Comparison among Modified Acid-Fast Stain and Some Immunological Methods in Diagnosis of *Cryptosporidium parvum* in Kut City

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مقارنة بين طريقة الصبغة المحوّرة المضادة للقصر بالحامض وبعض الطرق المناعية في تشخيص طفيلي Cryptosporidium parvum في مدينة الكوت

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الخلاصة :

أجريت هذه الدراسة للفترة بين تشرين الأول 2011 ولغاية كانون الثاني 2012 حيث تم فحص 100 عينة براز لأشخاص يراجعون مستشفى الكرامة التعليمي في مدينة الكوت ويعانون من أسهال مائي وألم في البطن . أعطت 34 عينة منها نتائج موجبة بعد فحصها بطرق الصبغة المحورة المقاومة للقصربالحامض 30 (30%) ، البطاقة المناعية 28(28%) وفحص الجسم المضاد المتألق المناعي المباشر34 (34 %). أخذت عينات عشوائية من ألأطفال الذين يعانون من ألأسهال الحاد المستمر بعمر (1 – 144) شهر . كان انتشار طفيلي *Cryptosporidium parvum* معنويا اعلى في ألأطفال دون سن السنة (59%) مقارنة بالأطفال بين (7-12) سنة حيث كانت (4%) . معدل ألأصابة بالطفيلي كان أعلى في الذكوروبنسبة (17%) عما هو عليه في الأناث (13 %) . لم يلاحظ وجود فرق معنوي بين الأطفال بالنسبة للجنس .

Abstract :

A total of 100 children who attending to Al-Karamah Teaching Hospital at Kut city were suffered from watery diarrhoea and abdominal pain were examined. The present study was conducted from October 2011 to January 2012. A thirty-four stool samples in which *Cryptosporidium parvum* oocysts had been seen by modified acid –fast stain examination were investigated, positivity detected with the ImmunoCard method, and Direct Fluorescent Antibody(DFA) method was found to be (30%), (28%) and (34%) respectively.

Children aged one month to 144 months presenting with acute or persistent diarrhoea were selected randomly. prevalence of *Cryptosporidium parvum* infection was significantly higher (59%) in children under one year old compared to children between (7-12) years old (4%). The infective rate of *Cryptosporidium parvum* in males was higher 17% than females 13% . Also, there was no significant difference among children in gender.

Key word : *C.parvum* , Human , Feces, Diagnosis

Introduction :

parasitic infections are considered as a serious public health problem [(1)]. Moreover , in several cases may increase host susceptibility to predate or decrease the competitive fitness of the individual and may be more prevalent in populations living in human modified habitats [(2)].

Cryptosporidiosis, also known as crypto, is a parasitic disease caused by *Cryptosporidium*, a protozoan parasite in the phylum Apicomplexa. It is spread through the fecal-oral route, often through contaminated water ; the main symptom is self-limiting diarrhea in people with intact immune systems. In immunocompromised individuals, such as AIDS patients, the symptoms are particularly severe and often fatal. Despite not being identified until 1976, it is one of the most common waterborne diseases and is found worldwide. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue [(3)].

The clinical syndrome of cryptosporidiosis are fever, diarrhea and large volumes of fluid loss from the gastrointestinal tract [(4)].The action of disease depends on the immune status of the host [(5)]. The high resistance of *Cryptosporidium* oocysts to disinfectants such as chlorine bleach enables them to survive for long periods and still remain infective [(6)]. Some outbreaks have happened in day care related to diaper changes [(7)]. The aims of present study to identify outbreaks of *Cryptosporidium parvum* and to compare among three methods for diagnosis of it.

Materials and methods :

Materials

Glass slides, methanol, ethanol, distilled water, wash bottle, HCl, carbol fuchsin, methylen blue, plan tube, EDTA tube, stick, syringes, ImmunoCard kit, direct fluorescent antibody (DFA) kit.

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. All immunoassay kits were used with unconcentrated, preserved stool specimens (10% formalin).

1.Modified Acid-Fast Stain

One gram of 100 human fecal samples were concentrated using flotation technique before staining [(8)]. 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 [(19)].

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. Children without diarrhea in the previous 72-hour period were matched for age and sex and recruited as the controls.

