

Epidemiological Study for Cryptosporidium sp in Some Areas of Dohuk Province/Iraq

دراسة في وبائية طفيلي البويغات الخبيثة
Cryptosporidium sp
في بعض مناطق محافظة دهوك/العراق



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Abstract

This study included the detection of the prevalence of cryptosporidiosis in children in Some Areas of Dohuk Province and referred children to Bardarash 's, Khazer's health centers and in adults referred to Azadi hospital in different ages whose suffer gastroenteritis. method of staining with Modified Zeil- Neelsen stain was used to detect cryptosporidiosis and using Malachite green oxalate and Methylene Blue as a differential stain.

The infection rates of 77 stool samples were compared whence (sex, age, stool sample type and research period). the results showed the total infection rates with cryptosporidiosis in tested samples was (29.87%) for both sexes. It was recorded (29.72%) of infection among males children and (30%) was recorded among females children, the results didn't show a significant correlation between gender and infectivity rate ($p \leq 0.05$). The infection is limited only to children in ages (3 months-7 years) for both sexes Where the infection rate for children between (3 months-2 years) was 42.42% for both sexes and in proportion 39.13% for children in (3 years-7 years) old for both sexes with significant correlation ($p \leq 0.05$), where one infection recorded between females in 7 years old in rate 2.5% Where the number of infections is decreasing whenever a person age increased, Therefore, no parasitic infection was recorded in children and adults who were of ages (8 years-33 years). The results revealed significant correlation ($p \leq 0.05$) between fecal status and infectivity rate, where infection rate in diarrhea samples was 45.45% while the rate was 18.18% in samples of normal stool. The results also showed an increase in incidence rate of injury in the months (May and June) and the proportion was 45.45%- 50% respectively. While the lowest rate of the injury was (18.18%) for both of the months (January and February) and the results didn't show a significant correlation between study period and infectivity rate ($p \leq 0.05$).

Introduction

The types of *Cryptosporidium* sp. are protozoan parasites that belong to the Apicomplexa, which are generally found in global water(1). These parasites cause a disease known as cryptosporidiosis, which causes acute watery diarrhea or steatorrhea with colic(2,3), Accompanied by nausea, abdominal pain, loss of appetite and sometimes mild fever. Often, the disease will end automatically except in people

with an immune deficiency as AIDS patients, causing severe cholera-like intestinal colic (4,5). These parasites infect the gastrointestinal tract of the vertebrates Including birds, fish, mammals, and reptiles, where the oocysts of these parasites are resistant to environmental conditions (6,7). Most infections of this parasite occur among humans (Anthroponotic), with parasitic infection by the zoonotic animal. The respiratory tract infection in this parasite was recorded in many vertebrates (8,9,10).

In view of the increasing infections of *Cryptosporidium* in human and animal, researchers have been pushed to study this parasite, including studies on the epidemiology of cryptosporidiosis in humans and animals (12,11), studies on the treatment of cryptosporidiosis in laboratory mice (14,13) and studies on spread the oocysts of the parasite in some water sources (16,15). Scientific research has been showing the rareness of studies on *Cryptosporidium* parasite in the province of Dohuk, as well as the absence of epidemiological study in its suburbs especially in the district of Bardrash on stool samples, so this manuscript is new addition to the studies of researchers in the country and so the aim of this manuscript is to study the epidemiology of *Cryptosporidium* sp and investigation of oocysts of the parasite in some areas of Dahuk, especially the district of Bardrash.

Materials and Methods

samples collection:

In the period from the beginning of January 2016 until the end of July 2016 a total of 77 stool samples were collected from children aged 3 months and 12 years and adults aged 18 to 33 years, and from both males and females suffering from gastroenteritis, diarrhea and those attending Azadi Teaching Hospital Duhok and the health centers in Bardrash district that located in the southeast of Dohuk province and are characterized as a small town surrounded

by villages and areas whose population relies on livestock, agriculture and the use of well water in watering and spend their daily drinking and washing needs. Parents were given clean, numbered plastic cans to collect samples indicating the age and sex of each child or person infected and the date of collection of the sample, the samples were stored in a Potassium dichromate solution at a concentration of 2.5% (prepared by dissolving 25 g of potassium dichromate in a liter of distilled water).

For the purpose of detecting parasite oocysts, the sample of each feces sample was examined by preparing smears stained by Modified Zeihl Neelsen Stain (ZN STAIN KIT) according to (13) using two differential stains for each prepared smear, these stains are (Methylene Blue and Malachite green oxalate).

