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Incidence of *Cryptosporidium* Spp. among children who attend to Sebha Medical Center

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Abstract:

The present study was undertaken to investigate the prevalence of *Cryptosporidium spp.* infection among paediatric patients attending Sebha Medical Center for their health problems. A total of 150 faecal specimens from various localities of Sebha region, and 50 stool samples from normal healthy controls were screened for oocysts of *Cryptosporidium spp.* using Modified Ziehl-Neelsen and Giemsa´s staining techniques. The overall infection rate was 10%. This is the first report of *Cryptosporidium spp.* infection in Sebha. The intensity of *Cryptosporidium spp.* was slightly more in Modified Ziehl-Neelsen (10.0%) than Giemsa´s staining techniques (8.6%) and this difference was not statistically significant (P> 0.05) by analysis of variance .

In general, boys were more infected with *Cryptosporidium spp.* (5.3%) than girls (4.7 %), difference between them was not statistically significant (P > 0.05).

No, statistically significant difference of prevalence of *Cryptosporidium spp.* was found among different age groups of paediatric patients (P > 0.05). However, infection among both symptomatic (80%) and asymptomatic (20%) children and difference between them was significant (P< 0.05).

Source of drinking water was significantly (p< 0.05) associated with the incidence of Cryptosporidiosis among paediatric patients.

It is concluded that *Cryptosporidium spp.* may be an important pathogen associated with gastrointestinal symptoms among children in Sebha region, Libya.

Key words: *Cryptosporidium*, children, Ziehl-Neelsen, Giemsa, Libya

Introduction:

Cryptosporidium is an infectious Coccidian parasitic protozoon, which has ability to cause diarrheal disease in human and animal called Cryptosporidiosis [1, 2].

This parasite accidentally transmitted by the faecal-oral route through the contamination water, food, fomites and rarely by inhalation to infect the lung [3, 4]. *Cryptosporidium* occurs most frequently in young children (under age of 10 years) [1, 6].

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Cryptosporidium species is zoonotic obligate intracellular parasites can infect the epithelial cells in small intestine mainly in the jejunum and ileum of human digestive system and other mammals, then multiply and shed their oocysts in high number to the environment via feces [5].

Cryptosporidium oocysts have thick wall and resistance to chemicals, so they can survive out of the host body for months until it is start a new life cycle in a new host [1].

The symptoms of *Cryptosporidium* infection will develop usually between 1 to 12 days with the average 7 days mainly in healthy immune systems people after exposure to parasite infective stage [3, 4]. Watery diarrhea, stomach cramps, nausea, poor appetite, and weight loss are the common symptoms of this infection [6, 7]. Dehydration can be happened in children and pregnant women [3]. Some infected people may not have any symptoms or a few episode of diarrhea [2].

Children (under the age of 10 year) and people who have weak immune system are highly susceptible *Cryptosporidium* infection [3]. Healthy people generally recover within one to four weeks, while immunocompromised individuals may be develop long and sever illness [6, 7].

High percentage of *Cryptosporidium* prevalence was recorded among children 25% in Uganda [9], 21% in Pakistan [8], 17% in Egypt [10] and 2.7% in Tunis [11].

There are a few studies done in Libya about the prevalence of *Cryptosporidium* spp., Among gastroenteritis children Bugharara *et al* [12] who record 3.19% infected by *Cryptosporidium* spp., 7.54% in Benghazi [13] and 6% in Tripoli [14].

The diagnosis of *Cryptosporidium* spp. depends on detection of oocyst in the stool samples. Different methods are used for this purpose such as ELISA, Ziehl-Neelsen Method, Auramine-Rhodamine Method, and Giemsa stain Method [5]. Ziehl-Neelsen Modified still the most common method used to detection the *Cryptosporidium* Oocyst in the stool samples [15].

In the present study, attempts were made to study the incidence and possible epidemiological risk factors associated with Cryptosporidiosis among random selected pediatric patients attending to Sebha Medical Centre.

