

Cryptosporidiosis in Children in Duhok City / Kurdistan Region / Iraq

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Abstract

This is the first study to detect cryptosporidiosis among children in Duhok city/Kurdistan region of Iraq. The study included 332 stool samples of children using Modified Ziehl Neelsen Method (MZN) and 122 of these were randomly selected to detect *Cryptosporidium* by ELISA. By MZN, the infection rate of *Cryptosporidium* oocysts was 66.95% in children; 44.68% in immunocompromised (ICD) and 22.27% in immunocompetent (ICT) group. Among ICT children, the infection rate was highest among less than one year of age (39.34%) in diarrhoeic group, while in non-diarrhoeic group, it was highest among 1-4 years age (28.57%). Among ICD children, the relations were non-significant among ages. Out of 122 stool samples of children, 26 (21.31%), and 30 (24.59%) were positive by MZN and ELISA, respectively. In conclusion, ELISA was more sensitive and specific than MZN which were 82.5% and 90.91% respectively. This study indicates that asymptomatic infection is common among different age groups of children.

Keywords: Cryptosporidiosis, ELISA, Children.

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Introduction

Cryptosporidium species are monoxenous eukaryotic obligate intracellular protozoan parasites (Phylum: Apicomplexa) infect humans, ruminants, carnivores, birds, reptiles, amphibians and fish¹ causing gastrointestinal tract symptoms. *Cryptosporidium* is commonly seen in children and ICD individual such as patients with AIDS.²

Researches throughout Iraq have recorded and documented the presence of *Cryptosporidium* and its distribution in animals and humans, as well as its importance as one of the diarrhoea causing agents.^{3,4} The present study was performed to detect cryptosporidiosis among children in Duhok city.

Material and Methods

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The present study was carried out at the College of Veterinary Medicine, Duhok University; Kurdistan Region during the period November 2010 to October 2013. A total 332 stool samples were collected from diarrhoeic and non-diarrhoeic children of different ages and both sexes who were attending the Hevi Paediatric Hospital and Hospital of Thalassemia in Duhok city, Iraq.

The formol-ether concentration method was carried out for all samples which were examined microscopically by MZN. From these examined samples; 122 stool samples were selected randomly for examining by copro-antigen ELISA kit.⁵ Stool samples were divided into 2 portions, one concentrated by formol-ether concentration technique⁶ and stained by MZN.⁷ The rest of stool samples were frozen at -20°C for ELISA to determine *Cryptosporidium* antigen in stool by using kit produced by DRG Instruments Company, Germany, EIA- 3467.

The Chi-square test and Fisher Exact test were used to analyze the association between infection rate and the explanatory variables including age and sex. In all cases, the values were considered to be statistically significant when the P-value was less than 0.05 ($p < 0.05$).

The following methods have been used in Tables and Figures for expression of significance of values:

- ◆ Different superscript small letter within each column indicate significant differences among values.
- ◆ Similar superscript small letters within each column indicate no significant differences among values.
- ◆ Different superscript capital letters within each row mean significant differences among values.
- ◆ Similar superscript capital letters within each row indicate no significant differences among values.
- ◆ Absence of superscript capital letters within rows and absence of superscript small letter within each column mean there are no significant differences among values.

Results

The infection rates in children showed 53 (22.27%) to be positive out of 238 samples in the ICT group and 42

Table-1: The infection rate of *Cryptosporidium* oocysts among ICT and ICD children by MZNM according to age.

Age group (years)	ICT Children			ICD Children		
	No. of stool samples	No. of Positive samples	%	No. of stool samples	No. of positive samples	%
< 1	79	25 ^a	31.65	14	5	35.71
1-4	53	17 ^a	32.08	32	17	53.13
5-8	62	8 ^{A,b,c}	12.90	38	16 ^B	42.11
9-12	44	3 ^{C,c}	6.82	10	4 ^D	40
Total	238	53 ^E	22.27	94	42 ^F	44.68

Table-2: The infection rate of *Cryptosporidium* oocysts among diarrhoeic and non-diarrhoeic ICT children by MZNM according to age.

