CLINICAL MICROBIOLOGY

Significance of Cryptosporidium in acute diarrhoea in North-Eastern India

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In a hospital-based study, stool samples from 2095 patients of all ages were examined for different fungal, protozoal and bacterial enteropathogens over a period of 2 years (July 1994–June 1996). Cryptosporidium was detected in 151 specimens (7.2%) and was the third commonest pathogen found. The highest prevalence of this organism was in the group aged 16–45 years and during the rainy months (July–Oct.). Diarrhoea caused by the protozoon was of mild to moderate severity and features of dysentery were absent. Amongst other enteropathogens, Candida albicans was the most frequently isolated, followed by enteropathogenic and enterotoxigenic Escherichia coli, Salmonella spp., Campylobacter jejuni, Entamoeba histolytica, Giardia duodenalis (lamblia), Shigella spp., Vibrio cholerae and Aeromonas spp.

Introduction

Cryptosporidium parvum, an apicomplexan protozoon, causes acute diarrhoea in the young of several animal species [1] and life-threatening chronic diarrhoea in immunocompromised individuals [2]. The global prevalence of this parasite can be ascertained by the reports from all continents [3]. In Asia, the organism has been detected in 2–20% of all the cases of acute diarrhoea [4, 5]. In India, the few reports that are available indicate a prevalence rate between 4 and 13% [6–9]. Notwithstanding the above reports, the detection of cryptosporidium in India is limited to major research laboratories and does not feature in protocols for routine investigations in most clinical laboratories. In a community-based study at Varanasi, the prevalence of cryptosporidium in children under 5 years of age suffering from acute diarrhoea was 3.8% [10]. However, hospital-based data are not available on this parasite from this part of the Gangetic plain. Against this background, it was decided to determine the prevalence and seasonality of cryptosporidium in diarrhoeal patients of different age groups who reported to the hospital and to assess the need or otherwise to search for this organism during routine microscopy of diarrhoeal stool samples.

Materials and methods

A single stool specimen was collected from each of the 2095 patients with acute diarrhoea who attended the outpatient department or wards of Sir Sunderlal Hospital, Banaras Hindu University, Varanasi between July 1994 and June 1996. The population included children, adolescents, adults and the elderly; 1455 patients (69.5%) were male and 640 (30.5%) were female.

Acute diarrhoea was defined as three or more loose stools in the preceding 24 h. Stool samples were collected in sterile disposable containers and transported to the laboratory within 2 h for immediate processing.

Processing of specimens

Detection of cryptosporidium. Oocysts of the parasite were sought by direct smear as well as by formol-ether concentration. The smears were dried in air and then fixed by acid alcohol (HCl 3% in methanol) for 3–5 min followed by staining with aqueous safranin 1% for 1 min with gentle heating from below until steam appeared. After washing in tap water, slides were counter-stained with methylene blue for 30 s. Oocysts appeared bright orange, usually with a clear halo against a blue background [11].

Detection of other parasites. The presence of other parasites was established by direct microscopy of fresh
Isolation of enteropathogenic bacteria. Stools were inoculated directly on to MacConkey agar (MA), desoxycholate citrate agar (DCA), thiosulphate citrate bile salt sucrose agar (TCBS) and campylobacter blood plates after incubation for 48 h. Inoculation was directly on to MacConkey agar (MA) and DCA from alkaline peptone water after incubation for 4–6 h and from selenite-F broth on MA and DCA plates after incubation for 48 h.

Suspect colonies were identified by a battery of test substrates as described in the WHO manual [11] and further identified by serological agglutination.

Isolation of C. albicans. Inoculation was directly on to Sabouraud's dextrose agar with chloramphenicol 0.05 g/L in duplicate and cultures were incubated for up to 7 days at 22°C and 37°C. A heavy to confluent growth of C. albicans not associated with any other diarrhoeagen was considered to be enteropathogenic [12–14].

Statistical analysis

The data were analysed with an SPSS package on an IBM-compatible computer. The different variables were tested by the χ² test.

Results

Cryptosporidium was observed to be the third commonest diarrhoeagen, with a detection rate of 7.2% (151 of 2095) (Table 1). It was detected as the sole enteropathogen in 73.5% (111 of 151) of the specimens whereas in the other 26.5% (40 of 151) it was mixed with other pathogens. Other pathogens isolated in this series were: C. albicans (11.8%), Escherichia coli (EPEC 1.9% and ETEC 6.2%), Salmonella spp. (6.7%), Campylobacter jejuni (4.1%), Entamoeba histolytica (2.1%), Giardia duodenalis (lambia) (1.7%), Shigella spp. (1.5%), Vibrio cholerae O1 (1.5%) and Aeromonas spp. (1.0%).