Materials and Methods:

Specimen collection:

A total of 200 stool sample were collected between December 2010 to May 2011, 150 sample from children who attend to paediatric ward in Sebha Medical Center and 50 sample (Control) from healthy children.

Stool samples were collected and preserved in 10% Formalin-saline. All information about life style, clinical status and consent were tacking from patient's family.

Examination of stool specimens:

All stool samples were prepared by formalin ether concentration methods and examined two slid after staining one by Modified Ziehl-Neelsen stain [16] and the second one by Giemsa stain [17] in order to compare the two staining methods. For the detection of *Cryptosporidium* oocysts.

Statistical analysis:

Statistical analysis was carried out by using windows (version 15.0 SPSS Inc, Chicago IL). A probability (p) value of less than 0.05 was considered as significant whenever appropriate.

Results and Discussion:

Cryptosporidium spp. is a common parasite can infect gastrointestinal tract and cause enteritis [2, 4, 11], and they are mostly isolated from stool samples of diarrheal children in many areas around the world [6].

Cryptosporidium spp oocysts were detected in 15 (10%) children stool sample from a total 150 stool sample were examined by Modified Ziehl-Neelsen stain, and 13 (8.6%) in samples

that stained by Giemsa stain (Figure 1), No, significant differences (p = 0.69) were found between the stained methods for the detection *Cryptosporidium* oocysts in both patient and control stool samples. This result is similar to *Shehata et al* [18], *Abdel-Hafeez et al* [19] and *Mathew et al* [1] results. *Garcia et al* [20] and *Perez-Schael et al* [21], who found the Ziehl-Neelsen stain is best method than Giemsa stain to detected the *Cryptosporidium* oocysts, this results is agree with our results.

The prevalence of this parasite in our study is 2% in control group by using the two staining methods. This is low percentage compared with children who attend to Paediatric Department in Sebha Medical Center. These findings were similar to Lacorix *et al* [22] and Bugharara *et al* [12], who reported a low infection of *Cryptosporidium* among normal healthy controls, While Khalil *et al* [23] and Uppal and Natarajan [24] did not record any *Cryptosporidium* oocyte in the stool samples of control group.Our results were higher than the results of Bugharara *et al* [12], Al-Hamaida *et al* [13] and Kara *et al* [14] (Table 1), and less than the percentage reported by Ali *et al* [25]. These differences may due to many factors, such as the population life style, type of drinking water and personal hygiene [2, 13]. The prevalence of this parasite in Libya is low in compared with other countries around the world such as Kuwait [26], Iraq [5] and Iran [6].

Out of the 150 study subjects, 80 were males and 70 were females (Table 2). Among the males 8 (5.3%) were positive for *Cryptosporidium* by both staining technique, and the females positive samples were 7 (4.7%) and 5 (3.3%) by using Ziehl-Neelsen stain and Giemsa stain, respectively. No, significant differences (p = 0.714) were found between the staining methods for the detection *Cryptosporidium* in both genders. Our results are similar to that were recorded by other studies [1, 5, 8]. However, *Laubach et al* [6] and *Saneian et al* [27] found a significant difference between both genders [6, 27].