The method of preparing the slides and dyeing them with the Zeihl Neelsen Stain:

Each fecal sample was examined by preparing smear stained by Nielsen stain, after washing it with distilled water to get rid of preservative (potassium dichromate). Amount of feces was taken by wooden sticks as much as the head of a matchstick and sprayed on two clean glass slides and left to dry on the air without using the flame, then the smear was fixed with absolute alcohol for 5 minutes and left to dry. The two slices were then dyed with red carbol fuchsin for 15 minutes and the two slices were washed with tap water and sulfuric acid was added at 2% concentration for 20 seconds. The two slices were washed again with tap water and one of the slices was stained with Malachite Green for five minutes (prepared by melting 5 grams of dye in 100 ml of distilled water). The other slice of the same sample was dyed with Methylene Blue for five minutes and then washed with tap water and then left to dry and examined under the microscope with a magnification force of X40 and then under the oily lens with a magnification force of X100.

Results

There was no difference between slides that were stained by green malachite stain and by methylene blue stain. The oocysts appeared in spherical or oval form, dyed red or pink and surrounded by a distinct translucent halo (figure1,2).

Fig. 1: *cryptosporidium* oocysts in human faeces smear stained with Zeihl Neelsen, and green malachite stain as a differentiation stain. under magnification force X 1000.

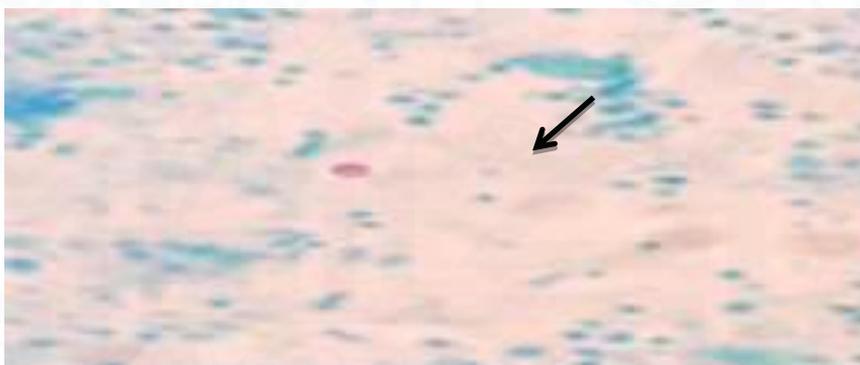
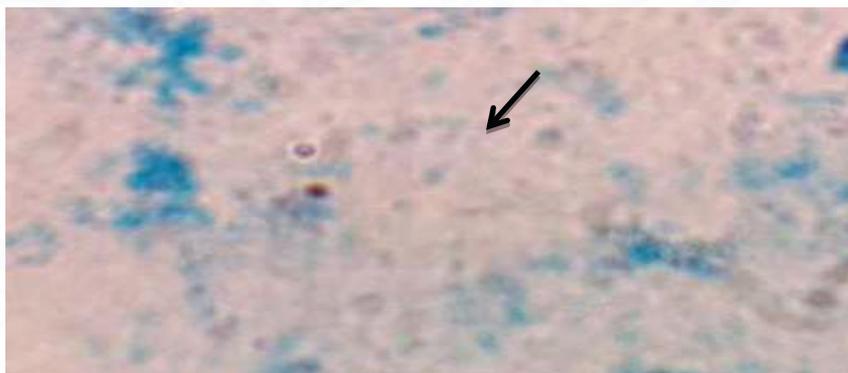


Fig. 2: *cryptosporidium* oocysts in human faeces smear stained with Zeihl Neelsen, and methylene blue stain as a differentiation stain. under magnification force X 1000.



1- Prevalence of *Cryptosporidium* and its correlation to the sex of the infected person:

The results showed the infected samples by *Cryptosporidium* oocyst was 23 samples (the percentage

was 29.87%) in both sexes and the percentage of infection was very similar among males and females, whereas the percentage of infection in males was 29.72%, while in females was 30% with a significant difference ($P < 0.05$) as shown in Table (1).

2- Prevalence of *Cryptosporidium* and its correlation to the age of the infected person:

No infections were recorded in the center of Dohuk city, where samples were collected from patients who were hospitalized at Azadi Teaching Hospital, who were over 10 years old and of both sexes. In the district of Bardrash and its suburbs, no injuries were recorded between children and persons over 7 years of age and the infection was confined among children between (3months-7years) of age for both sexes, where the percentage of infection was (42.42%) between the ages (3months-2years) while the percentage of the infection was (39.13%) for children aged (3 years - 7 years) and for both sexes and no significant difference was recorded. One infection among females aged 7 years was recorded and the percentage of the infection was (2.5%). The number of infections decreased with increasing age, so no parasitic infection was recorded in children and adults aged between (8 years - 33 years), as shown in Table (2).

3- Prevalence of *Cryptosporidium* and its correlation to the stool state of the infected person:

The results indicated in Table (3) showed an increase in the percentage of infection in diarrheal samples compared with the samples of normal feces where the percentage of infection in the case of diarrhea was (45.45%) and the percentage of infection in normal feces was (18.18%) and there was no significant difference between the two percentages.