2. 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. No reaction in the test window and a positive control line was interpreted as a negative result.

3.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 [(9)].

Results and Discussion :

Results

A total of 100 children who attending to Al-Karamah Teaching Hospital were suffered from watery diarrhoea and abdominal pain were examined. Samples of feces were stained by modified Ziehl-Neelsen and examined by microscopy for detecting of *C. parvum*. An overall prevalence of *C. parvum* 30 /100 (30 %) was appeared in table (1).

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

Name of Parasite	No. of Examined sample	No. of infected sample	Percentage %
C. parvum	**100**	**30**	**30**

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 year, while the lowest (4%) was appeared in age group 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 %
> 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

P-value	C.S
0.044	Non significant

Also the results of modified acid-fast stained smears were compared with those of ImmunoCard and DFA tests (Table 3). The modified acid-fast stained results were appeared that 30(30%) were positive for *Cryptosporidium* oocysts and 70 (70%) were negative. The ImmunoCard results revealed that there were 28 (28%) positive for *Cryptosporidium* oocysts and 72 (72%) were negative, while the DFA test was gave 34(34%) positive and 66(66%) was negative. Two specimens were negative for *Cryptosporidium* oocysts using modified acid-fast stained smears but generated positive results using the ImmunoCard and DFA tests.

Table 3. comparison Between Modified Ziehl-Neelsen , ImmunoCard,and DFA for 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%)

P-value	C.S
0.641	Significant

Discussion

Intestinal parasites are very common in developing countries and *Cryptosporidium* has revealed to be one of the most common parasites[10]. 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 [11].

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 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 [12].

According to the results of the present study *C.parvum* had an overall prevalence of 30 /100(30 %). Increased numbers of cases of *C. parvum* infection in this area were associated with contaminated drinking water supplied to these population [13]. 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 [14].The infection prevalence of *C. parvum* on average was similar to what was reported by Elwin *et al*.,(2009)[15] 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)** [16] 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 in Ramadi City [17]**.** Also, the study in Korea **noted that infection was more prevalent in infants under one year [18]. The present revealed that** no significant difference (P _ 0.05) was noted between males (17%) and females**(13%).** No such sex-associated prevalence was observed in the present study.

This lack of difference in the prevalence rates of *Cryptosporidium* parvum in children was in agreement with a study in Philippines where the gender of the children did not influence the rate of infection with this parasite [19]. **Also, our results were in agreement with** [18] and [20]. The possible reasons for the absence of sex-related difference in the prevalence among the children could be explained by the observation that all children irrespective of their sex participate equally in the domestic animals contact. Besides, the hygienic practices exercised by children of both sexes were also essentially similar.

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 we obtained may not be false-positives but may represent recently cured cases. In this study, the ImmunoCard test was high sensitive than the modified Ziehl-Neelsen stain for the detection of *Cryptosporidium*. In high-prevalence populations, test such as the ImmunoCard test, with a high sensitivity as described, should be used as screening test of diagnosing cryptosporidiosis. Compared with the modified Ziehl-Neelsen stain and DFA tests, the The ImmunoCard test had the advantage of being less time-consuming and simpler to carry out, and did not require specialised equipment [21].

The direct fluorescent-antibody (DFA) technique offers the highest combination of sensitivity and specificity and is considered the gold standard by many laboratories[22]. However, it does not provide a stained slide that can be archived. It requires special equipment (fluorescence microscope) and commercially available test kits. As a result, after application of DFA technique, *C. parvum* oocysts were determined in thirty four of the hundred feces samples (34%). The DFA has been shown
to be more sensitive than the modified acid-fast stain, particularly when the organism burden is low. They are more efficient and less labor-intensive procedures for detecting *C .parvum* that require less technical skill for interpretation [22][23]. Stephanie *et al*.,(2003) reported that the prevalence of *C. parvum* infection was 32.5% by using DFA[24] and Garcia *et al*.,(2003) reported 21% by using DFA and ImmunoCard [25], also our results were lower than the 25.9% detected in AIDS patients with chronic diarrhoea from Addis Ababa hospitals [26] and 8.5% reported previously in Dar es Salaam [27]. 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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