https://doi.org/10.31871/IJNTR.5.9.3
International Journal of New Technology and Research (IJNTR)
ISSN: 2454-4116, Volume-5, Issue-9, September 2019 Pages 14-26

Prevalence of Intestinal Cryptosporidiosis in Malaysian Children with Malignancies

Lubna Mohamed Elbeshti, Fawzia Shawesh, Altayeb Elazomi, Rukman Awang Hamat

***Abstract*— *Cryptosporidiumparvum* is an opportunistic parasitic agent that has a world-wide distribution. This parasite can be severe and very difficult to manage in immunocompromised patients especially in children with malignancies. However, data on immunocompromised children in Malaysia is very much lacking. A cross-sectional study was conducted over ten months in Institute of Pediatrics, Hospital Kuala Lumpur. A self-administered questionnaire was used and medical records were obtained. Stool samples were examined for the *Cryptosporidium* oocyst by using two different techniques i.e. modified Ziehl-Neelsen stain and Immunochromatographic (ICT) assays (RIDA-Quick *Cryptosporidium* R-Biopharm, Germany).One hundred and five stool samples were collected from children with different types of malignancies. All stool samples were negative for *Cryptosporidium*oocysts by two different techniques. (33.3%) from those patients had history of admission to other wards, (29.5%) had history of animal contact, (24.8%) had history of swimming in public swimming pool. In terms of precautionary measures practiced, (80.9%) and (75.2%) washed their hands before and after eating, or after going to the toilet respectively. In addition, preventive measures that were also observed: (16.2%) had history of admission to day-care center, (2.9%) had history of drinking tap water, and (0.9%) had history of travel. This study documented a zero prevalence rate of cryptosporidiosis amongst children with malignancies despite higher prevalence rates being reported in other developing countries. Our results may suggest that the children with malignancies are at low risk of acquiring cryptosporidiosis because of good personal hygiene, good infection control and practices in the hospital, and improve water supply system.**

***Index Terms- Cryptosporidium*,Cryptosporidiosis, immunocompromised,. Ziehl-Neelsen, opportunistic.**

I. INTRODUCTION

Cryptosporidiosis, which is caused by an intracellular protozoa of the genus *Cryptosporidium*, is still considered as an emerging infectious disease and causing of a wide spectrum of clinical diseases in both immunocompetent and immunocompromised individuals worldwide. Historically, the first case of human cryptosporidiosis was reported in a young child who presented with self-limited enterocolitis in 1976 (Nime*et al*., 1976). Heighten attention on the importance of the disease derived from several reported cases of severe infections with significant morbidity and mortality in AIDS epidemics in 1980's (Current, 1984; Casemore*et al*., 1985), and later from a massive outbreak in Milwaukee, Wisconsin in 1993 where 403 000 people were involved (MacKenzie*et al*., 1995). Now, epidemiological data on the prevalence of cryptosporidiosis and its transmission in HIV patients has been rapidly accumulating, and several risk factors have also been identified as an important transmission route for cryptosporidiosis.

The prevalence of cryptosporidiosis in HIV patients with diarrhea has been reported to range from 0 to 100% (Hunter & Nichols, 2002). The differences in the prevalence of cryptosporidiosis are believed to be due to differences in the study methodology, geographical location, type of study population, and sensitivity and specificity of laboratory methods or stage of the disease (Hunter & Nichols, 2002).

Direct contact with water contaminated with faeces or droppings of infected humans or animals, or hand-to-mouth transfer of oocysts from contaminated food or surfaces have been implicated in transmitting the parasite (Sulaiman*et al*., 1998; Quiroz *et al*., 2000; Havelaar*et al*., 2000). Recently, the use of highly active antiretroviral therapy (HAART) in HIV/AIDS patients has been associated with complete eradication of *Cryptosporidium* in gastrointestinal tract by immune reconstitution. The underlying mechanism for this is thought to be directly related to the replenishment of $CD4^+$cells in AIDS patients, rather than due to anti-parasitic activities of these drugs (Carr *et al*., 1998; Miao et al.,)).

Despite the general consensus on the opinion regard ıe devastating consequences of *Cryptosporidium* infection in patients with HIV/AIDS and the importance of preventing them from infection, it does not seem to be a shared understanding of the risks to other groups of immunocompromised patients, especially children with malignancy. In these patients, cryptosporidiosis can also be severe and even fatal (Hunter & Nichols, 2002).

