Cryptosporidiosis in immunocompetent children

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SUMMARY Cryptosporidial oocysts were identified by modified Ziehl-Neelsen stain in the stools of seven (3·2%) of 213 children with acute or chronic diarrhoea and one (0·9%) of 112 controls. All children with cryptosporidia were immunocompetent. Four of the index cases had a short illness (3–14 days) with watery diarrhoea, vomiting (2), and abdominal pain (2). Two index cases had chronic diarrhoea for over four months and failure to thrive. Both had a small intestinal enteropathy; one had cryptosporidial oocysts in stool specimens two months apart and the other had cryptosporidial schizonts attached to the jejunal mucosa. One index case had a colitis of indeterminate cause. Four of the index cases had recently travelled abroad. There had been an outbreak of gastroenteritis in the family of one of the index cases, and three affected sisters and an asymptomatic brother had oocysts in their stools.

Cryptosporidial infestation seems to be associated with acute gastroenteritis and sometimes with chronic diarrhoea and small bowel damage in immunocompetent children.

Cryptosporidia are coccidian parasites which have been known to be pathogenic to animals since 1907.1 The first description of disease in humans, however, was not until 1976.2 Most reported cases of cryptosporidiosis have occurred in immuno-deficient patients, in whom cryptosporidial infestation causes prolonged watery diarrhoea, which is often fatal.3-5 There have been several recent reports of cases of acute gastroenteritis associated with cryptosporidia occurring in apparently immunocompetent adults and children. Casemore and Jackson have found cryptosporidial oocysts in the stools of five (2·5%) of 196 apparently immunocompetent children with acute uncomplicated diarrhoeal episodes. One patient, a 1 year old child, had recurrent diarrhoea and failure to thrive.4 Tzipori et al7 found cryptosporidial oocysts more commonly in the stools of children (4·8%) with gastroenteritis than adults (1·6%) and the prevalence of infection was higher in the Australian summer and early autumn.7

In this paper we report the preliminary results of a prospective study of stool examinations for cryptosporidial oocysts from children with diarrhoea as compared with a control group who did not have diarrhoea.

Patients and methods

The index cases were children with acute gastroenteritis or prolonged diarrhoea attending the Queen Elizabeth Hospital for Children between September 1983 and February 1984. They were aged between 2 weeks and 11 years 8 months (mean age (SD) = 18·8 (24·6) months; median age = 4 months). The controls were hospital inpatients without diarrhoea also aged between 2 weeks and 11 years 8 months (mean age (SD) = 18·5 (25·7) months; median age = 4 months).

In September and October only watery stools sent to the laboratory were examined for cryptosporidial oocysts, but from November onwards stools were tested from all children with diarrhoea—that is, increased frequency of loose or watery stools. Stools were collected soon after admission and examined for parasites by a concentration method and for viruses by electron microscopy. They were cultured for salmonella, shigella, enteropathogenic Escherichia coli, and Campylobacter spp by standard techniques and for Yersinia enterocolitica on yersinia selective agar (Oxoid). In addition, stools from the family outbreak (see below) were investigated for the presence of enterotoxigenic E coli by Dr B Rowe of the Division of Enteric Pathogens, Central Public Health Laboratory, Colindale, London, NW9.

Cryptosporidial oocysts were identified by a modified Ziehl-Neelsen technique similar to that described by Henriksen et al8 and Casemore et al9 in

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that strong carbol-fuchsin was used without the application of heat. The faecal smears were air dried and fixed by passing the slide briefly through a flame. They were stained for 10 min with strong carbol-fuchsin without heating and then decolourised in 10% H₂SO₄ until the background material was free of carbol-fuchsin (about 15–60 s). They were washed well with tap water and counterstained with 1% aqueous methylene blue for 30–60 s. Finally, they were washed again with tap water and left to dry in an upright position (avoiding the use of blotting paper).

