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Cryptosporidial infection among children with diarrhea in Sebha, Libya.

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ABSTRACT

Human cryptosporidiosis is a major cause of diarrhea in developing countries and generally causes watery diarrhea in immunocompetent patients or chronic severe diarrhea in immunocompromised individuals. The present study was undertaken to investigate the prevalence of Cryptosporidium spp. infection among pediatric patients with diarrhea, attending Sebha Medical Center for their health problems. One hundred children were included in the study, in the age group from 1 to 10 years old, selected from Outpatient clinic Sebha Medical Center, Sebha. All the stool samples were screened for presence of Cryptosporidium oocysts, using Modified Ziehl-Neelsen staining techniques. Ten children (10%) in the study group were found positive for Cryptosporidium infection using Modified Ziehl Neelsen staining method. No, significant differences (x2=6.678, p = 0.410) were found between boys (7.69%) and girls (12.50%) for the detection Cryptosporidium oocysts. Significant difference (x2=18.264, p=0.021) was found between the different age groups of children for the diagnosis of cryptosporidiosis. Modified Ziehl-Neelsen staining technique was proved to be simple and useful for routine detection and identification of Cryptosporidium spp. oocysts in fresh fecal samples.

عدوى خفية الأبواغ بين الأطفال المصابين بالإسهال في سبها ، ليبيا

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الكلمات المفتاحية:
خفية الأبواغ
إسهال
سبها
ليبيا

الملخص

يعد داء خفيات الأبواغ سببًا رئيسيًا للإسهال في البلدان النامية ويسبب عمومًا إسهالًا مائيًا في المرضى ذوي الكفاءة المناعية أو الإسهال الحاد المزمن لدى الأفراد الذين يعانون من نقص المناعة. أجريت هذه الدراسة للتحقيق في انتشار طفيليات الأبواغ. تم تضمين مائة طفل في دراستنا مترددين على المختبر المرجعي سبها ومصابين بالاسهال ، في الفئة العمرية من 1 إلى 10 سنوات. تم فحص جميع عينات البراز بحثًا عن وجود الكيسة البيضية للكريبتوسبوريديوم، باستخدام صبغة Ziehl-Neelsen المعدلة. تم العثور على عشرة أطفال (10 ٪) في مجموعة الدراسة إيجابية لعدوى خفية الأبواغ. لا توجد فروق ذات دلالة إحصائية (P = 0.624) بين الأولاد (2.08٪) والفتيات (2.88٪). لم يتم العثور على فروق ذات دلالة إحصائية (P = 0.37) بين الفئات العمرية المختلفة للأطفال لتشخيص مرض كريبتوسبوريديوسيس. تعد عدوى خفيات الأبواغ أحد الأسباب المهمة للإسهال بين الأطفال ، وقد ثبت أن تقنية الصبغ بصبغة Ziehl-Neelsen المعدلة مفيدة في تشخيص الكيسات البيضية للكريبتوسبوريديوم في عينات البراز الطازجة.

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Introduction

Cryptosporidium spp. is an important opportunistic obligate, zoonotic intracellular protozoa, causes gastroenteritis in both adults, and children worldwide. The disease is self-limited in immunocompetent persons but potentially posing life-threatening diarrhea in immunocompromised individuals (23).

Microscopic examination of stool using acid fast staining, with or without stool concentration, is the most frequently applied screening technique for cryptosporidiosis. Acid-fast staining microscopy is cheap and accessible in poor rural settings; it is the best parasitological method in terms of sensitivity and specificity and shows good performance when compared with immunological and molecular methods for the identification and classification of Cryptosporidium oocysts (35).

By 2016, Cryptosporidium was the fifth leading cause of diarrheal mortality, responsible for approximately 44.8 million episodes of diarrhea and 48,300 annual deaths in children below the age of five [17]. Cryptosporidial infections are higher in tropical regions (0.5 to 19.1%) than in temperate regions (up to10%) among hospitalized pediatric diarrheal in different parts of the world [23] [26] [34] [2] [16]. Cryptosporidium is second only to rotavirus as a contributor to moderate-to-severe diarrheal disease during the first 5 years of life [18]. It has been estimated that 2.9 million Cryptosporidium-attributable cases occur annually in children aged < 24 months in sub-Saharan Africa [33] and infection is associated with a greater than two-fold increase in mortality in children aged 12 to 23 months [18].