There are relatively few studies, which investigate on the prevalence of *Cryptosporidium* in children with cancer in developing countries. Moreover, the epidemiology and clinical characteristics of cryptosporidiosis in these children in those countries are still scarce and probably under-reported. For instance, the only available data from Malaysia was published in 1999, which reported only 2% of children with cancer had cryptosporidium oocysts (Menon *et al*., 1999). In India, the prevalence of cryptosporidiosis in children with malignancies was 0.3% and 1.3%, respectively (Rudrapantna*et al*., 1997; Sreedharan, *et al*., 1996).

Nonetheless, higher prevalence of cryptosporidiosis (22%) was reported in Iran among the similar immunocompromised group (Berenji*et al*., 2007).

Interestingly, *Cryptosporidium*oocysts can be detected in asymptomatic children with cancer. This was reported in 22% of those children with different types of cancers in the United States (Pettoello-Mantovani*et al*., 1995). In addition, the parasite was also detected in 6.4% of healthy children in that

Lubna Mohamed Elbeshti, Department of parasitology, Faculty of Medicine, University of Zawia, Libya.
Fawzia Shawesh, Department of Medical Laboratories, Faculty of Medical Technology, University of Zawia, Libya .
Altayeb Elazomi., Department of Medical Laboratories, Faculty of Medical Technology, University of Zawia, Libya
RukmanAwang Hamat, Department of Faculty of Medicine and Health Sciences ,University Putra Malaysia

country. Thus, this can be a potential reservoir for disease transmission among the patients. Previously, nosocomially acquired cryptosporidiosis has been reported in hospital staff (Koch *et al*., 1985; Martino *et al*., 1988; Ravn*et al*., 1991; Casemore*et al*., 1994), and spread from a patient to another patient has also been documented (Unga*et al*., 1990; Ravn*et al*., 1991; Casemore*et al*., 1994). However, this does not attract much attention from the researchers. Thus, considering the characteristics of this parasite such as its robustness towards chlorine and acid (Carpenter *et al*., 1999; Dillingham *et al*., 2002), and low infective dose that can probably be as low as 10 oocysts are required for inducing infection (Okhuysen*et al*., 1999), one could imagine how such high numbers of immunocompromised children in a close setting such as in hospitals would be affected during nosocomial outbreaks. This further complicated by the absence of effective treatment regimen for the disease in immunocompromised children with cancer. Henceforth, in this current study, the object is to determine the prevalence and contributing factors of cryptosporidiosis among children with different types of malignancies and to investigate factors that might contribute to this infection in a single centre hospital. Thus, preventive measures can promptly be applied to minimize the risk of infection and limit the spread of the disease within this group of children. The main aim of this study was to estimate the prevalence of *Cryptosporidium*in children with different types of malignancies. Also to identify contributing factors involved in the acquisition of *Cryptosporidium*oocysts among children with different types of malignancies.

II. MATERIAL AND METHODS

Study Design and Location

The study was designed for a cross sectional study in Oncology ward, Institute of Pediatric, Hospital Kuala Lumpur. As well as in Laboratory of Parasitology of Faculty of Medicine and Health Sciences, UPM and Laboratory of Parasitology, UKM. This study was conducted over 10-month durations from November 2009 until August 2010. One hundred and five stool samples were collected from children (56 boys, 49 girls) between the ages of 3 months and 17 years (mean age: 2 years). The majority of those children were the Malays (75.2%), followed by the Chinese (11.4%), Indians (8.6%) and others (4.8%).Most of these children have different types of malignancies.The most common type of lympho-hematopoietic malignancieswas acute lymphoblastic leukemia (38.1%), followed by acute myeloid leukemia (8.6%), suspected leukemia (8.6%), lymphoma (7.6%), and chronic myeloid leukemia (1.9%). Whereas, among non-lympho-hematopoietic malignancies, brain tumor represented 11.4% of cases, followed by retinoblastoma (5.7%), hepatoblastoma (3.8%), Wilm's tumor (2.9%), pleuropulmonaryblastoma (1.9%) and right adrenal cortical tumor (0.9%). All other the information got from medical records. It was included type of malignancies, laboratory parameters, and treatment.

Fresh stool samples were collected from patients into wide mouth screw cap clean containers.

Stool samples were transported in a cool box to Laboratory of Parasitology, Faculty of Medicine and Heath sciences, UPM, and divided into two parts, first part for Immunochromatographic (ICT) assays, i.e. RIDA-Quick *Cryptosporidium* (R-Biopharm, Germany). Second part frozen at -20 ºC and kept until transported to Laboratory of Parasitology, UKM for staining.