The presence of cryptosporidial oocysts in the initial stool samples was kindly confirmed by Dr J Cohen, Wellcome Fellow in Infectious Diseases at the Royal Postgraduate Medical School, Hamme-Smith Hospital.

Serum immunoglobulin concentrations were measured in all children positive for cryptosporidia by the Department of Immunology, Institute of Child Health, London, using laser nephelometry. Lymphocyte transformation was determined by a micro method, measuring the incorporation of ³H-thymidine into phytohaemagglutinin stimulated lymphocytes. T cell numbers were measured by erythrocyte rosetting.

**Results**

Cryptosporidial oocysts were found in stools from seven (3·2%) of 213 patients with diarrhoea and from one (0·9%) of 112 controls (χ² = 1·71, p > 0·05). The control patient with cryptosporidia had asthma and chickenpox but was otherwise well and never developed abdominal pain, vomiting, or diarrhoea; cryptosporidial oocysts were detected for two weeks and then disappeared. There were numerous oocysts in his first stool sample.

The clinical features of the index cases are shown in Table 1. Four of the index cases (patients 1, 2, 3, and 7) had short lived illnesses with watery diarrhoea; two also had vomiting; and two of the older children complained of abdominal pain. Two index cases (patients 5 and 6) had prolonged diarrhoea and small intestinal enteropathy. One index case (patient 4) had a prolonged illness with bloody diarrhoea and mucus which started on holiday in Cyprus. *Escherichia coli* 0111 was present on stool culture, and cryptosporidial oocysts were present in the stools. Antibody titres to *Yersinia enterocolitica* remained between 1/40 and 1/80 over a four month period, but stool cultures for yersinia were consistently negative. The relevance of the raised yersinia antibody titres is uncertain. Colonoscopy and biopsy showed a colitis of indeterminate cause with no features of Crohn’s disease or ulcerative colitis. Electron microscopy of the colonic biopsy did not show cryptosporidia. Salazopyrine was started and the colitis resolved after three months.

**Patients with small intestinal enteropathy**

Two of the index cases had failure to thrive and chronic diarrhoea lasting for more than four months.

Patient 5 had been on holiday in France when he, his parents, and his 2 year old sister developed acute diarrhoea. The rest of the family had short illnesses, but the patient’s diarrhoea persisted and his weight fell from the 75th to the 10th percentile. He presented in October 1983 after nine weeks of watery diarrhoea. He had been exclusively breast fed for four months and had been given soya milk ever since, until four days before being seen, when he was given cow’s milk. Stools showed moderate numbers of cryptosporidial oocysts but no other pathogens were found. A proximal small intestinal biopsy performed in October showed mild villous atrophy without crypt hyperplasia, features which are consistent with a postenteritis enteropathy, cow’s milk intolerance, or soya milk intolerance (Fig. 1). Electron microscopy showed cryptosporidial schizonts adhering to the intestinal mucosa (Fig. 2). Cow’s milk or soya milk intolerance were considered likely diagnoses and the patient was started on Pregestimil and solids free of cow’s milk. There was no immediate improvement after milk elimination, but the

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**Table 1 Clinical features of index children with cryptosporidiosis**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of diarrhoea</th>
<th>Other symptoms</th>
<th>Travel abroad</th>
<th>Other pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 yr 9 mo</td>
<td>F</td>
<td>3 dy</td>
<td>Vomiting, abdominal pain</td>
<td>None</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>1 yr 3 mo</td>
<td>F</td>
<td>6 dy</td>
<td>Anaemia</td>
<td>Turkey</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>1 yr 2 mo</td>
<td>M</td>
<td>7 dy</td>
<td>Vomiting</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>4</td>
<td>3 yr 6 mo</td>
<td>F</td>
<td>3 mo</td>
<td>Bloody diarrhoea, mucus</td>
<td>Cyprus</td>
<td>E.coli 0111 (raised yersinia antibody titres)</td>
</tr>
<tr>
<td>5</td>
<td>1 yr 1 mo</td>
<td>M</td>
<td>4 mo</td>
<td>Failure to thrive</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>6</td>
<td>9 mo</td>
<td>M</td>
<td>5 mo</td>
<td>Failure to thrive</td>
<td>Nil</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>7</td>
<td>8 yr 10 mo</td>
<td>F</td>
<td>14 dy</td>
<td>Vomiting, abdominal pain, fever</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>
diarrhoea gradually resolved over several weeks. Five months after the onset of the illness he was still on a cow’s milk free diet and his weight was up to the 25th percentile.

