CRYPTOSPORIDIOSIS AMONG CHILDREN WITH ACUTE GASTROENTERITIS IN THE PEDIATRIC WARD IN THE GENERAL HOSPITAL, PENANG

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Abstract. Stool samples from 836 cases with diarrhea and acute gastroenteritis from the Pediatric ward, Penang General Hospital, were examined for *Cryptosporidium* oocysts. A dimethyl sulfoxide modified acid fast technique was used for the identification of the parasites. 36 samples or 4.3% were found to be positive for *Cryptosporidium*. The prevalence of infection was higher (2.39%) in children with diarrhea and vomitting than in children with acute gastroenteritis alone (0.8%). Stool examination and cultures from the *Cryptosporidium* positive samples revealed no other parasites, rotavirus or enteropathogenic bacteria. This suggests that *Cryptosporidium* may be an important agent in the causation of diarrhea in young children. A routine laboratory examination for the detection of *Cryptosporidium* in the search for causal agents of childhood diarrhea in our environment may, therefore, be significant.

INTRODUCTION

Cryptosporidium species is a coccidian parasite which was first recognised by Tyzzer (1970), as a protozoan parasite infecting animals. Cryptosporidium was subsequently described as a cause of diarrhea in calves (Pohlenz, 1978). The first case of human cryptosporidiosis was reported in 1976 from microscopic examination of intestinal biopsy (Nime, 1976). Since then, a large number of cases have been observed in both immunocompromised immunocompetent patients and the disease has been described as a zoonosis (Schultz, 1983).

Cryptosporidium is identified through the demonstration of its oocysts using the dimethyl sulfoxide modified acid fast stain technique (Melinda, 1984). This technique has been found to be simple, fast and reliable for the detection of the oocyst in stool samples.

The etiologic agents of acute diarrhea in a significant number of patients in Malaysia are not known. Microscopic examination of fecal samples for *Cryptosporidium* is not routinely done in our laboratory. As a result, cryptosporidiosis has been left undiagnosed and its prevalence is undetermined. The objective of the study was to determine the prevalence of *Cryptosporidium* in children with acute gastroenteritis and to correlate the results with their clinical features.

MATERIALS AND METHODS

A total of 836 selected stool samples received in the Microbiology laboratory, School of Medical Sciences, USM between January to December 1988 for stool culture was examined for cryptosporidium oocysts with a rapid dimethyl sulfoxide modified acid fast stain (Melinda, 1984). Fecal material was smeared over a 2.5 by 3.0 cm area of clean, flamed glass slides and air dried. The slides were prefixed in a coplin jar with absolute methanol for 5 to 10 seconds, stained in carbol fuchsin-DMSO solution in a coplin jar for 5 minutes. Each slide was then rinsed individually in gently running tap water until excess solution was washed off (10-30 seconds per slide). Slides were next placed in a decolorizer counter stain jar for one minute or until a green background appeared. Slides were then rinsed individually under running tap water for 10 seconds, drained, blotted, and placed on a rack until thoroughly dry. The slides were then examined under oil immersion at 100x. Positive and negative control samples supplied by the Department of Parasitology, University

Kebangsaan Malaysia were also stained simultaneously by the above procedure. Specimens were considered positive for cryptosporidium oocysts if they contain 4 to 5 μm cysts with typical internal vacuoles and material clumped to one side, and stained brilliant pink against a pale green background. The medical records of patients with positive smears were used for clinical analysis.

RESULTS

Out of the 836 fecal samples studied 36 (4.3%) were found to be positive for *Cryptosporidium* oocysts, 27 (3.2%) for rotavirus, 123 (14.7%) for enteric pathogen and 37 (4.4%) for other parasites as shown in Table 1. 20 specimens positive for *Cryptosporidium* were from children with symptoms of diarrhea, fever and vomitting, 9 positive specimens were from children with diarrhea and acute gastroenteritis and 7 positive specimens were from acute gastroenteritis children alone. None of the dysentery and watery stool children were found to be positive. Stool examination and cultures from the *Cryptosporidium* positive samples revealed no other parasites, rotavirus or other enteropathogenic bacteria.

All the patients with cryptosporidiosis were children aged under 2. The disease was found in both sexes and in all races. 20 of the patients were moderately to well nourished. The incidence of *Cryptosporidium* in the children with symptoms of diarrhea, fever and vomitting was higher than those with acute gastroenteritis alone or those with other symptoms as shown in Table 2.

Table 2

Clinical features of patients excreting *Cryptosporidium* oocysts.

Selected clinical symptoms	*Cryptosporidium* oocysts	%
Acute gastroenteritis alone	7	0.8
Diarrhea, fever and vomitting	20	2.4
Diarrhea and acute gastroenteritis	9	1.0
Dysentery and watery stool	0	0

DISCUSSION

Cryptosporidium sp. seems to be a parasite of significance in young children with gastroenteritis in Penang. Although only 4.3% of the patients with acute gastroenteritis in our study were shown to be excreting *Cryptosporidium* oocysts, nevertheless these findings support previous observations that the disease is prevalent in young children and asymptomatic bacterial diarrhea.

The role of *Cryptosporidium* in causing disease of infected children is unclear, however *Cryptosporidium* seems to be an emerging zoonosis (Shultz, 1983) and acquisition by the patient through animal contact seems most likely. Fecal-oral transmission from animals to animals and from animals to human beings has been proved and almost assuredly transmission from human to human occurs (Aderson *et al,* 1982; Baxley *et al,* 1983).

The rare reports of *Cryptosporidium* sp. in Malaysia may be partly explained by the laboratory diagnostic methodology adopted. The laboratory should make a deliberate attempt to stain specifically for the oocysts to ensure that the cases do not go undetected. We prefer the technique

Table 1

Prevelence of *Cryptosporidium* among children with acute gastroenteritis

Total sample examined	*Crypto-sporidium*	Rotavirus	Other parasites	Enteric pathogen
836	36	27	37	123
%	4.3	3.2	4.4	14.7

of dimethyl sulfoxide-modified acid fast stain (Melinda, 1984) because it is simple, quick and reliable for the detection of cryptosporidium oocyst in the large number of samples.

The observation suggest that *Cryptosporidium* sp. may be an important agent in the causation of diarrhea in young children and perhaps in asymptomatic bacterial diarrheal patient.

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REFERENCES

Aderson BC, Donndelinger T, Wilkins KM, Smith J. Cryptosporidium in a veterinary student. *J Am Vet Med Assoc* 1982; 180 : 408.

Baxley D. Hart CA, Taylar C. Human cryptosporidiosis : a possible case of hospital cross infection. *Br Med J* 1983; 287 : 1760.

Melinda AB. Rapid dimethyl sulploxide-modified acid fast stain of *Cryptosporidium* oocysts sp. in stool specimens *J Clin Microbiol* 1984; 19 : 952-3.

Nime FA, Burek JD, Page DL, Hoelscher MA, Yarley. Acute enterocolitis in a human being infected with the protozoa *Crytosporidium. Gastroenterology* 1976; 70 : 592-8.

Pohlenz J, Moon HW, Cheville NF, Berwick WJ. Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. *J Am Vet Med Assoc* 1978; 172 : 452-7.

Shultz MG. Emerging zoonosis. *N Engl J Med* 1983; 308 : 1285-6.

Tyzzer EE. A sporozoan found in the septic glands of common mouse. *Proc Soc Exp Biol Med* 1970; 5 : 12-3.