A self administered questionnaire was given to one parent or guardian after stool sample was taken from the patients. The questionnaires consist of four parts,

i. The first part consists of sociodemographic data of the patient such as age, gender, ethnicity, number of sibling. The age of the patients was categorized.
ii. The second part of questionnaire consists from five parts. This was about the information on the patient. This includes main complaint at admission, duration of admission (date of admission, date of discharge), history of underlining medical illness prior to admission, history of malignancy.
iii. The third part of questionnaires consists of eight parts. This was about the symptoms related to intestinal cryptosporidiosis. This includes fever, diarrhea, foul smelling stool, stool mixed with mucus, stool mixed with blood, vomiting, nausea, cramping abdominal pain.
iv. The fourth part of the questionnaire consists from eight parts. This was about the risk factors of intestinal cryptosporidiosis. Which includes source of drinking water, washing the hands before and after eating, washing the hands after using the toilet, history of swinging in swimming pools, history of animal contact, history of admission to day-care centers, history of admission to other wards and history of recent travel.

Immunochromatographic (ICT) assays RIDA- Quick *Cryptosporidium* (R-Biopharm, Germany) procedure

The procedure was done according to manufacturer's guideline. About 50mg of fresh stool sample was placed into a tube containing 1ml of extraction buffer then homogenized on a vortex mixer.

The sample was allowed to settle done for 3 minutes. A red test band along with the blue control band was positive. Whereas the blue control band appears negative.

Statistical Methods

Data analysis was performed by using Statistical Package for Social Sciences (SPSS) version 18. All important data were entered and have been calculated. Clinical parameters were obtained from medical records. We could not determine the association between the variables according to the negative results.

III.RESULTS

Detection of Intestinal Parasites in Stool samples Collected from Children with Malignancies

Of 110 stool samples collected, no *Cryptosporidium*oocysts (0%) were detected through modified Ziehl-Neelsen Stain and RIDA-Quick *Cryptosporidium* (R-Biopharm, Germany) in this study. Similarly, none of 97 samples (0%) that were screened by routine stool examination for the ova and cyst were positive for helminthes and other protozoa. The negative result screened by RIDA-Quick *Cryptosporidium* (R-Biopharm, Germany) is shown in Figure 1. If the result is

positive for *Cryptosporidium*, the red band will appear just below the blue band (control).

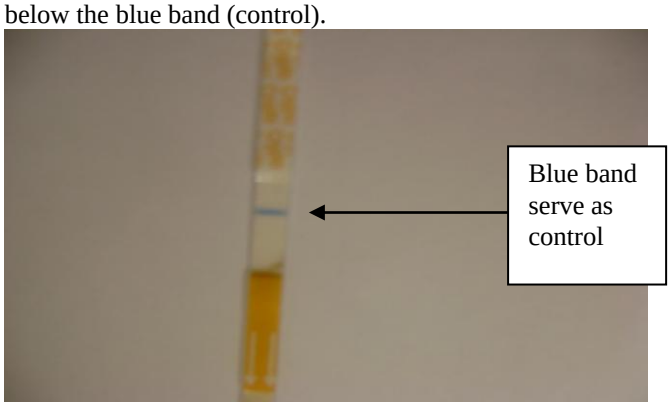

Figure 1: The negative Result of RIDA-Quick *Cryptosporidium* Stripe

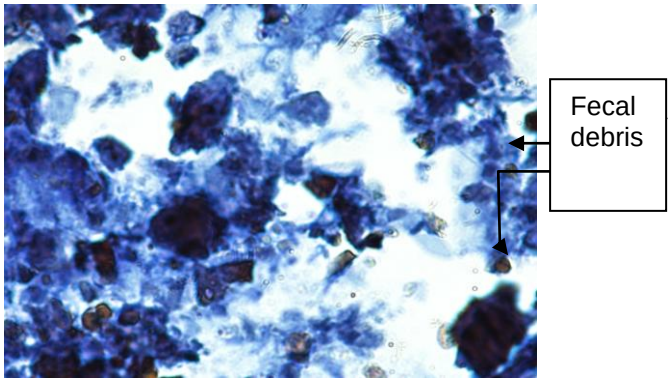

Figure 2: The negative Result of a fecal Smear Stained with modified Ziehl-

Neelsen stain (The photo was taking under magnification100×)

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Socio-demographic Characteristics of Children with Malignancies

Of 110 stool samples collected, 105 had a complete data on the socio-demographic characteristics (Table 4-1). The age of the respondents ranged from 3 months to 17 years old with the mean age of 2 and standard deviation of 0.88 years old. Among 105 children, [56 (53.3%)] were males, whereas [49 (46.7%)] were females. Malays represented the majority of the respondents, which were [79 (75.2%)] followed by the Chinese [12 (11.4%)], Indians [9 (8.6%)] and others [5 (4.8%)]. The other ethnics were from KadazanDusun [3 (2.9%)] and Bengali [2 (1.9%)]. Meanwhile, sixty three (60%) and [42 (40%)] of the respondents came from small and large families, respectively.