Patient 6 originally presented in July 1983 aged 7 months with acute diarrhoea; an adenovirus was detected in his stools by electron microscopy. This was before we had started routine laboratory examination of stools for cryptosporidial oocysts, but fortunately the stools were stored at -20°C. Diarrhoea persisted on cow’s milk, and he was seen again two months later. Stools from the initial episode in July and from September both contained cryptosporidial oocysts. His weight fell from the 25th percentile to below the 3rd percentile. A small intestinal biopsy was performed in November using a paediatric capsule with two ports (TC Components Ltd, Hampton, Middx). The biopsy showed a patchy enteropathy: one specimen was histologically normal but the other showed mild shortening of the villi with increased intraepithelial lymphocytes and inflammatory cells in the lamina propria (Fig. 3). No cryptosporidia were seen in the duodenal juice or by electron microscopy of the small intestinal mucosa. The diarrhoea gradually resolved without eliminating cow’s milk, and his weight is now above the 10th percentile.

FAMILY OUTBREAK
Patient 7 presented with a two day history of watery diarrhoea, vomiting, and fever. Her 13 year old sister had had an identical illness three weeks earlier and the mother and two other sisters had similar illnesses two weeks later. The father and brother were not affected and neither was their pet dog. The family had not been abroad. The father refused to give a stool specimen, but stools were obtained from the rest of the family and the dog (Table 2). Oocysts

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**Fig. 1** Jejunal mucosa (patient 5). Enteropathy with mild partial villous atrophy and increased intraepithelial lymphocytes. Haematoxylin and eosin. × 130.

**Fig. 2** Cryptosporidial schizont adhering to enterocyte (patient 5). Original magnification × 32 600.
were present in stools from three affected sisters and the unaffected brother. All stools were negative for viruses, bacterial pathogens, (including enterotoxigenic E. coli), and parasites.

Four of the index cases developed acute diarrhoea while travelling abroad or within one week of returning. None, except patient 7, had pets or gave a history of contact with farm animals. Most of the positive cases in the period under study were detected in September and October (Table 3).

**IMMUNOLOGY**

All affected children had normal total white cell counts, normal absolute lymphocyte counts (>2 x 10^9/l), and no neutropenia. T cell numbers were virtually identical to those of controls (30–50% of total lymphocytes), and ³H-thymidine uptake by phytohaemagglutinin transformed lymphocytes was, in all cases, normal. All serum immunoglobulin values fell within the 95% reference ranges for age, except for one child who had a raised total serum IgM.

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**Table 2 Clinical features in family outbreak**

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Symptoms</th>
<th>Duration of illness</th>
<th>Time between onset of illness and collection of stool specimen</th>
<th>Cryptosporidial oocysts detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>31</td>
<td>Watery diarrhoea, abdominal pain, fever</td>
<td>21 dy</td>
<td>11 dy</td>
</tr>
<tr>
<td>Father</td>
<td>30</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Sister</td>
<td>13</td>
<td>Watery diarrhoea, nausea, abdominal pain, fever</td>
<td>21 dy</td>
<td>28 dy</td>
</tr>
<tr>
<td>Sister</td>
<td>11</td>
<td>Watery diarrhoea, vomiting, abdominal pain, fever</td>
<td>14 dy</td>
<td>4 dy</td>
</tr>
<tr>
<td>Patient 7</td>
<td>8</td>
<td>Watery diarrhoea, vomiting, abdominal pain, fever</td>
<td>10 dy</td>
<td>2 dy</td>
</tr>
<tr>
<td>Sister</td>
<td>6</td>
<td>Watery diarrhoea, vomiting, abdominal pain</td>
<td>7 dy</td>
<td>5 dy</td>
</tr>
<tr>
<td>Brother</td>
<td>2</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Dog</td>
<td>4</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not applicable.
NT = not tested.
Oocysts: + = present; − = absent.
Table 3  Timing of cryptosporidial oocyst detection

