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Research Article

Diarrhea caused by *Cryptosporidium parvum* in Kut, Iraq using different methods

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Abstract: Cryptosporidiosis induce a self-limiting diarrhea in immunocompetent individuals but might be fetal in immunocompromised AIDS patients. Diagnosis of Cryptosporidiosis is not possible through routine stool examination, treatment and control of the disease is difficult. In this study the causative agent of diarrhea in children referred to a university affiliated hospital in Kut, Iraq during October 2011 to January 2012 was assessed. A total of 100 children who suffered from watery diarrhea and abdominal pain were recruited. Stool samples were collected and examined using Direct Fluorescent Antibody (DFA), modified acid fast stain and ImmunoCard methods. The results showed that 34 of the samples were positive for *Cryptosporidium parvum* oocysts using modified acid fast stain, and from 34 positive ones 30 of the samples were positive with ImmunoCard and 28 of the samples were positive with DFA. The rate of *Cryptosporidium parvum* was significantly ($p < 0.004$) higher in children aged less than one year than the children older than one year. The rate of *Cryptosporidium parvum* infection in male was higher than female (17% *vs.*13%).
Keywords: *C. parvum*, Human, Feces, Diagnosis

INTRODUCTION

Cryptosporidiosis is a worldwide zoonotic disease caused by various genotypically and phenotypically diverse species of *Cryptosporidium* which is a protozoanparasite in the Apicomplexa phylum. Cryptosporidiosis is considered as emerging pathogen by CDC, the disease is mostly asymptomatic and not noticed but might be presented with fever and mild to severe diarrhea which is self-limiting in immunocompetent individuals and life threatening in immunocompromised individuals like AIDS patients [1]. It is estimated that about 6% of diarrhea in immunocompetent individuals and 24% of diarrhea in AIDS patients are caused by *Cryptosporidium*. The organism was first isolated from a mouse in 1907 and later on from different animal species but only decades later when a large human waterborne outbreak occurred in 1993 in Milwaukee, it was considered as a serious waterborne disease [2]. The disease spread through the fecal-oral route, often from contaminated water. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) [3], once ingested, exist in the small intestine which resulted in an infection of intestinal epithelial tissue [4]. Control and treatment of Cryptosporidiosis is difficult and diagnosis of diarrhrea caused by cryptosporidiosis is not possible through routine stool examination unless specific request order by the physician. The clinical syndromes of cryptosporidiosis are fever, diarrhea and large volumes of fluid loss from the gastrointestinal tract [5]. Cryptosporidiosis outcome depends upon the immune status of the host [6]. The high resistance of *Cryptosporidium*oocysts to disinfectants such as chlorinebleach enables the parasite to survive for a long period of times and still remain infective [7]. Outbreaks happen in day care related to diaper changes [8]. Diagnosis of Cryptosporidiosis is done by detection of the parasite and identification of Cryptosporidial oocysts in fecal specimens is done by modified acid-fast staining which is the most common method of diagnosis of Cryptosporidios. Other methods such as immunodiagnostic techniques like Immunofluorescence, ELISA method and Immunocard test, and molecular techniques including PCR are used for diagnosis of Cryptosporidiosis. The aim of present study was to investigate whether *Cryptosporidium parvum* is the causative agent of diarrhea in the region and to compare the three methods; DFA, modified acid fast stain and ImmunoCard for diagnosis.

MATERIALS AND METHODS

Patients

This study was carried out from October 2011 to January 2012 in Al-Karamah Teaching Hospital which is affiliated to Wasit University, Kut, Iraq. The protocol was approved by the Ethical Committee of Wasit University, the study objective and the procedure was explained to the parents/guardians and those who agreed to participate were recruited. A total of 100 fecal samples were collected from the children aged 1 month to 12 years presenting with acute or persistent diarrhea. The samples were examined as soon as received by naked eyes and immunoassay tests were used with unconcentrated, stool specimens preserved in 10% formalin.

