Prevalence of Cryptosporidium parvum Among Children At Wasit Province

Abdulsadah Abdulabbas Rahi & Huda Hadi Raheem

أنتشارطفيلي *Cryptosporidium parvum* بين الأطفال في محافظة واسط

عبدالسادة عبدالعباس راهي هدى هادي رحيم

الخلاصة :

هو طفيلي معوي مهم يتواجد في الرضّع والأطفال*Cryptosporidium parvum* طفيلي في محافظة واسط . أجريت هذه الدراسة للفترة من شهر أيلول لغاية شهر كانون الأول من العام 2011 في مستشفى الكرامة التعليمي في محافظة واسط . تم أختيار ألأطفال عشوائيا" من عمر شهر واحد – 12 سنة من الذين يعانون من نوبات الأسهال الحاد أو المزمن . شخّص الكيس البويضي للطفيلي في عينات البراز بأستخدام طريقة القصر بالحامض المحوّرة.

فحصت 100 عينة براز من ألأطفال (55 ذكر و45 أنثى). أعطت 50 (50%) من العينات نتائج موجبة .كانت ألأصابة بالطفيلي أكثر معنويا" (59%) في ألأطفال دون عمر السنة مقارنة بالأطفال بين عمر(7-12)سنة حيث كانت بنسبة (4%). معظم ألأطفال المصابين كانوا يعانون من فقر الدم . كان معدل الأصابة للطفيلي في الذكورأعلى (27%) عمّا هو عليه في الأناث (23%). كذلك لاتوجد فروقات معنوية بين الأطفال من ناحية الجنس .

Abstract :

Cryptosporidium parvum is an important enteric parasite among infants and children in Wasit Province. The present study was conducted from September to December 2011 in Al-Karamah Teaching Hospital in Wasit Province. Children aged 1 month to 12 years presenting with acute or persistent diarrhea were selected randomly. The oocyst of C. parvum was investigated in stool specimens using a modified acid-fast staining method.

A total of 100 feces samples of children (55 male and 45 female) were examined. Fifty (50%) specimens were oocyst positive. The prevalence of Cryptosporidium infection was significantly higher (59%) in children under one year old compared to children between (7-12)years old(4%). Most of the infected children were had anemia. The infective rate of Cryptosporidium parvum in males was higher 27% than females 23% . Also, there was no significant difference among children in gender.

Key Words : Human, *Cryptosporidium parvum* , Modified acid-fast stain

1. Introduction:

Protozoans are unicellular organisms which include a number of pathogenic eukaryotes that infect human and animals usually through food and water contamination [(1)(2)]. Nearly, all the protozoan parasite have complex life cycle requiring both intra-and extracellular stage [(2)].

Cryptosporidium parvum, Giardia lamblia, Entamoeba histolytica, and Cyclospora sp. are widespread in the environment which causing of water- and food-borne diseases [(1)].Cryptosporidium spp. are waterborne, obligate intracellular protozoan parasite that infects epithelial cells lining the small intestines of human and over 170 differenthost species causing enteric disease [(3)].There are more than ten species of cryptosporidium , Cryptosporidium parvum and Cryptosporidium hominis are the two species responsible for the most cases of human cryptosporidiosis worldwide [(4)(5)].

The infection in immunocompromised patients is much more severe. It may often be life threatening. Passage of fluid, up to 12 liters per day, has been reported. Multiple pathways of Cryptosporidium transmission have been implicated. These include animal to human, water contami-nation and person-to-person. The latter may include contact between members of the same household, day care centers, and homosexual men[(6)]. Diagnosis of Giardia and Cryptosporidium infections has been done through a number of invasive and non-invasive techniques. The non-invasive techniques, microscopic examination of stools has been the most common. However, this method relies on an experienced technician and subsequent observation of intact organisms. Because of the historically low proficiency of correct microscopic examinations and intermittent excretion of organisms, alternative diagnostic methods have been investigated [(7)].

One important alternative has been the development of an antigen capture enzyme linked immunosorbent assay (ELISA) for use with stools. These tests have shown comparable sensitivity to experienced microscopic examinations, are fairly simple to perform and do not require the observation of intact organisms [(8)].The aim of present study is to determine the prevalence of Cryptosporidium parvum among children at Wasit Province.

2. Materials and methods :

2.1 Materials

Glass slides, methanol, ethanol, distilled water, wash bottle, HCl, carbol fuchsin, methylen blue, plan tube, EDTA tube, stick, syringes.

2.2 Methods

This study was carried out during the period from October 2011 to January 2012 in Al-Karamah Teaching Hospital of Kut city. A total of 100 fecal samples taken from children aged between 1 month to 12 years presenting with acute or persistent diarrhea. Fecal samples were collected in clean and label containers and examined as soon as received by naked eye for consistency.

Samples were concentrated using either flotation or sedimentation techniques before staining (10).The oocyst of C. parvum was tested by using modified acid-fast staining method which was a sensitive and specific path for the identification of Cryptosporidium in stool (9). Ordinary light microscope with 100 magnification power was used with oil immersion lens. In this technique, the oocysts appear as pink to red, spherical to ovoid bodies on a blue or purple background. Also blood samples were collected from patients with cryptosporidiosis to determine hemoglobin level (Hb%) by the Sahli's system (Spain), and White blood count (WBC's count) by thin blood films (11).

