CRYPTOSPORIDIOSIS IN SULAIMANI PEDIATRIC TEACHING HOSPITAL AND COMPARISON OF DIFFERENT DIAGNOSTIC METHODS FOR ITS DETECTION

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Abstract

The present study aimed to investigate the presence and prevalence of *Cryptosporidium* among children in Sulaimani Pediatric Teaching Hospital and to determine the best method for its diagnosis.

The study started from the 1st of Jun. to the 1st of Sept. 2012. Two hundred fifty stool samples were collected from children of 6 month to 12 years of age from both genders who attended the hospital. Stool samples were inspected by modified acid-fast stain as a standard method, direct wet mount, Crypto-Strips method and enzyme linked immunosorbant assy (ELISA).

Modified Ziehl-Neelsen technique revealed 38 (15.2%) positive cases for *Cryptosporidium* oocysts, of 22 males and 16 females, 4-6 years of age group was more susceptible to infection with *Cryptosporidium* and highly significant relationship was found between the genders and between different age groups of infection with a highly significant difference between rural and urban area.

The highest rate of infection was found in the soft type of stool samples with a significant difference between them.

The prevalence of *Cryptosporidium* was 13.6% by using direct wet mount, 6.8%, for ELISA and 4.4% by Crypto-Strips method in comparison with MZN method.

It is concluded that cryptosporidiosis found to be endemic in Sulaimani city for the first time and the modified acid-fast stain was the most reliable technique for its diagnosis.

Keywords: Stool samples, *Cryptosporidium* oocysts, Sulaimani, Iraq

1- Introduction

Cryptosporidiosis is a zoonotic gastrointestinal disease, caused by protozoa of the genus *Cryptosporidium* within the phylum *Apicomplexa*. (Chen *et al.*, 2002).

It causes clinical disease in both humans and animals; species names are based primarily on the animal species serving as hosts (Xiao *et al.*, 2004). Clinical illness is characterized by watery diarrhea, which can be accompanied by abdominal cramps, loss of appetite, low-grade fever, nausea, vomiting, and weight loss; however, asymptomatic infection occurs frequently (Hellard *et al.*, 2000). *Cryptosporidium* can also cause an opportunistic infection in human immunodeficiency virus (HIV)infected patients who might experience life-threatening infection with profuse, watery, cholera-like diarrhea (Kaplan *et al.*, 2000).

The infection is transmitted by the fecal-oral route and results from the ingestion of *Cryptosporidium* oocysts through the consumption of fecally contaminated food, water or through direct person-to-person or animal-to-person contact. The oocysts are infectious immediately as soon as being excreted in feces. The infectious dose is low; feeding studies have demonstrated that the ingestion of as few as 10-30 oocysts can cause infection in healthy persons. Infected persons have been reported to shed 10^8-10^9 oocysts in a single bowel movement and to excrete oocysts for up to 50 days after cessation of diarrhea (Okhuysen *et al.*, 1999).

The prevalence of *Cryptosporidium* in developing countries varies between 4-30%, while in developed countries ranges from 0.6 to 20% (Zu *et al.*, 1992 b; Das *et al.*, 1993). In Iraq, Mahdi *et al.* (1996) were first pointed to the status of cryptosporidiosis in children in Basra. In Baghdad, a prevalence rate of 6% with *C. parvum* was found (Al-Janabi, 2005), while in Arbil the rate of infection was 13.33% in children aged less than three years (Alsake, 2004).

2- Materials and Methods

A total of 250 stool samples were obtained randomly from in-patients and out-patients attending the Pediatric Teaching Hospital during the period 1st Jun. 2012 to 1st Sept. 2012. Questionnaire was organized to each patient. The population included in the present study was patients of 6 months to 12 years of age, complaining from diarrhea and abdominal pain.

All patient samples were obtained freshly and put in a dry, clean, sterile, and screw caped plastic container, and each container was labeled with number and name of the patient. Stool samples were divided into two parts: one portion was fixed and preserved in 10% formalin for direct wet mount using saline and iodine, and the other portion of stool sample was preserved without formalin, as frozen form for ELISA test, in the same time

of collection, each stool specimen was examined by the following techniques:

1- Microscopic examination: The colour, consistency, and presence of blood, mucus, ova and the parasites were recorded. Stool specimen was then inspected by using direct wet smear technique using saline and iodine solution for the presence of oocysts of *Cryptosporidium* (WHO, 1991).

A smear of stool specimen was prepared by an applicator stick, and spread by rolling the stick over the middle part of the slide. Left to dry then fixed with absolute methanol, by adding few drops (2-3 drops) and left to dry then stained with modified cold Zeihl-Neelsen (Fayer and Xiao, 2008). The slides then examined using the oil immersion lens (100X).