Table 1: Socio-demographic Characteristics among 105 Children with malignancies

Socio-demographic data	n	%
Age		
Less than 1 year	12	11.4
1-6 years	60	57.1
7-10 years	18	17.1
11-14 years	14	13.3
More than 14 years	1	1
Gender		
Male	56	53.3
Female	49	46.7
Ethnicity		
Malays	79	75.2
Chinese	12	11.4
Indians	9	8.6
Others	5	4.8
Number of siblings		
1-3	63	60
More than 3	42	40

Clinical Characteristics and Laboratory Parameters of Children with Malignancies

With regard to the clinical information obtained from the medical record (Table 4-2), most of the children admitted to oncology wards had fever [88 (83.8%)]. This was followed by diarrhea [57 (54.3%)], vomiting [47 (44.8%)], nausea [43 (41%)], offensive stool [39 (37.1%)], mucus in stool [35 (33.3%)] and abdominal pain [20 (19 %)]. Only 4 (3.8%) of them had blood in their stools. With reference to the type of diarrhea, 53 (50.5%) and 4 (3.8%) of them had acute and chronic diarrhea, respectively.

In addition, 64 (71.1%) of the children had anemia with hemoglobin (Hb) level below 11g/dl, whereas 30 (33.3%) of them had leucopenia with the total white cell count (WBC) below 5×10^9 /L. Forty two (46.7%) of them had neutropenia with the number of neutrophils less than 1 to $\times 10^9$ /L. Meanwhile, 75 (71.4 %) of them underwent chemotherapy. Fifty two (49.5%) and 48 (45.7%) of them received antimicrobials and steroids, respectively

Table 2: Description on Clinical Information of Children with Malignancies

Clinical information	n	%
Gastrointestinal manifestations (N=105)		
Fever	88	83.8
Diarrhea	57	54.3
Acute	53	50.5
Chronic	4	3.8
Vomiting	47	44.8
Nausea	43	41
Abdominal pain	20	19.1
Offensive stool	39	37.1
Mucus in stool	35	33.3
Blood in stool	4	3.8
Laboratory parameters (N=90)		
Anemia	64	71.1
Leucopenia	30	33.3
Neutropenia	42	46.7
Others (N=105)		
Febrile neutropenia	7	6.7
On chemotherapy	75	71.4
On antimicrobials	52	49.5
On steroids	48	45.7

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Type of Malignancies Diagnosed in Children in Oncology Wards

Among 105 children, majority of them [68 (64.8%)] were diagnosed with lympho-hematopoietic malignancies, whereas [37 (35.2%)] of them were diagnosed with non-lympho-hematopoietic malignancies (Table 3). Acute lymphoblastic leukemia (ALL) was the most common lympho-hematopoietic malignancies diagnosed in these children, which constituted 40 (38.1%) of them. The remaining types of malignancies comprised acute myeloid leukemia (AML) [9 (8.6%)], lymphoma [8 (7.6%)] and chronic myeloid leukemia (CML) [2 (1.9%)]. However, 9 (8.6%) of them were suspected to have leukemia. Among non-lympho-hematopoietic malignancies, brain tumor [12 (11.4%)] was the most commonly diagnosed, followed by retinoblastoma [6 (5.7%)], hepatoblastoma [4 (3.8%)], Wilm's tumor [3 (2.9%)], osteosarcoma [3 (2.9%)], pleuropulmonaryblastoma [2 (1.9%)] and right adrenal cortical tumor [1 (0.9%)]. Six (5.7%) of them had hematological disorders such as haemophagocyticlymphohistiocytosis [3 (2.9%)], aplastic anemia [2 (1.9%)] and pure red cell aplasia [1 (0.9%)].

