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Prevalence of *Cryptosporidium parvum* among children in Iraq

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Abstract: Cryptosporidiosis is a parasitic disease caused by an apicomplexan protozoa of. *Cryptosporidium parvum* is the specific infective agent in human . The present study aimed to search for the presence of *C. parvum*and to determine the prevalence of this parasite among children in Kut city, Iraq. Six hundred stool samples were collected from children less than twelve years old from October 2011 to May 2012. Stool samples were inspected by modified Ziehl-Neelsen acid fast stain and ELISA. Results indicated that 203 cases gave positive results (33.83 %) and 397 cases gave negative results (66.17%) with Ziehl-Neelsen acid fast stain . The higher infection, 115 (19.17%) appeared in age (<1) year while the lower infection 37 (6.17%) appeared in age (1-6) years. There was association between anemia 66.01% (134/203), Packed Cell Volume (PCV) 66.01% (134/203), White Blood Cells Count (WBC's) 66.01% (134/203) that showed increase in number, and infection with cryptosporidiosis, respectively. The high percentage of positive cases (100%) was recorded in microscopic examination compared to 72.5% (129/178) of positive cases detected by ELISA assay. The present study is the first record of cryptosporidiosis among children in Wasit Province, Iraq. It demonstrated clearly a high prevalence rate of *C. parvum* among children of less than 12 years old in Iraq. ELISA technique will be of great value in the rapid and accurate diagnosis of *C. parvum* in human fecal materials.

Keywords: *Cryptosporidium Parvum*, Prevalence, Children, Stool, ELISA

1. Introduction

Cryptosporidiosis is a parasitic disease caused by an apicomplexan protozoon,*Cryptosporidium parvum* is the specific infective agent in human. The first case of human cryptosporidiosis was reported in 1976 [1].

Cryptosporidium spp. is a waterborne, obligate intracellular protozoan parasite that infects epithelial cells lining the small intestines of human and over 170 different host species causing enteric diseases [2, 3].

There are more than ten species of *Cryptosporidium*, *C. parvum* and *C. hominis* are the two species responsible for the most cases of human cryptosporidiosis worldwide [4, 5].

The most widely used technique for the diagnosis of *Cryptosporidium* is the detection of oocysts in a fecal smear [6]. The diagnosis depends on staining feces with stains especially prepared for *Cryptosporidium* oocyst [7]. A large number of staining techniques have been used to recognize *Cryptosporidium*oocysts. The most widely used have been the modified acid-fast procedures [8] which is the gold standard for the detection of *Cryptosporidium spp*. [9]. It differentiates red-stained oocysts from similarly sized and shaped green-stained yeast forms [8].

Serological studies using ELISA demonstrated that *C. parvum*infection is more common in developing countries (50-60%) than in developed countries (25-30%) [10]. This work aimed to throw light on prevalence rate of *C. parvum* among children in Iraq and assessment of their epidemiological and clinical aspects.

2. Materials and Methods

2.1. Samples Collection

This study was carried out during the period from October 2011 to May 2012 in Al-Karamah teaching hospital of Kut city, Iraq. A total of 600 stool samples were taken from children aged 1 month to 12 years who suffering from with acute or persistent diarrhea. The

patients were divided into three age groups. Fecal samples were collected in clean and labelled containers and preserved in frozen under – 22º C until used.

2.2. Direct Stool Examination

A total of 178 samples of stools were examined by microscope as direct identification of *Cryptosporidium* parvum infections through staining with modified Ziehl-Neelsen acid fast stain technique [11].

2.3. Hematological Methods

A total of 203 patients with cryptosporidiosis were tested for three blood tests [Hemoglobin level (Hb %), Packed Cell Volume (PCV) and White Blood Cells Count (WBC's count)]. Two ml of each blood sample were collected by syringes in EDTA tubes and levels of Hb, PCV, and WBC were measured by CELL-DYN Ruby (Abbott, USA).

2.4. Immunoassay Method [12]

A total of 178 samples of stools were examined by ELISA assay. The ELISA kits were used on the frozen stool specimens**.** The *Cryptosporidium parvum*-ELISA based antigen detection kit made by (Onestep Company, USA) was used according to manufacturer's instructions to screen 94 randomly selected stool specimens for *Cryptosporidium* inclusive of the ones positive by microscopy.