Modified Acid-Fast Stain

One gram of fecal samples was concentrated using flotation technique before staining [9]. The oocyst of *C. parvum* was checked using modified acid-fast staining method which is a sensitive and specific method to identify *Cryptosporidium* in stool [10]. Light microscope with oil immersion lens was used to search for oocysts which appear as pink to red, spherical to ovoid bodies on a blue or purple background. Stool samples from age/sex match children without diarrhea in the past 72-hour period were used as control.

ImmunoCard

The ImmunoCard *Cryptosporidium parvum* rapid assay was performed on one gram of unconcentrated formalin-fixed stool specimens as specified by the manufacturer (Meridian Bioscience, USA). Results were visualized after 10 min. A positive reaction appeared as a grey-black band visible at the *Cryptosporidium* area in the test window. Any reaction in the test window, regardless of color intensity was interpreted as a positive result.

Direct Fluorescent-Antibody Assay (DFA)

Concentrated fecal samples were examined by a direct fluorescent-antibody assay (DFA) for oocyst of *C. parvum*. For DFA, 10 µL of the concentrated specimen was smeared on a DFA well slide and allowed to air-dry. The immunoflouresans (IFA; Cellabs-Australia) was stained in accordance with examination and assessed under a UV microscope [10].

RESULTS

A total of 100 children (age 1 month to 12 years) suffered from watery diarrhea and abdominal pain who attended Al-Karamah Teaching Hospital were recruited. Samples of feces were stained using modified Ziehl-Neelsen and examined under microscope for detection of *C. parvum*. Out of 100 samples 30 (30%) samples were positive for *C. parvum*. As it is shown in table 1 the rate of *C. parvum* infection was the highest in infants with 17 (59%) positive samples and the lowest with 1 (4%) positive sample. There was no significant difference in occurrence of infection between the genders.

Table 1 shows the rate of *C. parvum* infection according to the age and gender. The highest infection infection rate was recorded in infants with 17 (59%) positive samples and the lowest was 1 (4%) positive sample in age group 7-12 years old.

Table 1: Patients with Cryptosporidiosis in Relation of Age & Gender

Age / Year	Male +Ve	%	Male -Ve	%	Female +Ve	%	Female -Ve	%	Total	%
< 1	10	10	24	24	7	7	18	18	59	59
(1-6)	6	6	14	14	6	6	11	11	37	37
(7-12)	1	1	0	0	0	0	3	3	4	4
Total	17	17	38	38	13	13	32	32	100	100

Table 2 shows the comparison of modified acid-fast stained smears and ImmunoCard and DFA tests. The modified acid-fast stained results showed 30 (30%) samples positive for *Cryptosporidium* oocysts while ImmunoCard results revealed 28 (28%) samples positive and DFA test showed 34 (34%) positive samples. Two specimens were negative for *Cryptosporidium* oocysts using modified acid-fast stained smears but generated positive results using the ImmunoCard and DFA tests.

Table 2: Comparison of Modified Ziehl-Neelsen, ImmunoCard and DFA for the detection of *C. parvum*

Results	No. of Specimens	modified Ziehl-Neelsen	ImmunoCard	DFA
C.parvum positive	30 (30%)	30 (30%)	26 (26%)	32 (32%)
C.parvum negative	70 (70%)	0 (0)	2 (2%)	2 (2%)
Total	100 (100%)	30 (30%)	28 (28%)	34 (34%)

DISCUSSION

Intestinal parasites are common in poor societies and among parasitic diseases *Cryptosporidium* is of important public health threat especially in developing countries [11]. *Cryptosporidium* was originally isolated from mouse in 1907 and later from many other animals and finally in 1976 was first related to human disease and waterborne outbreak. Human and several mammalian species are infected with *C. parvum* transmitted by the fecal-oral route. Outbreaks are described as a result of transmission in day care centers,

swimming pools, public water supplies, and other water sources [2].