2- Fresh stool samples were then subjected to antigen detection for cryptosporidium using:

(Crypto Uni-Strip kit) from Coris Bio Concept C-1505 (Belgium).

3- Frozen stool samples were subjected to antigen detection for cryptosporidium using: **(Cryptosporidium II ELISA kit)** from Tech lab Cat No 30406 (USA)**.**

Statistical Analysis was done for each returned questionnaire with SPSS (Statistical Package for the Social Sciences-version16.0) package software program for statistical analysis.

Descriptive statistics (numbers and percentage) were calculated for all variables, as well as analytical statistics was done to find the relations between the variables. The relation between variables was calculated by using the appropriate statistical tests by Chi-square.

3-Results: Atotal of 250 stool specimens were examined out of which 38 (15.2%) samples were positive for the oocysts of *Cryptosporidium* using modified Z-N staining (Figure 1).

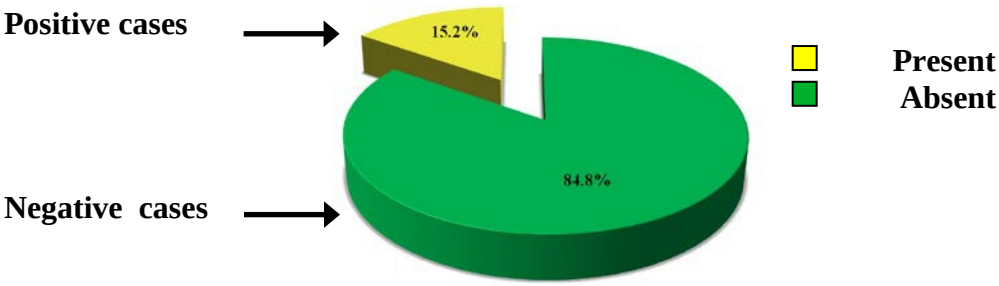

Figure 1: The rate of *Cryptosporidium* infection in Pediatric Teaching Hospital by MZN.

Over all, the prevalence of *Cryptosporidium* infection in male and female was (15.5%) male and (14.8%) respectively, with no significant difference in the total rate of infection with *Cryptosporidium* between males

and females (p>0.05), there was a highly significant difference found between the rate of infection among the children of urban areas (13.3%) and children of rural areas (32%) (Table 1).

Children of (4 - 6 years) age group showed the highest (16.7%) rate followed by the age group of (6 month to 3 years) at a rate of (15.6%), then lowest rate of infection (13% and 12.5%) was among those of (7-9) and (10-12) years of age respectively, with a highly significant difference between the rate of infection and the age groups (Table 2).

From the type of stool samples it was found that 23 samples were loose stool with prevalence rate of (17.3%), while 8 (8.3%) of them were watery and 7 (33.3%) were soft type of stool samples with a significant differences among them (p<0.05) (Table 3).

Table (1): The number of examined sample and the rate of infection with *Cryptosporidium* according to the gender and place of children in Pediatric Teaching Hospital (n= 250).

Characteristics		Exam. samples	The Result of ZN test		P-value
			Positive for *Cryptosporidium*		
		No.	No.	%	
Gender	Male	142	22	15.5	0.4899
	Female	108	16	14.8	
Place	Urban	225	30	13.3	0.0083
	Rural	25	8	32.0	
Total		250	38	15.2	

Table (2): The relationship between *Cryptosporidium* infection and the age of children.

Characteristics		Exam. samples	The Result of ZN test		P-value
			Positive for *Cryptosporidium*		
		No.	No.	%	
Age (years)	0.5-3	167	26	15.6	0.0016
	4 – 6	36	6	16.7	
	7 – 9	23	3	13.0	
	10 – 12	24	3	12.5	
Total		250	38	15.2	

Table (3): The number of examined sample and the rate of infection with *Cryptosporidium* according to type of stool

Characteristics		Exam. samples	The Result of ZN test		P-value
			Positive for *Cryptosporidium*		
		No.	No.	%	
Type of stool	Watery	96	8	8.3	0.0584
	Loose	133	23	17.3	
	Soft	21	7	33.3	
Total		250	38	15.2	

From the microscopic examination it was found that using direct wet mount by saline and iodine, the oocysts appear as a small spherical bodies and their sizes were about the size of some fungi but can be differentiated from fungi by their shapes which were oval. Also, the oocysts of *Cryptosporidium* contain granules inside them, while in iodine preparation the oocyst were colorless with the appearance of sporozoites inside some of them. (Figure 2).