2.5. Statistical Analysis

The suitable statistical methods were used in order to analyze and assess the results. These were used to accept or reject the statistical hypotheses. All the statistical analysis were done by using Pentium-4 computer through the Minitab program and Excel application [13].

3. Results

3.1. Prevalence of Cryptosporidium Infection According to the Age

Table (1) shows the prevalence of *Cryptosporidium* infection according to the age. Out of 600 samples, 203 cases gave positive results (33.83 %) and 397 cases gave negative results (66.17%) by using Ziehl-Neelsen acid fast stain. The higher infection 115 (19.17%) appeared in age (<1) year while the lower infection 37 (6.17%) appeared in age (1-6) years.

Table 1: *Prevalence of C. parvum Infection in relation to the age groups*

Age group (year)	No. of samples	Positive cases	%	Negative cases	%
(<1)	247	115	19.17	132	22
(1-6)	117	37	6.17	80	13.33
(7-12)	236	51	8.5	185	30.83
Total	600	203	33.83	397	66.17

P-value: 0.193
C.S: Non Significant

3.2. Prevalence of Cryptosporidium Infection According to the Gender

Table (2) represents the prevalence of *Cryptosporidium* infection according to the gender. The infection was recorded 33.74% (109/323) in the males and 33.93% (94/277) in females. No significant differences were recorded between males and females.

3.3. Levels of Some Hematological Parameters in Relation to the Patients 'Age

Table (3) shows the prevalence of positive cases of Hb, PCV and WBC's in relation to the age. The abnormal cases were recorded 134/203 (66.01%) and the normal cases were 69/203 (33.99%). The higher rate of abnormal cases (36.95%) were recorded in age (<1) year and the lower rate (13.79%) were in age (1-6) years.

The rates of infection related to abnormal cases were: 36.95%, 13.79%, 15.27% appear in age (<1) year, (1-6) years, (7-12) years, respectively. The rates of infection related to normal cases were: 19.7%, 4.43%, 9.85% appear in age (<1) year, (1-6) years, (7-12) years, respectively.

Table2: *Distribution of Cryptosporidium parvum infections in relation to the gender*

-Ve Cases (%)	+Ve Cases (%)	No. of Samples ♀	- Ve Cases (%)	+Ve Cases (%)	No. of Samples ♂	Age Group (year)
68(24.55)	52 (18.77)	120	64(19.81)	63(19.5)	127	**(<1)**
37(13.36)	20(7.22)	57	43(13.31)	17(5.26)	60	**(1-6)**
78(28.16)	22(7.94)	100	107(33.13)	29(8.98)	136	**(7-12)**
183(66.07)	94(33.93)	277	214(66.25)	109(33.74)	323	**Total**

P-value: 0.791
C.S: Non Significant

Table3: *Some hematological parameters levels in relation to the age in patients with cryptosporidiosis*

Age (years)	No. of +Ve cases	Abnormal cases (Hb, PCV,WBC)	%	Normal cases (Hb, PCV,WBC)	%
(<1)	115	75	36.95	40	19.7
(1-6)	37	28	13.79	9	4.43
(7-12)	51	31	15.27	20	9.85
Total	203	134	66.01	69	33.99

P-value: 0.308
C.S: Non Significant

3.4. Levels of some hematological parameters in relation to the patients ' gender

Table (4) shows the prevalence of positive cases of Hb, PCV and WBC's in relation to the gender. The abnormal cases were recorded 134/203 (66.01%) and the normal cases were 69/203 (33.99%). Higher rate of abnormal case were recorded in male (36.45%) compared to female (29.56%).

The rates of infection related to abnormal cases were 36.45%, 29.56% appeared in males, females respectively. The rates of infection related to the normal cases were

17.24%, 16.75% appeared in males and females, respectively.

3.5. ELISA Assay

Table (5) shows the comparison between microscopic examination and ELISA assay in the diagnosis of cryptosporidiosis cases. High percentage of positive cases (100%) was recorded in microscopical examination while 72.5% of positive cases were detected by ELISA assay.

Table4: *Some hematological parameters levels in relation to the gender in patients with cryptosporidiosis*

Gender	No. of +Ve cases	Abnormal cases (Hb, PCV, WBC)	%	Normal cases (Hb, PCV, WBC)	%
(Male)	109	74	36.45	35	17.24
(Female)	94	60	29.56	34	16.75
Total	203	134	66.01	69	33.99

P-value: 0.135
C.S: Non Significant

Table5: *Comparison between Microscopic and ELISA Methods*

Type	No. of Samples	+	%	-	%
Microscopic Examination	178	178	100	0	0
ELISA Assay	178	129	72.5	49	27.5

P-value: 0.065
C.S: Non Significant

4. Discussion

The size and extent of the problem of cryptosporidiosis in Iraq is not well characterized. These data provide important information on the occurrence and determinants of the most important intestinal parasitizes in Iraq.

