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Cryptosporidiosis among children with diarrhoea in three Asian countries: A review

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ABSTRACT

This review focuses on studies concerning cryptosporidiosis in three Asian countries. *Cryptosporidium* spp. infection was investigated in children < 12 years old afflicted with diarrhoea and admitted to the paediatric hospitals in Iraq, Jordan and Malaysia. Most of the patients complained of abdominal pain, watery diarrhoea and mild-to-severe dehydration. Stool samples were collected from children and five methods were used to detect oocysts of *Cryptosporidium* spp. including: direct wet mount, Sheather's sugar flotation, formalin-ether sedimentation, modified Ziehl–Neelsen and direct fluorescent antibody (DFA). The infection rate was 8.56, 37.3 and 4.6 in Iraq, Jordan and Malaysia, respectively. A combination of formalin ether sedimentation and acid fast stain was used to detect *Cryptosporidium* oocysts in Iraq. The DFA test showed the highest sensitivity for samples of children in Jordan. In Malaysia, direct wet mount, formalin-ether sedimentation, modified Ziehl–Neelsen and DFA gave the same results (4.62%) while Sheather's sugar flotation was 3.85%. Source of drinking water appeared to be an important risk factor in transmission of infection. In Jordan, the high rate of infection was recorded in rainy season (January–May).

1. Introduction

Cryptosporidium is a coccidian protozoan parasite found in the brush-border of the enterocytes of the small intestine in many vertebrates, including humans [1]. Cryptosporidiosis is recognized as a cause of diarrhoeal illness in man and animal [1]. The first case of human cryptosporidiosis was reported in 1976, and there are increasing numbers of documented infection with *Cryptosporidium* spp. since then. Now, it is considered a common enteric pathogen in humans and animals worldwide [2]. Cryptosporidiosis can induce self-limiting diarrhoea in immunocompetent people or severe and prolonged diarrhoea in immunocompromised patients, such as those with AIDS, transplant recipients, those are receiving chemotherapy for cancer, and patients with immunosuppressive infectious disease [3]. In developing countries, *Cryptosporidium* mostly infects children below five years of age and peaks in children below two years of age [4,5]. However, in industrialised countries, cryptosporidiosis also occurs in adults due to foodborne or waterborne outbreaks [6,7].

Diagnosis of the infection requires the detection of the oocysts in stool. Owing to the small size (4–6 μm) of the oocysts, the routine wet mount preparation and concentration methods have limited value for detection of oocysts in stool samples, which can easily be confused with other materials [8].

In Iraq, cryptosporidiosis was reported in children with severe diarrhoea and dehydration. Latif (unpublished data), Mahdi *et al.* [9] and Mahdi and Ali [10] showed that the infection rate in children under five years was 8.56%, 8.8% and 9.7% respectively. *Cryptosporidium* spp. oocyst was identified using modified Ziehl–Neelsen staining method.

In Jordan, Nimri and Batchoun found that 2.5% of asymptomatic children in the 6–14 years old age group were infected with *Cryptosporidium* spp. and an infection rate of 1.5% in symptomatic children with diarrhoea and other clinical symptoms [11]. Another study on elementary school children found the infection rate to be 7% [12]. The highest reported prevalence of cryptosporidiosis in Jordan is 37.3% and was found in paediatric patients [13].

In Malaysia, the documented prevalence of *Cryptosporidium* infection ranged within 0.9%–23% [14–21]. However, very few of these studies have focused solely on paediatric cases, with HIV-positive intravenous drug user accounting

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for the highest prevalence (23%). Using both microscopy and PCR techniques, Menon *et al.* showed that 0.9% of children hospitalized with acute diarrhoea in Kelantan was positive for *Cryptosporidium parvum* [14]. Ludin *et al.* reported a prevalence of cryptosporidiosis of 4.3% in Penang [15]. The reported figures in Malaysia are relatively low compared to Jordan (37.3%) [13], Nicaragua (35.7%) [22], and Ethiopia (12.1%) [23].

Now, there is no doubt that cryptosporidiosis poses a threat to health in both human and animals worldwide [24]. The aim of this review is to highlight on the prevalence of cryptosporidiosis among children with diarrhoea in three different Asian countries (Iraq, Jordan, and Malaysia). In addition, the review was focused on some epidemiological factors concerned with the infection.

