ORIGINAL ARTICLE

The Prevalence of Cryptosporidiosis in Children and Adults at University Hospital, Kuala Lumpur

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Summary

A total of 320 faecal specimens obtained from 295 patients (192 children and 103 adults) with diarrhoea and 47 faecal specimens from healthy adults were screened for *Cryptosporidium* oocysts. Faecal specimens were first screened by modified Ziehl Neelsen stain and specimens with oocysts were confirmed by direct immunofluorescence technique. *Cryptosporidium* oocysts were detected in 4 children but not in normal healthy adults or in diarrhoeic adults.

Key words: Cryptosporidiosis.

Introduction

Cryptosporidium, a coccidian protozoa of the gastrointestinal tract, is now recognised as an important cause of acute self-limiting diarrhoea in immunocompetent patients and life-threatening diarrhoea in immunocompromised patients[1]. Numerous species affect humans, farm animals and birds[2]. The majority of infection in man is probably associated with *Cryptosporidium parvum*. Human cases of cryptosporidiosis have been reported in various parts of the world. The incidence appears to be highest in the tropics. A frequency of 13% in children with diarrhoea has been reported in Southern and Eastern India[3,4] and 7.3% in children in Thailand[5]. In Malaysia, 4.3% of the stool samples collected from children with diarrhoea were found to be positive for *Cryptosporidium*[6]. In temperate countries, the incidence of cryptosporidiosis in children varies from 1.1% in Spain[7] to 1.4% in the United Kingdom[8] and 2.1% in France[9].

The route of transmission has been associated with drinking contaminated water with oocysts[10], person-to-person spread[11] and contact with infected animals[12].

The aim of this study is to determine the frequency of detecting *Cryptosporidium* oocysts in the faeces collected from normal healthy (adults and children) patients having acute diarrhoea.

Materials and Methods

Faecal specimens

Between July 1990 and May 1991, 320 faecal specimens were obtained from 295 patients with acute diarrhoea from the University Hospital. The stools received in the Department of Microbiology, Faculty of Medicine, University Malaya, were then screened for *Cryptosporidium* oocysts. The 295 patients

included 192 children below the age of 10 years (mean age 5.5 years old; made up of 117 boys and 75 girls) and 103 adults (mean age 36.5 years old; made up of 66 males and 37 females). A total of 47 faecal specimens obtained from 47 normal healthy adults (mean age 27.5 years old; 30 males and 17 females) were also screened for the oocysts and served as control during the study.

Detection of *Cryptosporidium* oocysts

A thin smear was made onto a slide with the watery faeces. In the case of formed or semi-solid faeces, the samples were mixed with normal saline to produce a more liquid emulsion. The smears were air-dried and fixed in methanol for 10 mins. The *Cryptosporidium* oocysts were identified after staining with modified Ziehl Neelsen stain[13]. Briefly, the smear was stained with cold strong carbol-fuchsin for 15 mins differentiated in 3% acid alcohol (3% HC1 in 95% ethanol) and counter stained with 0.25% malachite green for 1 min. In this stain, *Cryptosporidium* oocysts appear as round or oval structures (about 5μ) with a clear halo and a bright rose-pink mass in the cytoplasm.

Stools positive for *Cryptosporidium* was confirmed by the direct immunofluorescence technique, using *Cryptosporidium* Monofluo Kit (Diagnostic Pasteur) kindly supplied by Sanofi Malaysia. The oocysts appeared as apple-green round structures. The specimen was only considered positive when >5 apple-green fluorescent cryptosporidia was present per high power field.

Faecal specimens with *Cryptosporidium* oocysts detected by both direct staining and immunofluorescence methods were also cultured and examined for other common enteric pathogens.

Results

In this study, out of a total of 320 faecal specimens screened for *Cryptosporidium* oocysts, there was no oocyst detected in the diarrhoeal faeces collected from 103 adults. Among 192 faecal specimens collected from children, *Cryptosporidium* oocysts were only detected in the faeces of 4 children using both the modified Ziehl Neelsen and the direct immunofluorescence technique (Table I). The 4 children presented with diarrhoea. There were 2 males and 2 females and the age of the patients ranged from 5 months to 6 years.

Table I
Age, sex and associated enteropathogens in four children with cryptosporidiosis

			Results		
			Cryptosporidium oocysts		Other enteropathogens isolated
No	Age	Sex	Direct smear	Direct immuno.	
1	2 yrs	M	Positive	Positive	Nil
2	5 yrs	F	Positive	Positive	Nil
3	6 yrs	F	Positive	Positive	Nil
4	5 mths	M	Positive	Positive	Food poisoning *Salmonella*

Cryptosporidium was the sole pathogen detected in 3 of the 4 patients. In the fourth patient, *Salmonella*, a bacteria associated with food poisoning, was isolated. No *Cryptosporidium* oocysts were detected in the faeces of the normal healthy subjects.

Discussion

The results of this study suggest that *Cryptosporidium* may play a role in the aetiology of diarrhoea in children in Malaysia. Epidemiological surveys carried out in various parts of the world revealed a prevalence rate of 0.6% to 20% in developed countries and 4% to 32% in underdeveloped countries[14]. In this study, the prevalence was comparatively lower than studies undertaken in Thailand and India. Variable factors, such as the immunological status, nutritional and environmental factors, may have contributed to this difference in prevalence. In this study, all children were immunologically normal, well-nourished and stayed around the city metropolitan areas.

Most of the cases of cryptosporidiosis documented in the literature have been in children[15]. This also suggests that younger children are more susceptible to the infection. This study also supports the finding that cryptosporidiosis is an infection most often seen in children with diarrhoea. None of the adults examined, with or without diarrhoea, had *Cryptosporidium* oocysts. Children have been reported to be asymptomatic carriers of *Cryptosporidium*[16,17], but not adults. Thus, it may be hypothesised that cryptosporidiosis is uncommon among healthy adults with or without diarrhoea. Similar findings have been reported by Ghani *et al*[18].

When *Cryptosporidium* occurs in combination with one or more other enteric pathogens, it is difficult to evaluate the importance of *Cryptosporidium* in the causation of diarrhoeal diseases. The presence of food poisoning *Salmonella* in the fourth patient is not surprising, as *Salmonella* is the leading enteric pathogen isolated from children with acute diarrhoea in Malaysia[19].

How the patients in the present study acquired *Cryptosporidium* is not clear and this was not investigated. Probably, it could have been acquired through drinking water contaminated with oocysts or by contact with infected animals such as dogs or cats.

In all the studies undertaken so far, patients sampled have been those with diarrhoea, and this does not necessarily reflect the true prevalence in a community. As it is an ubiquitous parasite, it would be more appropriate if cross-sectional studies are carried out to determine the true prevalence and the spectrum of disease in both urban and rural areas.

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