Table 3: Type of Cancers or Disorders in Children with Malignancies

Type of cancers	n	%
Lympho-hematopoietic malignancies	68	64.8
Acute lymphoblastic leukemia	40	38.1
Acute myeloid leukemia	9	8.6
Chronic myeloid leukemia	2	1.9
Suspected leukemia	9	8.6
Lymphoma	8	7.6
Non- lympho-hematopoietic malignancies	37	35.2
Brain tumor	12	11.4
Retinoblastoma	6	5.7
Hepatoblastoma	4	3.8
Wilm's tumor	3	2.9
Osteosarcoma	3	2.9
Pleuropulmonaryblastoma	2	1.9
Right adrenal cortical tumor	1	0.9
Hematological disorders	6	5.7
Haemophagocyticlymphohistiocytosis	3	2.9
Aplastic anemia	2	1.9
Pure red cell aplasia	1	0.9

Factors that Influence the Acquisition of Cryptosporidiosis in Children with Malignancies

Factors that might influence the acquisition of potential cryptosporidiosis were investigated as shown in Table 4. In general, [85 (80.9%)] and [79 (75.2%)] of our respondents washed their hands before eating and after using the toilet, respectively. Moreover, during our work at the oncology ward we noted the parents or the guardians of those children washed their hands before prepare of the milk formula or give

the food to the children. Thirty-five (33.3%) had history of previous admission to other wards, and [31 (29.5%)] of them had history of contact with animals. Among children who had history of previous admission to other wards [20 (19%)], [8 (7.6 %)], [4 (3.8%)], [2 (1.9%)], [1 (0.9%)] had history of admission to the surgical wards, general ward, neurological ward, ophthalmology ward, intensive care unit respectively. Among children who had a history of animal contact, [17 (16.2%)], [7 (6.7)], [6 (5.7%)] and [1 (0.9%)] of them had a history of contact with cats, fish, dogs, and chicken, respectively. In addition, [26 (24.8%)] had history of swimming, among those children [19 (18%)], [4 (3%)], [3 (2.8%)] had history of swimming in swimming pools, river, sea respectively. Seventeen (16.2%) had history of admission to day-care centres, [74 (70.4 %) [9 (8.5 %)] [5 (4.7%)] they were looking after by their families, babysitters, grandmothers respectively. Surprisingly, only 3 (2.9%) had history of drinking unfiltered tap water the rest of them used boiling water. Moreover, only one patient (0.9%) had history of recent travel few weeks ago before admission to the hospital.

Table 4: Factors that Might influence the Acquisition of Cryptosporidiosis among 105 Children with Malignancies

Factors	n	%
Washing hands before eating	85	80.9
Washing hands after using the toilet	79	75.2
History of previous admission	35	33.3
History of contact with animals	31	29.5
History of swimming	26	24.8
History of admission to day-care centres	17	16.2
History of drinking unfiltered tap water	3	2.9
History of recent travel	1	0.9

IV. DISCUSSION

Cryptosporidium has been recognized as a significant cause of gastrointestinal disease in both immunocompetent and immunocompromised people worldwide (Current & Garcia, 1991; McCole*et al*., 2000). In healthy individuals, *Cryptosporidium* usually causes benign self limiting illness (Isaacs *et al*., 1985). Nonetheless, in severely immunocompromised people the disease can be chronic and life-threatening (Tzipori, 1987; Riggs *et al*., 1997; Langer & Riggs, 1999; Amadi*et al*., 2002). However, studies on *Cryptosporidium* infection in humans are mainly focused on immunocompromised adult cases (HIV/AIDS patients) and only few studies have investigated the prevalence and risk factors related to cryptosporidiosis among immunodeficient children especially children with cancer. Moreover, data on the potential source of parasite in this group of children have not been well explored despite few cases of severe infection, which include death have been documented in some reports (Foot *et al*., 1990; Tumwine*et al*., 2003). In addition, most of the currently available data of cryptosporidiosis in oncology patients are also derived from studies in adult population. For instance, few studies reported that cryptosporidiosis developed as a result of immunosuppressive therapy used in cancer patients (Mead *et al*., 1986); while others documented the provocative effect of cryptosporidiosis towards aplastic crisis in these patients (Casemore, 1988; Foot *et al*., 1990). Moreover, clinical manifestations and significant morbidity were reported to be similar to that observed in HIV/AIDS patients (Gentile *et al*., 1991; Tanyuksel*et al*., 1985; Sreedharan*et al*., 1996). Thus, in view of the devastating clinical consequences and the absence of effective drug for cryptosporidiosis, it is vital that more information is required about the disease in this group of children.