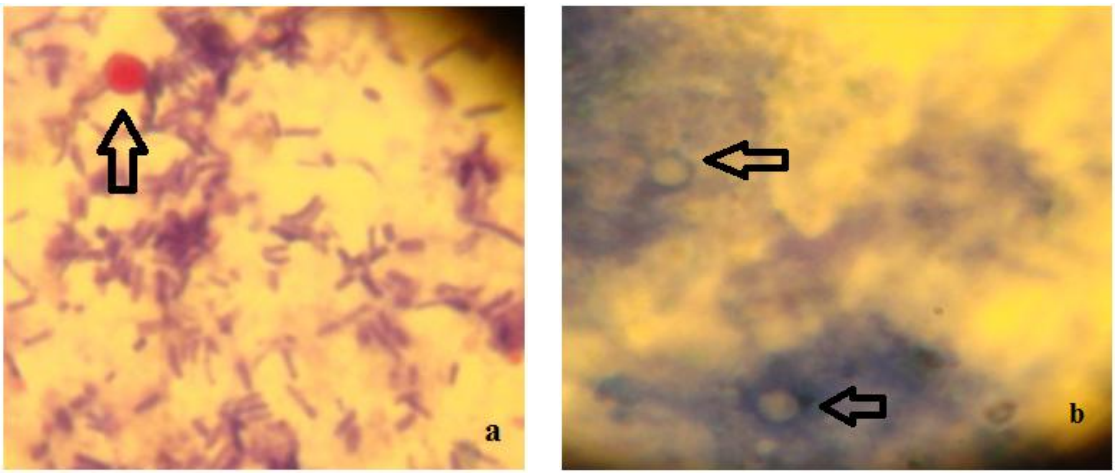

Figure 1: *Cryptosporidium* oocytes: a-by Ziehl-Neelsen stain. b-by Giemsa stain

Table 1: Studies about *Cryptosporidium* in Libya among children.

Reference	**Category**	**Prevalence (%) of *Cryptosporidium* In children stool samples**
Bugharara *et al* [12]	Children with diarrhea in Benghazi	**3.19**
Al-Hamida *et al* [13]	Children with diarrhea in Benghazi	**7.54**
Ali *et al* [25]	Children with diarrheal in Zleiten	**13.0**
Kara *et al* [14]	School children in Tripoli	**6.0**
Present (2012)	Children attending Sebha Medical Center	**10.0**

Age-wise distribution of *Cryptosporidium spp* showed the highest prevalence (4 %) in the 1-3 year and less than in other age groups (Table 2). No, significant differences were found between the age groups and two staining methods.

Many studies in the world showed that the children below 2 years of age are more susceptible to infected by *Cryptosporidium spp* [5, 8, 10], this may due to weak immune system, personal hygiene, uncontrolled contact with surrounding environment, play by contaminated soil or water and cross infection especially in children facilities [2, 6, 28, 29].

The factors associated with the presence of *Cryptosporidium spp* among children are shown in the Table 2. When these factors were correlated with the *Cryptosporidium spp*, a statistically significant relation was found between the presence of *Cryptosporidium spp* with drinking a storage water between, who drinking water after storage and from filtered water directly (P=0.004). These results were in agreement with other studies conducted worldwide [8, 13, 30, 31]. While Iqbal *et al* [8] and Al-Hamaida *et al* [13] did not found any significant differences between *Cryptosporidium* spp infection and drinking storage of water.

No, significant differences were recorded between *Cryptosporidium* infection and children with direct contact with domestic animals, and breast feeding and the other children, and between two staining methods. The finding of many different studies agree with our results in the absent of relationship between *Cryptosporidium* infection and direct contact with animals [18, 32].

The immunoglobulin transfer from mother to her children during breast feeding, so this process can protect the baby from *Cryptosporidium* infection [2, 6, 18]. In the present study, breast feeding did not reduc *Cryptosporidium* infection significantly; our results are agreed Iqbal *et al* [8] and Al-Hamaida *et al* [13] results.

Table 2: Factors associated with the prevalence of *Cryptosporidium* among children.

		Number	Number of positive samples (%) with Ziehl Neelsen Stain		Number of positive samples (%) with Gimsa Stain	
Total Number		150	15 (10%)		13 (8.6%)	
Gender						
Male		80	8 (5.3%)		8 (5.3%)	
Female		70	7 (4.7%)		5 (3.3%)	
Age groups						
Less than 1 year		81	5 (3.3%)		3 (2.0%)	
1-3		46	6 (4.0%)		6 (4.0%)	
More than 3 year		23	4 (3.3%)		4 (2.6%)	
Factors						
			Present (%)	Absent (%)	Present (%)	Absent (%)
Drinking storage water			11 (73.3%)	4 (26.7%)	9 (69.2%)	4 (30.8%)
Contact with animals			2 (13.3%)	13 (86.7%)	1 (7.7%)	12 (92.3%)
Breast feeding			4 (26.7%)	11 (73.3%)	2 (15.4%)	11 (84.6%)

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