DETECTION OF CRYPTOSPORIDIUM OOCYSTS IN ACUTE DIARRHEAL STOOLS

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ABSTRACT

Three hundred and fifty stool samples from patients with acute diarrhea and 30 samples from normal healthy controls were examined for the presence of various parasitic and bacterial enteropathogens by conventional techniques. Cryptosporidium oocysts were detected in 15 out of 350 (4.3%) fecal samples. In 10 cases (2.9%) it was identified as a sole pathogen while in 5 cases (1.4%) it was isolated in association with cysts of E. histolytica, Giardia lamblia and with Salmonella typhimurium. Ten out of fifteen cases were in the pediatric age group. None of the 30 control cases had Cryptosporidium oocysts in their stools.

Key words: *Acute Diarrhea, Cryptosporidum.*

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Cryptosporidiosis, a coccidial protozoan infection, well known in veterinary medicine as a cause of acute diarrhea in the young of several animal species has now been recognized as an important enteric pathogen in humans(1). It gives rise to a severe opportunistic infection in immunocompromised patients(2), and is known to produce a self limiting diarrhea in immunologically competent host in both developed(3-5) and developing countries(6-8). It has been suggested as one of the agents responsible for 25-30% cases of acute diarrhea in whom no etiological agent can be detected with the best available microbiological techniques(9). In the past, diagnosis of Cryptosporidiosis was possible only by intestinal biopsy. With the development of simple laboratory diagnostic techniques of examining stool samples directly as Iodine wet mount or after concentration and staining smears by the modified Kinyoun acid fast staining, it is possible to screen a large number of suspected cases. In this communication, the preliminary results of a prospective study on Cryptosporidiosis in patients with diarrhea are reported.

Patients and Methods

Freshly passed stool samples from 350 cases with diarrhea and 30 cases with no history of diarrhea attending the Outpatient Department of Lok Nayak Jai Prakash Narain Hospital, New Delhi, were screened for both bacterial and parasitic enteropathogens.

For identification of *Cryptosporidium* oocysts, fecal smears were prepared from stool samples concentrated by the formol ether concentration technique, fixed with methanol and stained by a modified Ziehl-Neelsen technique(1). Briefly, the smears

were treated with cold carbol fuchsin for over 30 minutes, washed and decolorised with 3% acid alcohol for 15 seconds, washed well in running water and counter stained with methylene blue, washed again, blotted dry and examined under oil immersion lens for small acid fast cysts, round to oval in shape and about 4-5 μm in size.

Stool samples from all patients were also processed for bacterial enteropathogens, like *Salmonella, Shigella, Esch. coli* (EPEC) and *Vibrio* according to standard procedures.

Results

In the present study, an etiological agent could be identified in 46.6% cases of diarrhea. The remaining 53.4% cases of diarrhea could have been due to viral enteropathogens or newer bacterial enteropathogens.

Cryptosporidium oocysts were present in 15 out of 350 (4.3%) patients with diarrhea. The highest isolation (10.0%) was seen in the 5-10 years age group. In the other age groups the isolation of *Cryptosporidium* oocysts showed little variation (*Table I*). However, no case of Cryptosporidiosis was seen in the 10-15 years age group. In 10 cases (2.9%), *Cryptosporidium* oocysts were identified as the sole pathogen while in the remaining 5 cases (1.4%) it was associated with other enteropathogens, namely the cyst of *Giardia lamblia* (3 cases), cyst of *E. histolytica* and with *Salmonella typhimurium* one case each. The other enteropathogens isolated either alone or in combination included *Salmonella* spp., *Shigella* spp., EPEC and *V. cholerae* (*Table I*). None of the 30 control cases had *Cryptosporidium* oocysts.

Discussion

The increasing number of report of gastroenteritis associated with Cryptosporidiosis places the parasite as an important enteropathogen.

Reports of *Cryptosporidium* oocysts excretion in the Western studies have indicated an incidence of 4-5% in cases with acute diarrhea. Mathan *et al.* in a study from rural Southern India showed Cryptosporidium in 13.1% cases of acute diarrhea and 9.8% in the control group. Such a high prevalence rate is probably due to the unsanitary living conditions prevalent in the rural areas studied and also due to the close association of subjects with farm animals(11).

In the present study, the source of infection was unknown. Since most of the cases under study came from areas in and around Delhi none of the cases had direct contact with farm animals and contact with domestic pets was unexceptional. The geographic distribution of cases did not correlate with any particular water supply. Reports from most developing countries like Costa Rica, Bangladesh and India describe peak rates of isolation of *Cryptosporidium* species during the warm, rainy, humid months(7,8,11) while seasonal patterns in developed countries have not been consistent(5,9,12,13). The present study also showed a higher isolation (10 out of 15 cases) of *Cryptosporidium* from April to September when it is very warm and humid. Three out of 15 cases with *Cryptosporidium* oocysts also had cysts of *Giardia lamblia*. Several workers have observed a coincidence of *Cryptosporidium* and *Giardia*(14,15). Jokipii *et al.* observed that the two infections were found together six times more often than expected on the basis of their individual frequencies(16).

TABLE I—*Enteropathogens Isolated from Acute Diarrhea Cases*

		Parasitic isolates				Bacterial isolates				
Age (Yrs)	No.	Crypto-sporidium	Giardia lamblia	*E. Histolytica*	Other helminthic ova	Salmonella spp.	Shigella spp.	*V. Cholerae*	EPEC	Total
≤1	95	3 (3.2)	6	5	1	11	0	1	6	33 (34.7)
1-5	56	2 (3.6)	12	10	5	6	2	2	3	42 (75.0)
5-10	50	5 (10.0)	10	5	5	2	1	2	0	30 (60.0)
10-15	25	0 (0.0)	3	3	6	1	3	2	1	19 (76.0)
15-25	45	3 (6.7)	3	5	4	0	0	0	1	16 (35.6)
>25	53	1 (1.9)	3	6	1	0	1	0	0	12 (22.6)
Unknown	26	1 (3.9)	1	2	2	2	1	1	1	11 (42.3)
Total	350	15 (4.3)	38 (10.9)	36 (10.3)	24 (6.9)	22 (6.3)	8 (2.3)	8 (2.3)	12 (3.4)	163 (46.6)

Figures in parentheses denote percentages.

Most diarrheal cases with *Cryptosporidium* oocysts did not have any distinctive clinical symptoms except the two cases who presented with mild but prolonged diarrhea and abdominal discomfort. None of the patients received any specific treatment. The role of *Cryptosporidium* as an important pathogen in persistent diarrhea in children needs further evaluation.

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