In the present study, all stool samples obtained from 105 children with or without diarrhea who were diagnosed with different types of malignancies did not reveal the presence of *Cryptosporidium*oocysts. In our study, only one stool smear was used for mZN staining and the remaining specimen was subjected for coproantigen testing. This is not surprising as our finding is in accordance with a prospective study done by Burgner*et al.,* (1999) in Australia. In their study, 149 stool samples from 60 children with malignancies who presented with diarrhea were stained (2 slides were prepared for each stool sample) with safranin–methylene blue technique and none were found to be positive for *Cryptosporidium*oocysts. Khalil *et al*.,(1991) also found that stools of 111 children with different types of malignancies who underwent immunosuppressive therapy were also negative for *Cryptosporidium*. The stool was also subjected to a single stool examination technique. However, the type of staining method used could not be traced due to limited available information. In another study, *Cryptosporidium* was also not detected in 97 stool samples of 37 immunocompromised children (24 had acute leukemia and 7 had lymphoma). In their study, stool sample was submitted for more than one and several staining techniques were used such as carbolfuchsin rapid negative stain, modified Ziehl-Neelsen, Giemsa, methylene blue and acid fast stains (Kern *et al*., 1987).

On the contrary, different prevalence of cryptosporidiosis among children with cancer have been reported in a few studies and children less than 2 years of age are frequently infected in both community and hospital settings (Tzipori*et al*., 1983; Mata *et al*., 1984; Bern *et al*., 2002; Simango&Mutikani, 2004; Priest *et al*., 2005). Recently, the highest prevalence was reported among 89 diarrheic children who were diagnosed with leukemia and lymphoma in Turkey (Tamer *et al*., 2008). In this study, they found that 12.4% and 7.9% of these children had cryptosporidiosis by using ELISA and Kinyoun's acid fast staining methods, respectively. In addition, 7 (14.8%), 3 (10%) and 1 (8.3%) of children with ALL, CML and solid tumors, respectively were positive for *Cryptosporidium* by using one of these methods. EL-Mahallawy*et al*., (2004) reported 9.6% of 104 diarrheic children with cancer had cryptosporidiosis in Egypt. In this prospective study, 10 of 104 stool samples were positive for

*Cryptosporidium*oocysts by using modified acid-fast stain and immunoperoxidase test with monoclonal antibodies.

Meanwhile, Tappeh*et al*., (2011) reported that 3 of 72 (4.2%) cancer children with or without diarrhea were found positive for *Cryptosporidium* in Pakistan. Among the positive ones, 2 and 1 of them were from rural and urban areas, respectively. In this case-control study, two samples were obtained from each child and were subjected to formol-ether concentration and modified acid fast staining techniques. Similar findings have been reported in two studies among children with cancer in Turkey and Iran. Aksoy*et al*., (2003) reported that 2 of 50 (4%) symptomatic or asymptomatic children with different types of malignancies were found positive for *Cryptosporidium*oocysts in their stool samples in Turkey. These children were diagnosed with leukemia (1) and lymphoma (1). In this study, modified formol-ether acetate concentration method was used and four smears were prepared for Trichrome (2 slides) and Kinyoun's acid-fast stains (2 slides). Meanwhile, a recent finding from a study conducted among 176 immunocompromised patients (children and adults) revealed that 2 of 48 (4.2%) children with cancer had *Cryptosporidium*. In this study, one stool sample was used from each patient for the detection of *Cryptosporidium*oocysts by ELISA technique.

In Malaysia, only one study has reported the prevalence of cryptosporidiosis in children with cancer (Menon *et al*., 1999). In this study, a total of 237 stool specimens were collected from 50 children with cancer (32 with leukemia and 18 with solid tumors) who were hospitalized for chemotherapy. Three consecutive stool samples were collected from each patient and stained with mZN. One of 10 samples collected from a child with retinoblastoma was positive for *Cryptosporidium* (2%). In this case, there was no history of contact with animal although 50% of all subjects had history of animal contact. Based on these data, variations in the prevalence of cryptosporidiosis found in our study and others could be explained by the differences in socio-ecological factors, research methodologies and detection methods involved. Thus, it is very difficult to compare all the available data because of the absence of a universal "reference standard method" used in all studies (Hunter & Nichols, 2002).

Nevertheless, the absence of *Cryptosporidium* in our study could be explained due to the number of sample obtained from our patient. We managed to get one single specimen from each child as the compliance of the parent was very poor. In addition, there was rapid turn-over of patients admitted in oncology wards and shorter duration of hospital stay. It has been known that children with cryptosporidiosis could intermittently release the *Cryptosporidium*oocysts in their stools although the underlying mechanism for this is still unknown (Navin*et al*., 1984). Thus, we believe that the chance of detecting *Cryptosporidium* would have been higher if more than one stool specimen had been collected from each child. However, issues regarding the number of stool specimens that should ideally be submitted have been continuously debated for many decades. It is believed that the intensity of cryptosporidialoocyst shedding can vary over time in immunocompromised patients (Kuhls, 2000). However, there are few reports documented that 1 or 2 specimens can still be used in immunocompromised patients if acid-fast stain is employed as a diagnostic staining method (Clavel*et al*., 1995; Blackman *et al*., 1997). In our study, mZN was used as a confirmatory staining technique. Many studies have used mZN stain as the gold standard for *Cryptosporidium* detection in clinical and research laboratories in many developing countries (Garcia *et al*., 1983;Arrowood& Sterling, 1989; Brett *et al*., 2003;Caccio&Pozio, 2006). In addition, the technique is relatively cheap, easy to perform without using special microscopes and able to simultaneously detect other pathogens such as *Isospora* and *Cyclospora*. This method is also able to differentiate green yeasts from red-stained oocysts (Kuhls, 2000). The sensitivity and specificity of this method are reported to be 40 to 90% and 50 to 85%, respectively (Chako*et al*., 2010).

