LOW PREVALENCE OF *CRYPTOSPORIDIUM PARVUM* IN HOSPITALIZED CHILDREN IN KOTA BHARU, MALAYSIA

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Abstract. The aim of this prospective study was to determine the prevalence of *Cryptosporidium parvum* in hospitalized children in Kota Bharu, Malaysia. Over a 19 month study period, 258 stool samples were examined from 159 children; 109 with diarrhea and 50 controls without diarrhea. Modified Ziehl-Neelsen staining method and a polymerase chain reaction (PCR) assay were used to detect *C. parvum* and the samples were also examined for the presence of other intestinal parasites. Only 1 of the 109 (0.9%) children with acute diarrhea was positive for *C. parvum* by microscopy and PCR. Thirty-one percent of children were infested with other intestinal parasites, the most common being *Ascaris lumbricoides* and *Trichuris trichiura*. In conclusion, we found *C. parvum* to be an uncommon infective agent in hospitalized children with or without diarrhea in Kota Bharu, Malaysia.

INTRODUCTION

Cryptosporidium parvum is an important cause of gastroenteritis in children worldwide, with prevalence rates varying from 1 to 4% in the developed world (Baxby and Hart, 1986) and 6 to 17% in the developing world (Salon *et al*, 1990; Enriquez *et al*, 1997). This coccidian protozoan usually causes a self-limiting watery diarrhea lasting 10 to 14 days in immunocompetent hosts. However, a protracted life-threatening illness can occur if the host is immunocompromised. Infection occurs following ingestion of oocysts, which are transmitted via the fecal-oral route and via contaminated water supplies. The source of infection is humans with animals such as cattle or domestic pets being possible sources (Juranek, 1995).

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Kelantan is a predominantly rural state in the northeast of peninsular Malaysia. Lai (1992) reported that 11.4% of hospitalized children with diarrhea in Kota Bharu, the capital of Kelantan State, were positive for *C. parvum*. This is much higher compared with prevalence data from other parts of Malaysia: 4% in Penang (Mat Ludin *et al*, 1991) and 2% in Kuala Lumpur (Ng and Shekhar, 1993). *C. parvum* isolates can be characterized at the molecular level (Morgan *et al*, 1997) and given that the prevalence of *C. parvum* was reported to be relatively high in Kota Bharu, a molecular epidemiological study was planned to determine the extent and nature of genetic variation in *Cryptosporidium* isolates from north east Malaysia. The aim of the present study was to confirm the high prevalence of *C. parvum* in children and collect *C. parvum* samples for the molecular epidemiological study.

MATERIALS AND METHODS

This was a prospective study conducted

from August 1996 to February 1998. One to 3 stool specimens were collected on consecutive days from each child admitted with diarrhea to the Universiti Sains Malaysia Hospital and the Kota Bharu General Hospital. Both hospitals serve a population from urban as well as rural areas. Diarrhea was defined as an alteration in bowel habit and the passage of loose or watery stools 3 or more times a day. Stool samples were also collected from a control group of hospitalized children without diarrhea. Informed consent was obtained from the parent or guardian of each child that participated in the study. A questionnaire was completed with information from the parent or guardian of each patient, documenting clinical information and exposure to animals, both livestock and pets.

The modified Ziehl-Neelsen staining method was used to examine for *C. parvum* oocysts by microscopy (Scott, 1998). Stool samples were also examined for ova and cysts of other intestinal parasites by direct microscopy. A portion of one stool sample per patient was stored in 5% potassium dichromate and sent to Australia (World Health Organization Collaborating Center for the Molecular Epidemiology of Infectious Diseases, Murdoch University) for screening "blind" for *C. parvum* with a polymerase chain reaction (PCR) assay (Morgan *et al*, 1998). Screening by PCR was performed for samples from 138 patients; 95 children with diarrhea and 43 controls. There were insufficient stool samples in 21 cases for preservation in dichromate solution for subsequent PCR screening.

RESULTS

During the 19-month study period, a total of 258 stool samples were collected from 159 children: 198 samples from 109 children with diarrhea and 60 samples from 50 controls.