2. Source of infection

Drinking water plays an important role in the transmission of infection. In Iraq, it is common practice to store commercial waters in barrels and containers for a few days before consumption [10]. This might lead to exposure to viable oocysts in the environment, thus contaminating the water. Many people in Jordan depended on untreated rainwater collected directly from the roof, then stored in metal or cement tanks. Treated water from water treatment plants sometimes got contaminated with burst sewage pipes due to bombings of streets and buildings in Iraq. Moreover, oocysts of *Cryptosporidium* spp. can survive in chlorine used for water treatment [25]. The low prevalence of infection in Malaysia could be attributed to the use of treated water, and the absence of household pets. Treated water supplies were available to approximately 99% of urban and 91% of rural populations [26]. Earlier studies in these areas have revealed no oocysts in treated water [27,28], although they were found in filter backwash water samples from treatment plants. Studies from other countries had reported high prevalence of *Cryptosporidium* spp. oocysts in water from wells and springs [13,22]. Other possible methods of transmission in Iraq are contacted with person suffering from diarrhoea, association with dogs and cats, or consumption of untreated milk [9]. People living in urban areas with proper amenities and no domestic pets, it is logical to conclude that the source of infection is probably anthroponotic. However, nothing concrete can be proven without further testing using genotyping method or molecular identification of species.

Seasonal or temporal trends associated with increased incidence vary from country to other. In Central America, South Africa, and India, the peak of incidence was reported in rainy season [29]. This review showed that the results were similar to that conducted in Kuwait regarding the seasonality of cryptosporidiosis in Kuwaiti children. The results of that study showed that the maximum numbers of cases were recorded during the rainy seasons [30].

3. Diagnosis

Stool samples were collected from children < 12 years old who were suffering from abdominal pain, watery diarrhoea and dehydration admitted to hospitals in Iraq (500/province), Jordan (300), and Malaysia (130). Up to five different methods were used for diagnosis of infection, including direct wet mount, Sheather's sugar flotation, formalin-ether sedimentation, modified Ziehl–Neelsen staining and direct fluorescent antibody (DFA).

In direct wet mount preparation, *Cryptosporidium* oocysts appeared spherical in shape with a thick cell wall and diameter ranged from 4 to 6 μm. In modified acid-fast, oocysts stained pinkish red with blue or green background, depending on the counter stain used. In fluorescent assay, oocysts fluoresce an apple green colour over a dark background.

4. Prevalence

The infection rate of cryptosporidiosis in children in Iraq was 8.6%–9.7% [9,10]. The high rate was in Baghdad (14.60%) and the lowest was in Babylon (2.20%) (Latif, unpublished data) (Table 1). These studies used direct wet mount and modified Ziehl–Neelsen staining method. Mahdi and Ali reported that combination of formalin-ether sedimentation and modified acid-fast stain methods has been regarded with high sensitivity and specificity, since it both concentrates the oocysts and differentiates them from other faecal matters [10].

In Jordan, out of 300 stool samples, 112 (37.3%) were positive for *Cryptosporidium* sp. Direct fluorescence test gave the highest rate of positive samples (37.3%) and statistically the most sensitive compared with the other diagnostic methods (Figure 1).

In Malaysia, out of 130 stool samples, 6 (4.62%) samples were positive for *Cryptosporidium* sp. All the children with positive results were below 4 years of age. Regarding the five tests used, direct wet mount, formalin-ether sedimentation concentration, modified Ziehl–Neelsen staining, and direct monoclonal fluorescent antibody tests gave the same result (4.62%) while Sheather's sugar flotation detect only positive in 5 children (3.85%). The comparative result between the five methods is

Table 1
Prevalence of *Cryptosporidium* spp. oocysts in stool samples from children in Iraq.

Province	No of positive samples	%
Niniva	72	14.40
Baghdad	73	14.60
Diala	14	2.80
Babylon	11	2.20
Basra	44	8.80
Total	214	8.56

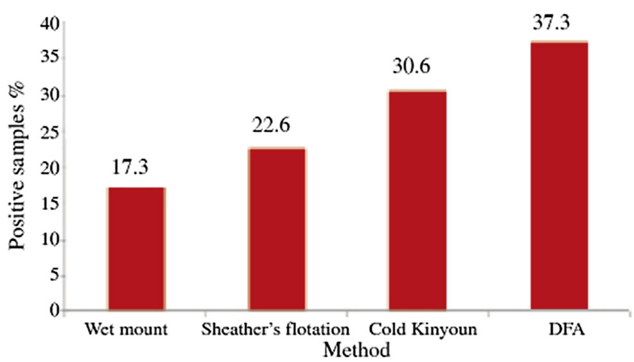

Figure 1. Comparison of four laboratory methods for identification of *Cryptosporidium* sp. oocysts in stool samples from children in Jordan [13].