Although cryptosporidium could be detected throughout the year, the detection rate was significantly higher (p < 0.05) during the rainy season (8.5%, 88 of 1036) than in the summer (6.7%, 44 of 659) and winter (4.8%, 19 of 400) months (Table 4). The monthly distribution, on the other hand, showed peaks in Dec. (11.6%), March (11.2%) and April (9.3%), which are in the dry season.

Of the 154 cryptosporidium-positive diarrhoeal stool specimens, 97 (63.0%) were semi-solid and 57 (37.0%) were liquid. The number of motions in cryptosporidium-associated diarrhoea ranged from 3 to 18 with an average of 7.6/day. Microscopic
examination of the cryptosporidium-positive diarrhoeal stools showed white blood cells in four (0.3%) and red blood cells in none.

Discussion

Cryptosporidium was the third most commonly detected diarrhoeagen amongst the various bacterial, parasitic and fungal enteropathogens which were looked for in the present study. It is interesting to note that this protozoon was not only the most frequently detected parasitic diarrhoeagen, but was also four and two times more frequent than *G. duodenalis* (*lamblia*) and *Ent. histolytica*, respectively (Table 1). Furthermore, its role as a diarrhoeagen is strongly suggested by the detection of this protozoon as the sole pathogen in c. 75% of the positive samples. Its role as a diarrhoeagen at high frequency has also been reported from hospitals in northern [6], southern [7], eastern [8] and western [9] India (range of detection 4.3–13.0%).

This is a comprehensive study on the prevalence of cryptosporidium in all age groups, unlike other Indian studies which have focused on children under the age of 5 years only. The most commonly infected age group was 16–45 years (Table 2). Although several reports indicate that children aged <24 months are the most susceptible, no significant difference (p > 0.05) in the occurrence of this organism was observed in different age subgroups of children under the age of 5 (Table 3).

This Gangetic plain is characterised by extremes of hot dry summers and cold humid winters interspersed with warm and humid rainy months. Cryptosporidium could be detected in significantly higher numbers (p < 0.05) during the rainy season than at other times (Table 4). A similar seasonal pattern has been reported in South Africa [15], Brazil [16], the Gambia [17] and Mexico [18]. In contrast, reports from coastal/subcoastal areas of India such as Calcutta [8] and Vellore [7] showed no significant seasonal variation in the detection of the parasite. The reason for this might be the prevailing high humidity throughout the year in these areas. However, peaks in the dry months of Dec., March and April deserve further exploration in this area.

<table>
<thead>
<tr>
<th>Season</th>
<th>Number of specimens examined (%)</th>
<th>Number of cryptosporidium detected (%)</th>
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<tbody>
<tr>
<td>Rainy (July–Oct.)</td>
<td>1036(49.5)</td>
<td>88(8.5)</td>
</tr>
<tr>
<td>Winter (Nov.–Feb.)</td>
<td>400(19.1)</td>
<td>19(4.8)*</td>
</tr>
<tr>
<td>Summer (March–June)</td>
<td>659(31.4)</td>
<td>44(6.7)</td>
</tr>
<tr>
<td>Total</td>
<td>2095(100.0)</td>
<td>151(7.2)</td>
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*χ² = 6.45, p < 0.05.

An absence of dysenteric features is confirmed by the failure to detect faecal leucocytes and erythrocytes in any of the 111 specimens positive for cryptosporidium only. However, 15 of the 40 specimens with mixed enteropathogens that included cryptosporidium were found to show such evidence of mucosal invasion. Although there is a possibility that some of the cases might be of viral origin, the present observation suggests a secretory mechanism in cryptosporidium-associated diarrhoea as proposed by some earlier reports [19, 20]. This pathogen caused diarrhoea of mild to moderate severity with an average stool frequency of 7.6 per day.

The importance of cryptosporidium-associated diarrhoea in the present study, which is likely to increase further in the Indian subcontinent with the spread of AIDS, makes it necessary to screen for the parasite in the routine diagnostic laboratory. Furthermore, there is an urgent need for the development of effective chemoprophylaxis and immunoprophylaxis for this diarrhoeagen, which are unfortunately unavailable at the present time.

References