There are more efficient methods such as the modified acid fast stain for detecting *Cryptosporidium* oocysts in diarrheal stools. This method is highly sensitive and specific, which is used as the primary test in our clinical laboratory for patient testing, particularly when the organism burden is low. Modified acid fast stain is more efficient and less labor-intensive procedures for detecting *C. parvum* that require less technical skill for interpretation [14]. Recent studies have found enzyme linked immunosorbent assays (ELISA) to be sensitive, cost-effective, simple and a rapid method for detection of *Cryptosporidium* in stool specimens [15].

Cryptosporidiosis is reported worldwide but its prevalence varies widely in different parts of the world [16]. The present study is the first record of cryptosporidiosis among children in Wasit Province, Iraq. The absence of reports of cryptosporidiosis in this area may be because a specific diagnosis method is not being used routinely during stool examination. Therefore, it seem reasonable to test apparently healthy people with undiagnosed chronic diarrhoea who are animal handlers, travelers to endemic areas, hospital workers, house-hold contacts of infected patient and children in day care centers [17].

The current study explores the prevalence of *C. parvum* among children ≤12 years old in Kut City, Iraq, during seven months. The results demonstrate clearly a high prevalence rate of *C. parvum*(33.83%) among children of less than 12 years old. The higher rates of infections with *C. parvum* may be explained by crowding condition, poor sanitary and hygienic conditions, and low dose of infection. Additionally, persons who infected shed up 10^5 to 10^7oocysts/g of feces both during infection and up to 3 weeks to 2 months, infective stage are highly resistant to environment condition and many common disinfectants that used to treat drinking water [18]. This result was comparatively closer to similar studies conducted around the globe [10,19, 20, 21].

The present study observed difference between prevalence values among age groups, the most cases of cryptosporidiosis occurred among children less than 1 year.The prevalence of *Cryptosporidium* in children below five years of age was 8.2% and 14.3% in children of India in the age group of six months to one year [22]. This may be explained by milk bottles contamination or un-breast feeders and creeping on a contaminated ground. Also the explanation for it remains hypothetical though it has been suggested that their immune functions are low so that a low dose of infection may result in cryptosporidiosis and that repeated low dose infections may induce immunity against *Cryptosporidium* which protects older children [16]. Additionally, youngest children tend to have relatively more

symptomatic disease than older [23]. The rate of infection in the present study is similar to other studies [24, 25].

The present study revealed that no significant difference ($P > 0.05$) was noted between males (33.74%) and females (33.93%) which may indicates that both sexes have equal chance of being infected. These results were in agreement with other studies conducted worldwide [26, 27, 28].

The widespread occurrence of anaemic and leukocytosis (66%) among the examined patients is worrisome but agrees with the earlier observation that about 30% of the world population is anemic [29]. Anemia is commonly caused by deficiency of iron in diet [30]. It is common knowledge that due to combined forces of ignorance and poverty the diets of many individuals and households in developing countries often lack many essential blood-building in gradients, including iron. These factors might have contributed to the high occurrence of anemia in the studied area. Similar observations were made by other researchers [31].

The present study was carried out to assess the epidemiological and clinical aspects of the cryptosporidiosis disease and also to estimate the agreement and correlation between direct smear by modified acid fast stain and ELISA for serodiagnosis of *C. parvum*. Since direct observation of oocysts is not a suitable diagnostic method to be carried out on humans due to small number, isolation of organism from other tissues is also a difficult and time consuming procedure, therefore the serological techniques appear to be the methods of choice [32]. ELISA immunoassays may result in an increased detection rate due to their high sensitivity [33]. Our result revealed that the prevalence of *C. parvum* infection was 72.5% by ELISA. This result was comparatively closer to similar studies conducted by many authors [10, 28, 34]. However, different results were obtained by other authors worldwide [16, 35].

5. Conclusion

The present study is the first record of cryptosporidiosis among children in Wasit Province, Iraq. It demonstrated clearly a high prevalence rate of *C. parvum* among children of less than 12 years old in the area of the study.

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