The discovery of *Cryptosporidium* oocysts can be improved if the stool specimen is subjected to concentration techniques. In our study, all stool specimens were concentrated the formalin-ether method. Weber*et al*., (1992) reported that there was an increased of the recovery of oocysts by using formalin-ether concentration technique as compared to sucrose flotation and zinc sulfate flotation techniques. Waldman *et al*., (1986) proposed that ether sedimentation was better than sucrose flotation, as ether extracted lipids from the samples, thus dispersing the oocysts into the aqueous phase for easier recovery. Moreover, the sugar flotation technique is more cumbersome to perform, and the presence of the sugar solution of Sheather's (Sheather's*et al*., 1923) could inhibit staining procedures (Casemore*et al*., 1985). Similarly, epidemiological data did not support the superiority of Sheather's sugar flotation for the detection of *Cryptosporidium* oocysts in stool specimens (McNabb *et al*., 1985).

Recently, the use of several copro-antigen tests or antigen detection assays have been employed as another diagnostic modality in detecting *Cryptosporidium* antigens in stool samples (Jex*et al*., 2008). In general, the specificity of these antigen detection assays such as EIA tests and dipstick kits is reported to be high (98–100%) (Robert *et al*., 1990; Ungar, 1990; Newman *et al*., 1993; Garcia & Shimizu, 1997; Chan *etal*., 2000; Katanik*et al*., 2001; Johnston *et al*., 2003), but the sensitivity of copro-antigen detection can be lower than most microscopic approaches (Johnston *et al*., 2003). Moreover, RIDA-Quick *Cryptosporidium* has more advantages than EIA tests as it is less time-consuming and simpler to perform, and do not require an ELISA microplate reader or other specialised equipment (Weitzel*et al*., 2006). For the diagnosis of *Cryptosporidium* infections, RIDA-Quick *Cryptosporidium* has been shown to perform better than EIA tests. The sensitivity and specificity of this method have been reported to be 98.8% and 100%, respectively (Regnath*et al*., 2006). In our study, an immunochromatographic assay (RIDA-Quick *Cryptosporidium*, R-Biopharm, Germany) was used to detect the *Cryptosporidium*antigens. Nevertheless, no *Cryptosporidium* oocysts were detected. This could be explained by the low number of antigens present in our stool samples, thus it might not be able to detect the *Cryptosporidium* antigens in our stool specimens. It has been known that low parasite densities might lead to false negative results (Ali & Hill, 2003). In addition, the stool specimens that we processed might not be suitable for the detection of *Cryptosporidium*antigens by using this test. Of 105, only [57 (54.3%)] children had diarrhea with loose watery stools. The

rest (45.7%) had no diarrhea and probably had solid or semi-solid stools. A recent finding reported that by using native stool samples (semisolid or solid), the distribution of parasites might not be even that would also contribute to the failure of the detection (Current & Garcia, 1991). This could also have contributed to the false-negative results obtained through the dipstick test in our study.However, it has been shown that the test might be useful if experienced practitioners of stool microscopy are not available, or for confirmation purposes. In addition, if only a single stool sample is available as observed in our study, the test can appropriately be used for the detection (Weitzel*et al*., 2006). Recent findings have suggested that this test can be used for the rapid and cost-effective screening of large numbers of stool samples (Garcia *et al*., 2003; Johnston *et al*., 2003). However, like other immunological methods, they do not allow the species or genotype of *Cryptosporidium* involved in the infection to be determined.