Age group (years)	Total No. of samples	Diarrheic group			Non-Diarrheic group		
		No. of samples	No. positive samples	%	No. of samples	No. of positive samples	%
< 1	79	61	24 ^{A,a}	39.34	18	2 ^{B,a,d,e,c}	11.11
1-4	53	39	13 ^{a,c}	33.33	14	4 ^c	28.57
5-8	62	37	4 ^b	10.81	25	4 ^{e,f}	16
9-12	44	12	1 ^{c,b}	8.33	32	1 ^{b,f}	3.13
Total	238	149	42 ^C	28.19	89	11 ^D	12.36

Table-3: The rate of *Cryptosporidium* oocysts among diarrhoeic and non-diarrhoeic ICD children by MZNM according to age.

Age group (years)	Total No. of samples	ICD Children					
		No. of samples	No. positive samples	%	No. of samples	No. of positive samples	%
< 1	14	5	2	40	9	3	33.33
1-4	32	14	6	42.86	18	11	61.11
5-8	38	21	11	52.38	17	5	29.41
9-12	10	8	3	37.5	2	1	50
Total	94	48	22	45.83	46	20	43.48

(44.68%) were positive out of 94 samples of ICD group. Highly significant differences were found among group results (Table-1).

It is obvious that the rate of infection with *Cryptosporidium* is according to age among ICT and ICD children of both sexes. In ICT individuals the highest infection rate 17/53 (32.08%) was found among the age group 1-4 years followed by less than one year 25/79 (31.65%). The lowest infection rate 3/44 (6.82%) was found among the age group 9-12 years. Significant differences were found between first two age groups compared with the third and fourth groups. In ICD individuals; the highest infection rate 17/32 (53.13%) was found among the age of 1-4 years; the lowest infection rate 5/14 (35.71%) was found among less than one year. No significant differences were found among these values in

the ICD group.

Among age group of ICT and ICD individuals; significant differences were found between third and fourth age groups. In general there was a significant high infection rate among ICD individuals as compared the ICT group regardless of age; which were (44.68%) and (22.27%), respectively.

The infection in diarrhoeic and non-diarrhoeic ICT children according to age is shown in Table-2. Among the diarrhoeic ICT individuals the highest rate 24/61 (39.34%) was found among the age group of less than one year old, whereas, the lowest rate 1/12 (8.33%) was found among the age group 9-12 years. Significant differences were found between the first age group with the third and fourth groups; there was a significant difference between

Table-4: The rate of *Cryptosporidium* oocysts of the ICT children by MZNM according to gender.

Age group (years)	Total No. of samples	No. of samples	ICT Children				%
			Male		Female		
			No. positive samples	%	No. of samples	No. of positive samples	%
< 1	79	45	9 ^{A,a,b}	20	34	16 ^{B,a}	47.06
1-4	53	25	8 ^{C,a}	32	28	9 ^{C,a,c}	32.14
5-8	62	21	6 ^{D,a,b}	28.57	41	2 ^{D,b,d}	4.88
9-12	44	26	2 ^{E,b}	7.7	18	1 ^{E,c,d}	5.56
Total	238	117	25 ^F	21.37	121	28 ^F	23.14

Table-5: The rate of *Cryptosporidium* oocysts of the ICD children by MZNM according to gender.

Age group (years)	Total No. of samples	No. of samples	ICD Children				%
			Male		Female		
			No. positive samples	%	No. of samples	No. of positive samples	%
< 1	14	6	2	33.33	8	3	37.5
1-4	32	15	8	53.33	17	9	52.94
5-8	38	18	4	22.22	20	12	60
9-12	10	3	2	66.67	7	2	28.57
Total	94	42	16	38.1	52	26	50

the second age group and the third group. Among the non-diarrhoeic ICT individuals the highest rate 4/14 (28.57%) was found among the age group of 1-4 years; and the lowest rate 1/32 (3.13%) was among the age group 9-12 years. The values have a significant difference between first age group and the fourth group as well as between the second age group and the third and fourth groups. Rates of infections between diarrhoeic and non-diarrhoeic groups, with regard to age had no significant differences except among the age group of < 1 year but the rate of infection in diarrhoeic group was more than the non-diarrhoeic one.