There have been several studies investigating the epidemiology and prognosis of Cryptosporidium infection in patients with malignant disease. For example, Tanyuksel *et al*. [12] reported on a study, done in Turkey, of 106 fecal samples from patients with diarrhea and various cancers. Cryptosporidium oocysts were detected in 18 (17.0%), of these 106 patients. Blanshard *et al*. [13] described the various presentations of crytosporidiosis in HIV- positive patients in London. In this population, cryptosporidiosis was diagnosed in some 5% of all patients with HIV infection and 21% of patients with AIDS. The authors studied the course of infection in 128 patients. Transient infections were found in 28.7% and were more common in the less strongly immunosuppressed patients. Fulminant disease, the passage of more than 2 liters of stool/day, affected 7.8% of patients but only those with a CD4 count less than 50/mm^3 [13]. Cryptospo-ridiosis remains among the most common causes of diarrhoea in patients with AIDS [14]. Cryptosporidiosis is now the most common cause of waterborne disease in the world [15].

Several methods are available for identification of Cryptosporidial oocysts in fecal specimens including modified acid-fast staining which detects oocyst wall, Immunocard test, fluorescein conjugated 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 used as the gold standard for detection of *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 required an experienced microscopists to check the slides, but it is affordable and allows at the same time to detect other parasites eg, Isospora and Cyclospora [16].

According to the results of the present study *C. parvum* showed an overall rate of 34 out of 100 (34 %) using modified acid-fast staining method. The high rate of infection with *C. parvum* in the Kut area might be associated with contamination of drinking water [17]. 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 is 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 [18]. The infection prevalence of *C. parvum* on average was similar to what was reported by Elwin *et al*. [19] in which the prevalence of *C. parvum* infection was recorded to be 46%. This is also in agreement with the report of Charles *et al*. [20] in which the prevalence of *C.parvum* in diarrheal children aged 5-8 years old was found to be 58%.

The present 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 in which the prevalence of *C. parvum* infection was higher among children under one year old in Ramadi City [21]. The results of the present study revealed no significant difference (P _0.05) between male (17%) and female (13%). Although the sample size is not enough to conclude this but is in agreement with a study completed in Philippines where the gender of the children did not influence the rate of infection with this parasite [22]. Also, our results were in agreement with Natividad *et al*. [23] and Ke-Xia *et al*. [24].

The possible reasons for the absence of sex-related difference in the prevalence among the children might be due to the fact that there is no difference in sex behavior in children and risk factors of *C. parvum* infection such as food consumption, domestic animals contact and etc. Besides, the hygienic practices exercised by children of both sex are also essentially similar. In developing countries, *Cryptosporidium* infections occur mostly in children younger than 5 years [18, 25-27]. Cryptosporidiosis remains among the most common causes of diarrhoea in patients with AIDS [14]. Cryptosporidiosis is now the most common cause of waterborne disease in the world [15].

The ImmunoCard test detects only intact *Cryptosporidium* oocysts, the rapid test detect antigen, which may persist after the patient stops shedding intact organisms. Therefore, the results of the current study is not false-positives but might represent recently cured cases but not in active disease like the current study. The ImmunoCard test showed to be highly sensitive compared with the modified Ziehl-Neelsen stain for the detection of *Cryptosporidium*. In high-prevalence populations, test such as the ImmunoCard test, with a high sensitivity should be used as screening test of diagnosis of cryptosporidiosis compared with the modified Ziehl-Neelsen stain and DFA tests. The ImmunoCard test is less time consuming and easy to perform and does not require sophisticated equipments [28].

The direct fluorescent-antibody (DFA) technique offers the highest combination of sensitivity and specificity and is considered the gold standard by many laboratories [29]. However, it does not provide a stained slide that can be archived and requires specialequipment. As a result, after application of DFA technique, *C. parvum* oocysts were determined in thirty

four of the hundred feces samples (34%). The DFA showed to be more sensitive than the modified acid-fast stain, particularly when the organism burden is low. The test is more efficient and less labor-intensive and requires less technical skill for interpretation [29]. Stephanie *et al*. [30] reported that the prevalence of *C. parvum* infection was 32.5% by using DFA and Garcia *et al*. [31] reported 21% by using DFA and ImmunoCard ,also our results were lower than the 25.9 % detected in AIDS patients with chronic diarrhoea from Addis Ababa hospitals [32] and 8.5% reported previously in Dar es Salaam [33]. The possible explanations for the discrepancy between the present and previous study finding might be the result of variation in sampling techniques used, variation in the environmental condition of the different study localities and different methods used for detection of cryptosporidiosis.

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