Another possible explanation that might contribute to the negative detection of *Cryptosporidium* is the immune status of the children in our study. Although patients with lympho-hematopoietic cancers are more prone to have devastating clinical outcomes compared to other type of cancers (Gentile *et al*., 1990; Travis *et al*., 1990; Tsaihong*et al*., 1990; Gentile *et al*., 1991), these unusual complications are believed to be directly related to the $CD4^+$ lymphocyte count, and patients with $CD4^+$ counts of less than 50 are at greatest risk for both severity of disease and prolonged carriage (Urban *et al*., 1996; Hoepelman, 1996; Theodos, 1998; Hunter & Nichols, 2002; Udgiri*et al*., 2004; Abubakar*et al*., 2007).

In our study, we could not comment on the level of immune status of children with malignancies as the $CD4^+$ count was not measured. Nevertheless, patients with leukemia or secondary to chemotherapy and steroids may have reduced phagocytic activity owing to neutropenic condition in their blood (Nelson *et al*., 1996). In addition, these patients might also have abnormalities in their cellular immune system (T-lymphocyte dysfunction) (Freifeld*et al*., 1997). In this condition, patients are relatively susceptible to opportunistic infections including cryptosporidiosis as the host defense mechanism is impaired. Studies in animal models (Heine *et al*., 1984) and in patients with AIDS (Soave *et al*., 1984; Pitlik*et al*., 1983) have proposed that impaired T-lymphocyte function may lead to persistence cryptosporidiosis. Meanwhile, a recent study by Mahdi *et al*., (2007) reported that the immunoglobulin levels (IgM, IgA and IgG) were also suppressed in patients with cancer and this might indicate the low antibody production by B-lymphocytes in response to particular antigen.

In general, based on these findings, it seems that the defect in the immune system of patients with hematologic malignancies is difficult to be characterized, as these cancers are associated with a broad spectrum of deficiencies involving both B and T cells. In our study, 33.3% and 46.7% of children had leucopenia and neutropenia, respectively, and only 6.7% of them had febrile neutropenia. In addition, 71.4% and 45.7% of them had received chemotherapy and steroids, respectively, as well. However, they seem to be at low risk of acquiring the disease. This might be related to the different degrees of immunosuppression in patients at the time of infection and during its course in our study or the immunodeficiency state might be transient and eventually returned to near normal immune function as proposed by Burgner*et al* (1999). Similar findings have been documented in a study conducted by Rudrapatna*et al*., (1997). In this study, stools from 1,029 cancer patients who received or not received chemotherapy were all negative for *Cryptosporidium*.

Nevertheless, it seems that despite difference prevalences of cryptosporidiosis documented worldwide, studies on risk factors that might contribute to the acquisition of cryptosporidiosis among children with cancer are still lacking. This could also might influence directly or indirectly the number of infected children with cryptosporidiosis as well. In our study, most of these children hospitalized in oncology wards washed their hands before eating (80.9%) and after using the toilet (75.2%). In addition, only 16.2% and 33.3% had history of previous admission to day-care centres and other wards, respectively. Thus, the risk of exposure to *Cryptosporidium* in over-crowded places and hospital settings might be low in our study although information on risk factors among hospital personnel would also be necessary to be included as well, but this was beyond the scope of the current study. It is well known that cryptosporidiosis can be spread by person to person route of transmission as documented by the occurrence of day-care centres outbreaks (Alpert *et al*., 1986; Combee*et al*., 1986), multiple family infections (Soave & Ma,1985), and sequential infections in hospitalized patients (patient to patient) and hospital staff (Koch *et al*., 1985; Ungar*et al*., 1990; Casemore*et al*., 1994).

In addition, asymptomatic carrier among our patients was not detected. Thus, the potential risk of nosocomial outbreak is very low in our study. It is believed that asymptomatic carriage among children is quite common and can be a potential reservoir for cryptosporidiosis (Zar*et al*., 1985; Hunter *et al*., 2004). Meanwhile, other potential sources of cryptosporidiosis can also play important roles for the disease transmission. Drinking contaminated water of various sources, contact with domestic animals or pets, swimming in contaminated water and travelling have been identified as important risk factors among immuno-compromised patients (Seaton, 1992; Glaser *et al*., 1998; Pieniazek*et al*., 1999; Morgan *et al*., 2000a; Emmerson, 2001; Puech*et al*., 2001; Merlani&Francioli, 2003; Kavanagh *et al*., 2005). In our study, only 0.9%, 2.9%, 24.8% and 29.5% had history of recent travelling, drinking unfiltered tap water, swimming and animals contact, respectively. Thus, this further minimized the risk of exposure to *Cryptosporidium* that might probably reflected by the absence of detection of this parasite. Recently, CDC has recommended measures to control and prevent this infection among people in the community as well as immuno-compromised individuals. This includes extensive hand washing, avoiding direct

ACKNOWLEDGMENTS

All authors contributed equally to the manuscript.

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