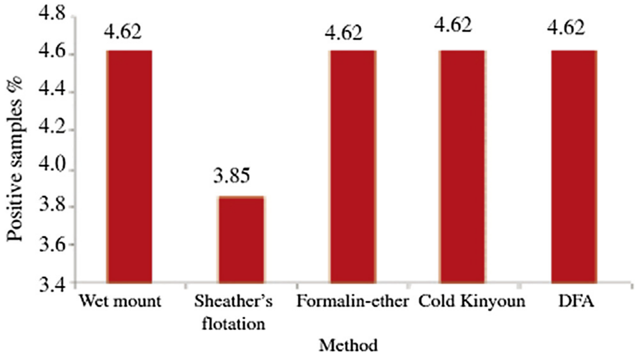

Figure 2. Comparison of five laboratory methods for identification of *Cryptosporidium* sp. oocysts in stool samples from children in Malaysia [21].

illustrated in Figure 2. There was no difference in the distribution of cases between males and females. All infected individuals lived in urban areas. These areas were supplied with tap water from water treatment plants and have structured plumbing. Four (66.67%) of the positive cases are Malays, while the rest are Chinese and Indians. None of the families owned animals (Table 2).

Jordan showed a high rate of infection (37.3%) followed by Iraq (8.6%) and Malaysia (4.62%). Although this figure is higher than those previously reported in Jordan [11,12], it was attributed to the specificity of tests used for detection of oocysts in stool samples.

In Malaysia, the prevalence of cryptosporidiosis in this study (4.62%) is similar to that of previous reports where the infection rate ranges between 0.9% and 11% [14–17,20]. A majority of infected cases were children < four years of age. This is in agreement with other Malaysian studies [14,16]. In Ireland [31], China [32] and Bangladesh [33], the highest frequency of cryptosporidiosis were reported in children below three years of age.

The infection rate of cryptosporidiosis varied according to the number of samples, diagnostic tests, availability of facilities and reporting systems. Infection rate of 13.5%–19.5% have been reported in Egypt [34], and 10% in Kuwaiti children [30]. Very high rates have been reported in Bedouin children (48%) [35], in Texas–Mexico border (70.2%) [36] and from the Republic of Korea (57%) [37].

In Jordan, the direct wet mount showed the lowest number of positive samples (17.3%) in comparison with other methods [38]. Positive samples increased to 22.6% by flotation concentration method. This method necessitated reading the results within 15 min of preparation because the oocysts tend to shrink and disappear if left for a long time. Moreover, the presence of Sheather's sugar solution inhibits the staining procedure [39].

Table 2

Demographic data of *Cryptosporidium*-positive children in Malaysia [21].

Age	Gender	Race	Locality	Source of water	Presence of animals
6m	Female	Malay	Urban	Tap water	Nil
1y 4m	Male	Malay	Urban	Tap water	Nil
1y 7m	Male	Indian	Urban	Tap water	Nil
1y 9m	Female	Malay	Urban	Tap water	Nil
2y	Female	Chinese	Urban	Tap water	Nil
3y 4m	Male	Malay	Urban	Tap water	Nil

m: Month; y: Year.

The acid-fast staining technique showed a higher rate of infection (30.6%). Oocysts appeared red to pink colour. DFA gave the highest rate of positive samples (37.3%). This method showed high sensitivity and was able to detect oocysts even when present in low number and large number of samples could be scanned.

Meanwhile, the outcome for the different techniques did not differ much in Malaysian study. The five methods showed the same results except for Sheather's sugar flotation method, which detect one less positive than others. This may be due to the distortion of oocysts over time because of osmotic effects, making it harder to identify. The best way that we would recommend to screen for *Cryptosporidium* spp. in hospitals is by using modified Ziehl–Neelsen staining since it is cheap, rapid and easy to interpret. DFA could be used to confirm the acid-fast positive samples or to diagnose cases of persistent diarrhoea with unknown cause.

Although some studies reported discrepancies in detection of oocysts between direct microscopy, concentration technique, acid-fast staining and DFA [13,40–42], it is also not uncommon for the results to be similar [43]. Menon *et al.* found that the prevalence of cryptosporidiosis by modified Ziehl–Neelsen was similar to that by PCR assay, which was considered more sensitive than conventional microscopy [14].

5. Conclusion

The infection rate was 8.56, 37.3, and 4.62 in Iraq, Jordan and Malaysia, respectively. A combination of formalin ether sedimentation and acid fast stain was used to detect *Cryptosporidium* oocysts in Iraq. DFA test showed the highest sensitivity for samples of children in Jordan. In Malaysia, direct wet mount, formalin-ether sedimentation, modified Ziehl–Neelsen and DFA gave the same results (4.62%) while Sheather's sugar flotation was 3.85%. Source of drinking water plays an important role in the transmission of infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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