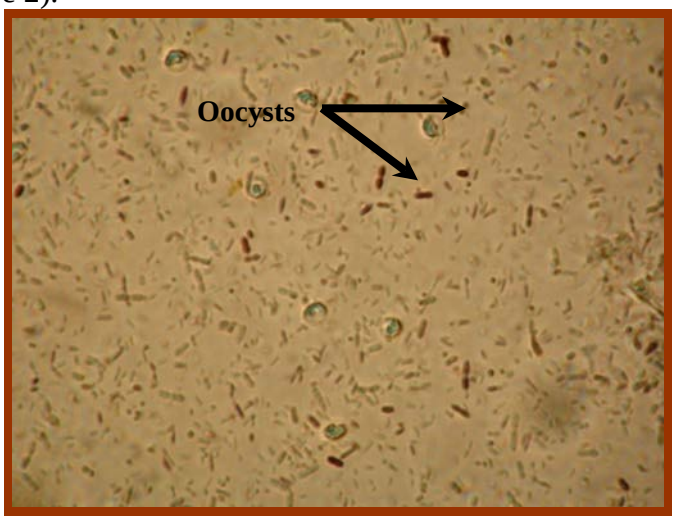

Figure (2): Oocyst of *Cryptosporidium* by direct wet mount (Iodine) with magnification 1000X.

Also from the results of using modified cold Ziehl-Neelsen stain the oocysts appear as small as 4µm, spherical in shape, stained with dark pink or red color against green background color. (Figure 3).

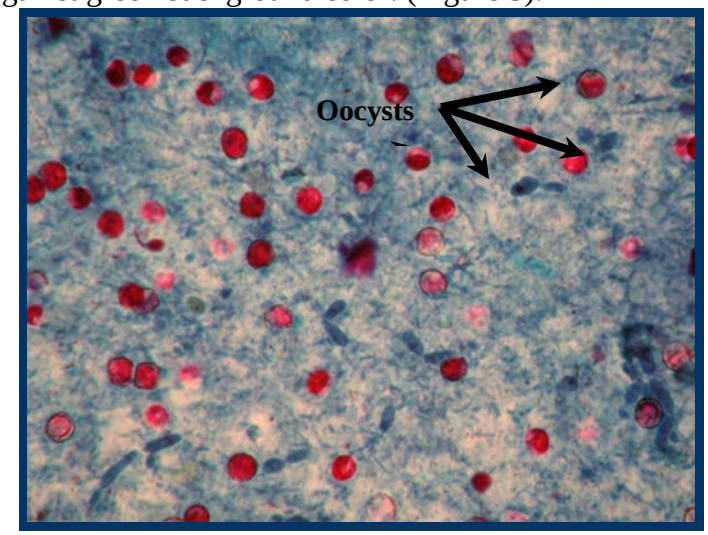

Figure (3): Oocyst of *Cryptosporidium* by Modified cold Ziehl-Neelsen stain with magnification 1000X.

The results of using the Crypto-Strip in *invitro* diagnostic test showed two red lines for positive samples while the negatives were with one red line only seen on the stips (Figure 4).

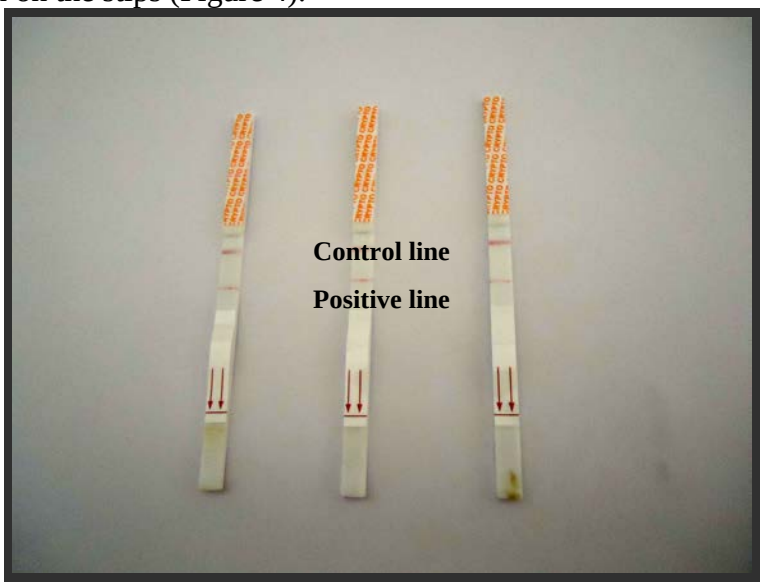

Figure (4): Positive *Crypto*-Strips which used for detection of *Cryptosporidium*.

The result of using four methods in examination of 250 stool samples recorded that the higher rate of infection was 15.2% by modified cold Ziehl-Neelsen stain then came direct wet mount method with prevalence rate of 13.6%, while by using ELISA test it was 6.8% and Crypto-Strips method 4.4% (Table 4).