The infective stage of Cryptosporidium spp., is known as oocysts, which are shed in the feces of a host and are immediately infectious to subsequent hosts. Routes of transmission of cryptosporidiosis include waterborne, person-to-person (i.e., the fecal-oral route), zoonotic and foodborne [27]. Numerous outbreaks have occurred worldwide as a result of contamination of drinking water with the oocysts of Cryptosporidium [7]. Direct person-to-person transmission may occur following the ingestion of oocysts in fecal matter, and is associated with poor hygiene. In the case of zoonotic species of Cryptosporidium, such as C. parvum, calves, rodents, puppies, kittens, and many other animals serve as important reservoir hosts in zoonotic transmission [27].

In some countries of the Arab world, Cryptosporidium species are shown to be an important cause of diarrhea in pediatrics and in immunocompromised patients [1] [32] [8] [9] [31] [24].

In Libya, Cryptosporidium has been diagnosed either alone or along with other intestinal parasites in various population groups, mostly among children. Modified acid-fast staining microscopy has frequently been used as the diagnostic method of choice. Cryptosporidiosis risk factors have been reported in Libya, including source of drinking water, contact with domestic animals and breast feeding (20).

There are a few studies about the prevalence of Cryptosporidium spp. done in Libya. [6] Recorded 3.19% infection of Cryptosporidium among children with gastroenteritis in the city of Benghazi. [3] Reported 7.54% Cryptosporidial infection in Benghazi among the children with diarrhea. [4] Found 13% cryptosporidiosis in children with diarrhea in Zliten. Comparatively low infection rate (0.9 to 2.1%) of Cryptosporidium spp. has been reported in school children in Tripoli, and Tripoli surrounding areas of [5] [25]. Kara et al [15] reported 6% infection of Cryptosporidium among school children in city of Tripoli. Lower incidence (2.3%) of this parasite has also been reported among adults and children in Brack, Wadi Al –Shati [22]. A study performed in Sebha city [20] showed 10% infection of Cryptosporidium spp. among children suffering from diarrhea . Saaed and Ongerth [29] reported 4% incidence of Cryptosporidium spp. in the stool samples of children with diarrhea in the Kufra city. In another study in the city of Kufra [30], observed that highest contamination of different fresh salad vegetables with both Cryptosporidium (80.8%) and Giardia (84.4%) and indicated that contamination of raw salad vegetables may cause a health risk to consumers of such products which are sold in Kufra city.

In the present study, Ziehl -Neelsen method was used to diagnose infection of Cryptosporidium spp. among children with diarrhea, who, were attending to Sebha Medical Centre, for their treatment.

Patients and Method:

Specimen collection:

This study was conducted on 100 children (52 boy and 48 girls) in Sebha city who, were attending Sebha Medical Center for their treatment. A stool sample was collected in screw capped plastic containers from each patient between June 2020 to October 2020 and specimens were preserved directly in 10% Formalin-saline. We collected demographic characteristics such as gender, age, area of residence and clinical data of each patient in the presence of clinicians in outpatient door of hospital.

Examination of stool specimens:

All stool samples were concentrated by formalin ether concentration and stool smears were stained in Modified Ziehl-Neelsen stain method was used [13] [10] for the detection of Cryptosporidium oocysts.

Data analysis:

Statistical analysis was performed with SPSS software version 16 (IBM, Chicago, IL, USA). Probability values were considered to be statistically significant when the calculated p-value was equal to or less than 0.05. The difference in parasitic contamination among the different categories was compared using the Pearson'schi-square test (x2).

Results

In the present study, Cryptosporidium oocysts were detected in the stool specimens using Zieh Neelsen staining method. Of 100 stool samples, only 10 children (four boys and six girls) showed infection of Cryptosporidium and results are presented in Table 1. There was insignificant difference observed in the infection rates of cryptosporidiosis between boys and girl (p=6.678). Age wise, infection of Cryptosporidium among children is shown in Table 2. A significant difference (x2=18.264, p=0.021) was found between the two age groups of children (21.87 % in 1to5 years and 4.41% in 6 to 10 years of age). Prevalence of cryptosporidiosis in different cities in Libya is presented in Table 3. Figure 1 showed oocysts of Cryptosporidium spp. stained with Ziehl Neelsen .

Table 1: Infection of Cryptosporidium among children with diarrhea.

No. Examined		No.Positive(%)	
Boys	Girls	Boys	Girls*
52	48	4(7.69)	6(12.52)

*x2 =6.678, p =0.410

Table 2: Cryptospridium infection among children according to age groups.

Age(Y)	No. Examined	No. Positive(%)
1-5	32	7(21.87)*
6-10	68	3 (4.41)

* x2 =18.264, p=0.021

Table 3: Prevalence of Cryptosporidium in different Libyan cities/ localities.