3. Results and Discussion :

3.1 Results

A total of 100 children who attended to Al-Karamah Teaching Hospital were suffered from watery diarrhoea and abdominal pain were examined. Samples of feces were stained by modified acid fast stain and examined by microscopy for detecting of C. parvum oocysts. An overall prevalence of C. parvum 50 /100 (50 %) was appeared in table (1).

Table .1 The Overall Prevalence of *C. parvum*

Name of Parasite	No. of Examined sample	No. of infected sample Male	Female	Percentage %
C. parvum	**100**	**27**	**23**	**50**

Table (2) shows the prevalence of *C. parvum* infection according to the age and gender. The highest infection(59%) was recorded in age group 1(**> 1**) year, while the lowest (4%) was appeared in age group 3 (7-12) years old. There was no significant difference in occurrence of infection between genders**.**

Table 2. Patients with Cryptosporidiosis in Relation of Age & Gender

Age / Year	Male +Ve	%	Male -Ve	%	Female +Ve	%	Female -Ve	%	Total	%
Group 1 > 1	20	20	14	14	12	12	13	13	59	59
Group 2 (1-6)	6	6	14	14	11	11	6	6	37	37
Group 3 (7-12)	1	1	0	0	0	0	3	3	4	4
Total	27	27	28	28	23	23	22	22	100	100

Table (3) shows the range of hemoglobin (Hb) in Patients with Cryptosporidiosis. The lowest hemoglobin (8-9) was appeared in 7/50 (14%) whose had severe anemic case,while the largest hemoglobin rate (11.1-12) was appeared in group 11/50 (22%).

Table.3 Hemoglobin Level in Patients with Cryptosporidiosis

Hemoglobin (Hb %)	No. of patients	%
8 - 9	**7**	**14**

9.1 - 10	**12**	**24**
10.1 -11	**20**	**40**
11.1 - 12	**11**	**22**
Total	**50**	**100**

therelationship between age and total count of leukocytes appears in table 4. The large number of WBCs was shown in group 1 and 2, while the low number was in group 3.

Table .4 WBC's Count in relation to the Age

Age (years)	No. of +Ve cases	Abnormal cases (WBCs more than 10^4 cell)	%	Normal cases (WBCs less than 10^4 cell)	%
1) >G1(	**25**	**14**	**56**	**11**	**44**
G2 (1-6)	**17**	**9**	**53**	**8**	**47**
G3 (7-12)	**8**	**3**	**37.5**	**5**	**62.5**
Total	**50**	**26**	**52**	**24**	**48**

3.2 Discussion

Intestinal parasites are very common in developing countries. *Cryptosporidium* has revealed to be one of the most common parasites[(12)]. Human and several mammalian species can be infected with *C. parvum* transmitted by the fecal-oral route. Outbreaks have been described as a result of transmission in day care centers, swimming pools, public water supplies, and other water sources [(13)].

Several methods are available for identification of Cryptosporidial oocysts in fecal specimens including modified acid-fast staining which detects oocyst wall, fluoresceinconjugated monoclonal antibody-based
detection of oocyst wall antigen, enzyme-linked immunosorbent assay (ELISA) which detects Cryptosporidial antigen and most recently polymerase chain reaction (PCR) which detects Crytosporidial DNA. Modified acid-fast stain of a fecal smear has been the gold standard for
detecting *Cryptosporidium* oocysts in stool. This method is commonly used in clinical microbiology laboratories to easily identify Cryptosporidial oocysts. Although the concentration and staining
procedures are time-consuming and also require an experienced microscopist to read the slides, it is inexpensive and allows the detection of other parasites (eg, *Isospora* and *Cyclospora*) at the same time [(14)].

According to the results of the present study *C.parvum* had an overall prevalence of 50 /100(50 %). Increased numbers of cases of *C. parvum* infection in Wasit province were associated with contaminated drinking water supplied to these population [(15)]. Because the 50% infectious dose is relatively low for *C. parvum*, ranging from approximately 10 to 1,000 for healthy humans, oocysts could be transmitted through low levels of contaminated water or food, followed by person-to-person transmission, especially among household members. Food-borne *C. parvum* infection

has been transmitted through ingestion of fresh-pressed apple cider, and risk factors for food-borne transmission have had been reported for consumption of stored cooked food and raw milk [16].The infection prevalence of *C. parvum* on average was similar to the results of Elwin *et al*.,(2009)[17] in which the prevalence of *C. parvum* infection was recorded (45.9%). This is also in agreement with the report of Charles ***et al.*, (2000)** [18] in which the prevalence of *C. parvum* in diarrheal children aged (5-8) years old was found to be 58%**.**

The study also revealed a significant positive correlation between incidence and intensity of infection among different age groups with peak values among under one year age group. The rate of infection in the present study is similar to other studies in Iraq such as Majidah,(2008) in who proved the prevalence of C. parvum infection was higher among children under one year in Ramadi City [19]**.** Also Jong-Yil Chai **et al.,(2001) noted that infection was more prevalent in infants under one year in Korea [20]. The present revealed that** no significant difference (P _ 0.05) was noted between males (27%) and females**(23%). These results were in agreement with** Jong-Yil Chai et al.,(2001) [20] and Ke-Xia Wang et al.,(2002) [21].

The widespread occurrence of anaemic among the examined patients is worrisome but agrees with the earlier observation that about 30% of the world population is anaemic[22]. Anaemia is commonly caused by deficiency of iron in diet according to the report of WHO [23]. 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 ingradients, including iron.These factors might have contributed to the high occurrence of anaemia in the study area.

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