4- Prevalence of *Cryptosporidium* and its correlation to the period of the study:

The highest rate of infection was recorded among children of both sexes in both May and June, with a percentage of infection (45.45% - 50%) respectively, while the lowest incidence of parasitic infection was in January and February, where the rate of infection was (18.18%) for both months with a significant difference ($P < 0.05$), Table(4).

Table(1) Prevalence of *Cryptosporidium* and its correlation to the sex of the infected person

Gender	No. Infected Samples	No. Not Infected Samples	Total Number	Infection Percentage
Males	11	26	37	29.72%
Females	12	28	40	30%
Total	23	54	77	Average 29.86%

Table(2) Prevalence of *Cryptosporidium* and its correlation to the age of the infected person

Age	No. Infected Samples	No. Not Infected Samples	Total Number	Infection Percentage
3months-2years	14	19	33	42.42%
3years-7years	9	14	23	39.13%
8years-33years	0	21	21	0%
Total	23	54	77	Average 27.18%

Table(3) Prevalence of *Cryptosporidium* and its correlation to the stool state of the infected person

Stool State	No. Infected Samples	No. Not Infected Samples	Total Number	Infection Percentage
Normal	8	36	44	18.18%
diarrhea	15	18	33	45.45%
Total	23	54	77	Average 31.81%

Table(4) Prevalence of *Cryptosporidium* and its correlation to the duration of the study

Duration	No. Infected Samples	No. Not Infected Samples	Total Number	Infection Percentage
January	2	9	11	18.18%
February	2	9	11	18.18%
March	2	8	10	20%
April	4	10	14	28.57%
May	5	6	11	45.45%
June	5	5	10	50%
July	3	7	10	30%
Total	23	54	77	Average 30%

Discussion

Cryptosporidium sp spreads all over the world, which causes cryptosporidiosis disease which affects all age categories of humans, especially the children. Studies and researches that conducted on the epidemiological study of this parasite at the level of the governorates of Iraq recorded a difference in the percentage of infection of the *Cryptosporidium*. The percentage of infection in the city of Ramadi and its suburbs was 39.13% (17), In Diyala city, the infection rate was 2.3% (18), In the city of Mosul, the infection of this parasite was 14.3% (19) and the infection in northern Baghdad was 14.78% (15). In addition, some studies and researches that conducted around the world have shown that parasite prevalence varies from country to country and from region to region according to the geographical variation for those countries. The prevalence of the parasite was 1-4% in Europe and North America, while it was 3-20% in the continents of Africa, Asia, Australia and South and middle of America(20). The infection with this parasite has been recorded in the neighboring countries of Iraq, including Iran, where the infection rate was 1.7% (21), While in Turkey, the infection

rate was 17%. The infection was 1.1% in the United States(22).

What was mention earlier (24, 23) in the description of oocysts of *Cryptosporidium* was very similar to what was seen in this manuscript where the oocysts were seen spherical or oval shape contain an objects are not clear surrounded by a transparent halo when moving the fine adjustment. The percentages of female and male infection with the oocysts were very close, whereas the incidence of infection in stool samples in the case of diarrhea was higher than that in normal feces. These ratios were identical to those of (15, 25). The difference in infection ratios among the target age groups was clear. This manuscript showed a decrease in the incidence of infection among children as the age of the child increased until no infection was recorded for the children over the age of 7 years due to Increasing the effectiveness of the immune system in the elimination of the parasite as the individual progresses age, and these results were similar with both (27,26) and also identical with the published results by (28) where it was reported that the highest incidence of *Cryptosporidium* in the children who are less than 5 years old in Saudi Arabia.

In this manuscript, the highest ratio of infection among children was recorded in May and June and there was no relation between the spread of parasitic infection and high or low temperatures during the seasons, The results were similar to those of (15), where the highest incidence of parasitic infection was in May and July in northern Baghdad and the ratio was 25.7% and 19.35% respectively, while (29) reported that the highest ratio of parasitic infection in Samarra was in the spring months April and March. The highest prevalence of *Cryptosporidium* oocysts in some water sources in Basrah city was during February and April as referred by (30). In contrast, the highest infection of

oocysts among the children in Taza district in Kirkuk was (71.4%) in September, as referred by (31).

The difference in the results between the different studies examined in the parasite epidemiology of oocysts is due to several reasons: differences in the number of samples examined in each study, different geographic regions where studies and researches were carried out, whether urban or rural, The educational level of the population of those areas and the density of the population, as well as the extent to which animal husbandry and family members are involved in one place. Security conditions which have seen in the country in 2014, may be played a role in the occurrence of parasitic infection, which led to displacement the population from one region to another and increased the overlap of the population with different categories and thus led to the deterioration of the economic situation of the country in general and the study area in particular then resulted in the occurrence of cryptosporidiosis among people in general and children in particular.

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