Table (4): Comparison between efficacy of different methods for diagnosis of *Cryptosporidium* infection in Pediatric Teaching Hospital.

The tests	No. of examined	The Results	
		No. of Positive	
		No.	%
Ziehl –Neelsen stain	250	38	15.2
Direct wet mount	250	34	13.6
ELISA test	250	17	6.8
Crypto-Strips method	250	11	4.4

3-Discussion

The results of the present study recorded a total prevalence rate of 15.2% for *Cryptosporidium* infection in children attending the Pediatric Teaching Hospital in Sulimani city. This indicates that Sulimani city is endemic with this parasite, the endimicity may be due to contaminated food and water with the oocysts of this parasite. This rate was higher than those recorded in Diala by AL-Ta'ey (1997) and in Basra by Mahdi and Ali (2002).

Alsake (2004) in Arbil and in Baghdad by Yaqoob *et al.* (2004), Al-Janabi (2005) and Al-Warid *et al.* (2012).

The prevalence rate also was higher than those reported from some neighboring countries such as those reported by Nemri and Hijazi, (1994), Iqbal *et al.* (2001), Dabirzadeh *et al.* (2003) and Mohammadi *et al.* (2006), this may be due to: number of patient's samples in different screening studies, differences in the nature of the areas, age of patients, diagnostic methods used, living conditions, socioeconomic criteria, nutritional status, immunological status, personal hygiene and the variation of temperature between the seasons in different locations.

The data also showed no significant difference between males (15.5%) and females (14.8%) infection with *C .parvum* , although the rate of infection in males was higher than females. This may be attributed to that boys at this age possibly are more exposed to *C. parvum* oocysts because of their more activities than the girls. This result agrees with the results of Mumtaz *et al.* (2010) and El-Helaly *et al.* (2012) in Egypt, but it disagrees with the results of the studies that were carried out in Kenya by Gatei *et al.* (2006) and in Iran by Khalili and Mardani (2009).

According to the children's residence, a significant difference was found in the prevalence rate between children in rural areas (32%) and those in urban areas (13.3%). This may be attributed to increased exposure to zoonotic infections, low socioeconomic standard and close contact with animals and soil which appears to be contributing factors that increase the risks infection with *C. parvum* in rural area (Youssef *et al.*, 2008). This agree with Rahouma *et al.* (2011) and El-Helaly *et al.* (2012). This also may related to risk of exposure to contamination from their environment (food, water and toys) and the lack of self-awareness, hygienic behavior and \or contracting the infection from their household (Ben *et al.* 2002). This result was similar to that reported by Guy *et al.* (2001), and in accordance with results of Maleki *et al.* (2005); Khalili *et al.* (2006) and Mohammadi *et al.* (2006).

It was found that out of 38 positive samples, (33.3%) were soft stool, (17.3%) were from loose stool sample, and (8.3%) were watery stool, this may be because of that the patient of watery diarrhea immediately received treatment after attending the hospital (esp. children) before stool examination. Current results may showed that stool texture isn't direct evidence for infection and that's very important in epidemiological status of this disease.

The results of using wet mount preparation with *Lugol's Iodine* stain showed that *C. parvum* oocysts do not stain with iodine and appear as transparent discs (Mahgoub *et al.*, 2004); this may be due to their thick cell

wall that resists staining with iodine because the thick-walled oocysts are only shedding with the stool.

The data of comparing the four methods used in detection of *Cryptosporidium* that

ZN method showed a high sensitivity rate in detection oocysts, this may be due to the physical and biochemical characteristics of oocysts that depend on storage conditions, which may affect detection (Inoue *et al.*, 2006). Staining techniques are commonly used but may be less suitable if oocyst structure is affected, as in case of frozen samples (Ward and Wang, 2001), the low rate of infection using ELISA and Crypto-Strips method may be because of the factors that affected the detection of *C. parvum* infection by both ELISA and Crypto-Strips method such as type of kits and the differences of species strains, genotypes, and subtypes used in the kits preparation. The lowest rate reported in this study may be due to using few amount of the stool, one loop for formed stool and two loops for liquid stool samples in the case of Crypto-Strips method and dilution of stool in the case of ELISA which may not be adequate for carrying enough number of the oocysts to be detected especially when few numbers are present in the specimens. This agree with the results recorded by Weitzel *et al.* (2006) and Tabash (2009) and disagrees with the results of El-Sweify (2011) who recorded similar efficacies for comparison of an acid-fast stain and ELISA and this may be due to the high sensitivity of ELISA test and the low antigens found in the stool because of dilution.

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