Diarrhea group

There were 60 males and 49 females with an age range from 2 months to 14 years (average 2 years). The majority of children had an acute diarrhea; only 9% (10/109) had prolonged diarrhea (lasting 2 weeks or more). *C. parvum* was only detected in one sample by microscopy and PCR from a child with diarrhea. The index case was a one-year old girl who had loose stools with mucous lasting 10 days. The stool frequency was 2 to 4 times per day. Numerous *C. parvum* oocysts were found in all 3 stool samples from this child who required intravenous fluids for rehydration. There was a positive history of contact with cattle as well as cats for this child. Fifty-two percent of children in this group with diarrhea gave a history of animal exposure. Ova from other intestinal parasites such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Blastocystis hominis* and *Giardia* were found in stool samples from 17 children (16%) and 8 had more than one infestation (Table 1). One child, a 2-month old girl, was known to be human immunodeficiency virus (HIV) positive. She had a chronic diarrhea lasting 2 months but her stools were negative for *C. parvum*.

Control group

The main diagnosis in the control group was thalassemia (36%), followed by typhoid. There were 31 males and 19 females with an age range from 2 months to 11 years (average 6 years). No child in the control group had diarrhea in the fortnight preceding admission

Table 1
The prevalence of intestinal parasites in children with and without diarrhea.

Parasite	Children with diarrhea (n=109)	Children without diarrhea (n=50)
	Number (%)	Number (%)
Cryptosporidium parvum	1 (0.9)	0 (0%)
Ascaris lumbricoides	11 (10)	10 (20%)
Trichuris trichiura	11 (10)	9 (18%)
Blastocystis hominis	2 (1.8)	3 (6%)
Giardia lamblia	1 (0.9)	2 (4%)

to hospital.

None of the 50 children in the control group had stool samples that were positive by microscopy or PCR for *C. parvum*. Sixteen children (32%) were positive for intestinal parasites and 7 had more than one infestation. Forty-four percent gave a history of animal exposure.

DISCUSSION

In this study of 109 hospitalized children with diarrhea, only one child was found infected with *C. parvum*. This child had a typical history of watery diarrhea with mucous and was significantly dehydrated. No other pathogens were found in the stool samples from this child to account for the diarrhea, strongly suggesting that *C. parvum* was the causative agent. None of the 50 children in the control group had stool samples that were positive for *C. parvum*. Asymptomatic excretion in children without diarrhea occurs particularly in developing countries (Iqbal *et al*, 1999). The limitations of our control group include a small number of children and on average, only one stool per patient was examined. Intermittent oocyst excretion is recognized and Clavel *et al* (1995) found that 3 stool samples were the optimal number necessary to detect the organism in immunocompetent patients. Children in the control group were also older than those with diarrhea and the frequency of cryptosporidiosis is reported to be highest in children below 2 years of age (Bhattacharya *et al*, 1997).

The 0.9% prevalence of *C. parvum* in this study is significantly lower than that reported by Lai (1992) of hospitalized children from the same area. The reasons for the discrepancy between the present study and previous study are unclear. One possibility is that the study undertaken by Lai could have been conducted during an outbreak of cryptosporidiosis in Kota Bharu, resulting in a high prevalence rate. The low proportion of cases with prolonged diarrhea in our study could be another possibility for the low prevalence observed, since a study in Thailand found a higher prevalence of *C. parvum* in children with prolonged diarrhea as compared with those with acute diarrhea (Jirapinyo *et al*, 1993).

Seasonality has also been reported to affect *Cryptosporidium* excretion (Clavel *et al*, 1996). However, as our study spanned a period of 19 months, a seasonal effect is unlikely. Another possibility for the low prevalence observed is that one of the methods that we used, modified Ziehl-Neelsen followed by examination by microscopy was not sensitive enough for detecting low numbers of oocysts in the samples. However, the samples were also screened by a PCR assay that is more sensitive than conventional microscopy (Morgan *et al*, 1998) and these PCR results confirmed the low prevalence rate for *C. parvum* in our study group.

In conclusion, we found *C. parvum* to be an uncommon infective agent in hospitalized children in Kota Bharu, with a lower prevalence rate compared with previous reports from Malaysia.

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