In ICD children (Table 3), the highest rate was 11/21(52.38%) among the diarrhoeic group of 5-8 years, whereas, the lowest rate 3/8(37.5%) was among the age group of 9-12 years. These rates have no significant differences. Among the non-diarrhoeic group, the highest rate 11/18 (61.11%) was among the age group of 1-4 years, whereas, the lowest rate; 5/17 (29.41%) was among the age group of 5-8 years. No significant differences were found. Comparing the significant relationship of infection rate between the diarrhoeic and non-diarrhoeic groups among both the ICT and ICD individuals, showed that the diarrhoeic and non-diarrhoeic groups from the ICD individuals had an infection rate higher than that of diarrhoeic and non-diarrhoeic groups in ICT children.

Table-4 shows the highest positive rate of 8/25 (32%) to

be in males in the age group of 1-4 years, and the lowest rate 2/26 (7.7%) in the age group 9-12 years, without significant differences except between the second and the last group of age. In females the highest rate 16/34(47.06%) was in the age group lower than one year, and the lowest rate 2/41(4.88%) in the age group 5-8 years, with a significant difference between the first age group and the third and the fourth groups, and between the second age group and the third group. The infection rate of males and females regardless of age, was similar among the ICT children.

Among the ICD children (Table-5), the highest rate in males was 2/3 (66.67%) among the age group 9-12 years and the lowest rate 4/18 (22.22%) among the 5-8 years age group. Among females the highest rate 12/20 (60%) was in the age group of 5-8 years and the lowest rate 2/7 (28.57%) in the age group of 9-12 years. These differences were statistically non-significant between males and females.

Discussion

This is the first study in Duhok city on the cryptosporidiosis in children. Infection among ICT children in this study was 22.27%. The result of this study disagrees with the data recorded in Iran, where the prevalence of the parasite in various regions was 4.1% in the West of Iran, 7% in Southeast 2.2% in the South, 7.7%

in North west, and 2.5% in central parts of the country.⁸

In Korea, the rate of infection was 3.3% among villagers in several rural areas.⁹ Another Korean study recorded a prevalence of 1.5%.¹⁰ Other rates of infection were reported in Egypt 17%, Uganda 5.9%, Kenya 25%, Turkey 3.5%, Pakistan 10.3% and Indonesia 8.2%.¹¹⁻¹⁵

The published data of many studies on humans, indicated the presence of differences in prevalence rates of *Cryptosporidium* infection among countries and regions within each country, which are attributed to the living pattern, hygienic conditions, socio-economic status, using of unsafe water, degree of environment contamination, and people's lifestyle.¹⁶

The highest rate of infection in the present study was among ICD individuals recorded as 44.68%. This result disagreed with these obtained by Roy et. al.¹⁷ who found the prevalence rate of 90% in patients with malignant diseases.

In our study, ICT individuals had the maximum infection rate which was the highest among the age group 1-4 years, followed by age group less than one year with no significant difference between the two values, 9-12 years age group had the lowest rate. These results agreed with the results of Mahdi et. al.,¹⁸ who found the largest infection rate in the ages of 1-12 months among children of Ramadi province. In contrast, the higher age group (4.5 years) was found to be the most susceptible in Kuwait¹⁹ which is contradictory to the present study.

In the current study, children between the age of 1-4 years were more susceptible of acquiring the infection as at this age children start walking and have a high level of inquisitiveness. They pick up objects from the ground, infect their fingers or even put them in their mouths. Thus they were exposed to many pathogens and causative agents and can get infected more than older children who can discriminate harmful from beneficial objects. This interpretation has been confirmed by Mohanad et. al.²⁰

In the present study a significant difference was observed between ICD individuals (44.68%) and ICT (22.27%) groups. This result is similar to the findings of Goncalves et. al.²¹ who found prevalence rates in children of Baghdad to be 32% and 21.2% among ICD and ICT groups respectively.

In diarrhoeic ICT individuals, the infection rate was significantly higher among the age group less than one year, followed by 1-4 years whereas, the lowest infection rate was among the age group 9-12 years, this result agrees with Kawan et. al.²²

In this study, the diarrhoeic group (28.19%) had a total infection rate significantly higher than non-diarrhoeic group (12.36%) and this result is same as the findings of Abdul Razak et. al.²³

In our study, no significant differences were noted between males and females ICD individuals, which agrees with the study by Hamed et. al.²⁴

Conclusion

In the current study, the diarrhoeic group had a total infection rate significantly higher than the non-diarrhoeic group, which indicates that asymptomatic infection is common among different age groups of children. The highest rate of infection in the present study was among ICD individuals in comparison to the ICT group.

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