City	Prevalence (%)	Reference
Benghazi*	3.19	Bugharara *et al* (6)
Benghazi*	7.54	Al-Ahmaida *et al* (3)
Zilten*	13.0	Ali *et al* (4)
Tripoli *	1.0	Ben Rashed *et al* (5)
Tripoli and surrounding areas**	0.9	Ben Rashed *et al* (5)
Tripoli**	6.0	Kara *et al* (15)
Brack**	2.5	Saada *et al* (28)
Tripoli*	2.1	Rahouma *et al* (25)
Sebha*	10.0	Mabrooka *et al* (20)
Brack**	2.	Mergani *et al* (22)
Kufra*	4.0	Saaed and Ongerth (29)
Sebha *	10.0	Present study

*= Subjects with gastroenteritis
**=Subjects without gastroenteritis

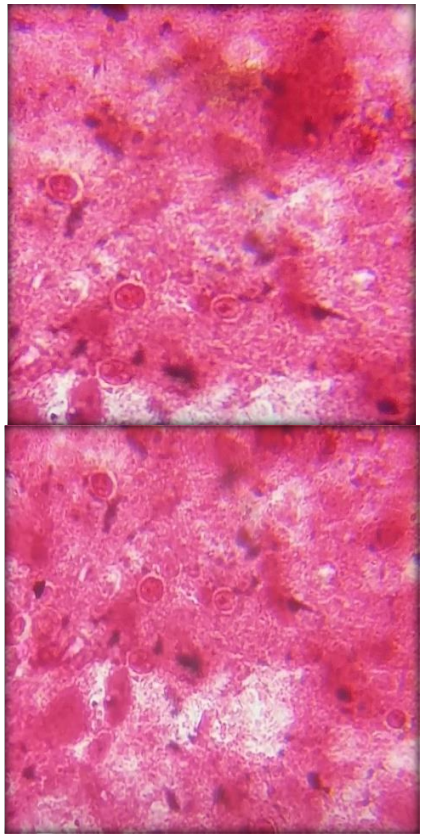

Fig.1: Cryptosporidium oocysts with Modified Ziehl Meelse

Discussion:

In Libya, human cryptosporidiosis has been investigated either alone or along with other intestinal parasites mostly among children attending hospital for their treatment [6] [4] [5] [20]. [4] Reported Cryptosporidium infection is one of the important causes of diarrhea in children below five years of age.Cryptosporidiosis has been reported in both patients with gastroenteritis, prevalence rate varies from 0.9 to13% with mean 3.4% [4] [5] and patients without gastroenteritis, incidence varies from 1 to 2.5% with mean 1.7% [5] [28] in different cities and localities in Libya.

In the present study, Cryptosporidium infection was diagnosed in 10% of cases presented with diarrhea. Similar results were found in another study in Sebha [20], who reported 10% infection of cryptosporidiosis among children presented with diarrhoea. Our results showed higher infection rate of Cryptosporidium spp. than the results of [6] [15] [22] [29] among children in the city of Benghazi, Tripoli , Brack Wadi Al-Shati and Kufra respectively. However, Ali et al [4] observed a slightly higher (13%) Cryptosporidium infection among children with diarrhoea in the city of Zliten. Moreover, another study in the Kufra city [30] found that 80.8% raw salad vegetables were contaminated with Cryptosporidium oocysts and may cause a health risk to consumers of such products .They suggested that the highest contamination of fresh salads with this parasite is likely caused by irrigating crops with fecal contaminated water. Out of the 100 studied subjects, 52 were boys and 48 girls. Among the boys, 4 (7.69%) and girls, 6 (12.5%) showed cryptosporidial oocysts. No, statistically significant difference (p = 0.410) was found for the detection Cryptosporidium in both gender. These results were similar that were recorded in other studies [31] [25] [21] [20] [17].

Age-specific distribution of Cryptosporidium spp in this study, showed significant differences (p=0.210). In the present study higher prevalence was found 1 to 5 years of age. These results were not in agreement with other studies conducted in Libya, who did not find differences in the prevalence of cryptosporidiosis in the different age groups of children [6] [3] in Benghazi city.

Finding of Cryptosporidium oocysts in the stool specimens in the present study is most probably the cause of their diarrhea among children. Similarly, several studies stated that Cryptosporidiu is an important enteric agent associated with diarrhea in children in Libya [3] [4] [16] [29].

Data of this study indicates Cryptosporidium spp. may be playing a minor role in causing diarrhea among the pediatric population mainly under the age of 10 years. The presence of this infection in children can be threatening to other susceptible people in this region. The detection of cryptosporidial infection among population with acute or chronic diarrhea is important for epidemiological, parasitological and control management point of view.

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