%) and it is nearly accordance with previous research carried out around Iraq, including in Basra Province (23.8%) by Salim,[12] and in Kirkuk city (22.68%) by Salman et al.[13] While, infection rates were stated in studies carried out in Baghdad province (47.33%) by Merdaw et al.[9] Differences in sample sizes, as well as cultural and geographic variations, could also contribute to the fluctuation in the infection rate focusing on different age groups, in addition to several diagnostic methods. The findings indicated a greater infection risk among boys in contrast to girls, and there was a significant difference (P <0.05) between the males and females. This was in line with a study done in Al-Najaf city by Salim and Al-Aboody,[14] reported a higher rate among boys as compared to girls. Boys may have a higher infection rate than girls for a variety of reasons, including the fact that they are more socially active than girls and that their outdoor activities bring them into close proximity to the contaminated play area, soil, and animals which increases the risk of parasite transmission. While, in Erbil City, Khoshnaw et al.[8] Reported a higher rate among girls as compared to boys. The current investigation showed age groups (5 years) recorded the higher rate of the infection, while the lowest rate of infection was among children from (<1 one year). These results were in agreed with a study conducted in Buner District, Pakistan, reported higher rates of infection among children less than 6 years by Khan et al.[15] The exact cause of this higher level is unknown, it could be due to immuno-physiological and immune response, as well as may be due to the fact that children were more exposed to the infective stage of the parasite by swallowing water and less awareness. Moreover, may be infected children of age groups had more close contact with domestic animals, as the oocyte of the Cryptosporidium released form infected animals and transmitted to the children.[16]

The study showed the sources of drinking water and the rate of *Cryptosporidium* infection, as the infectivity rate was high among children who drink both types of water (14.49%) than those drinking bottled water and municipal tap water at home (8.33%). These were in agreed with a study conducted in Benisuef in Egypt, by Abdel Gawad et al[17] who stated that tap water is associated with the higher rate of infection among people compared to mineral water gallon (55% vs. 12%). This could be oocysts survive chlorine treatment in water for months and can resist for 180 days in water and up to a year at 4 °C.[18] While, agreed with the study conducted in Wasit Province, Iraq by Alkhanaq and Thamer,[19] who reported a higher rate among people drinking bottled water than those drinking tap water.

Regarding residency, the rate of cryptosporidiosis was higher among children of east Duhok city kindergartens (12.26%) than west ones (9.26%). This may be due to the fact that resident's environment is thought to be favorable for intestinal parasite transmission, may be due to a lack of proper sanitary facilities and limited access to safe water.[20]

The study showed significant relationship between the consistency of stool and frequency of *Cryptosporidium* spp. while, higher percentage of infection was reported among diarrheic stool specimens. These results were in agreement with a study conducted by Koyee and Faraj,[21] reported the rates of infection were higher among diarrheal stool than formed stool. Also, a study conducted in Cameron reported higher rates of infection in diarrheic stools than non-diarrheic specimens by Tombang et al.[3] This could be due to the fact this parasite infects mostly immunocompromised individuals.

https://doi.org/10.15218/zjms.2025.048

Conclusion

A number of participants tested positive for Cryptosporidiosis. The study revealed children of various ages of both genders were susceptible to infection in different educational day care centers; the infection was prevalent under the age group of five years old. The Modified-Ziehel-neelsen Stain method was appeared more effective for detection of *Cryptosporidium* spp. In different educational day care centers. Consequently, active prevention programs have to be considered for the improvement of cleanliness and health education.

Competing interests

The authors declare that they have no competing interests.

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