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept 1983</td>
<td>3*</td>
<td>7</td>
</tr>
<tr>
<td>Oct 1983</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>Nov 1983</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>Dec 1983</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>Jan 1984</td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td>Feb 1984</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>213</td>
</tr>
</tbody>
</table>

*One was also positive in July 1983 when examined retrospectively.

Discussion

The increasing number of reports of gastroenteritis associated with cryptosporidiosis do not provide absolute proof of a causal relation. Current et al.\textsuperscript{10} described 12 veterinary workers who had cryptosporidial oocysts in their stools after handling infected calves; nine had diarrhoea but two were asymptomatic and another had malaise but no diarrhoea. We have described two asymptomatic children with oocysts in their stools. The number of oocysts does not necessarily correlate with severity of disease since one of these children had numerous oocysts in his stools.

Nevertheless, it is highly probable that cryptosporidia can cause acute gastroenteritis in immunocompetent children and adults. The family outbreak we describe was not associated with any other pathogens. The clinical picture was typical of other descriptions of cryptosporidial infestation, with watery diarrhoea and abdominal pain as prominent symptoms; stools collected early in the illness contained oocysts. We have also found that oocysts were no longer present in these patients’ stools as symptoms disappeared.

A proximal small intestinal enteropathy associated with cryptosporidia has not previously been described in immunocompetent patients. It is well described in immunodeficient subjects, however, in whom electron microscopy has shown cryptosporidia adhering to the jejunal mucosa.\textsuperscript{3,4,11} An enteropathy which is often patchy may be seen in prolonged diarrhoea after acute gastroenteritis (the postenteritis syndrome) and in cow’s milk sensitive and other food sensitive conditions.\textsuperscript{12} It is possible that our two patients with prolonged diarrhoea and failure to thrive had one of these syndromes: patient 6 had adenoviruses detected in the stools by electron microscopy at onset and patient 5 did not present until nine weeks after the onset of diarrhoea. Patient 5, however, showed a delayed recovery after milk elimination, unlike the immediate response seen in cow’s milk sensitive enteropathy, and patient 6 recovered without altering his diet.

Isaacs, Hunt, Phillips, Price, Raafat, Walker-Smith

The coccidian parasite isospora, which is closely related to cryptosporidium, causes watery diarrhoea, abdominal pain, and fever in man, which may last several months\textsuperscript{13}; chronic diarrhoea with malabsorption have also been described.\textsuperscript{14} Chronic diarrhoea and failure to thrive have not previously been described in immunocompetent individuals with cryptosporidiosis,\textsuperscript{15} but our evidence, albeit circumstantial, strongly suggests such an association. It is not clear why some children but not others should develop persistent infections.

We noted a possible association with travel abroad. A study from Finland found that 12 of 14 apparently immunocompetent adults with cryptosporidia associated diarrhoea had recently travelled to Leningrad and one to France.\textsuperscript{16} An association with foreign travel may partly explain the finding that five of our seven index cases first had cryptosporidia detected in their stools in September or October, although Tzipori et al.\textsuperscript{7} have also described an increased incidence of infection in the Australian summer and early autumn. When the study has been running longer the relation with season and travel may be more clear.

A case has recently been described of a nurse who was probably cross infected from a child with cryptosporidiosis.\textsuperscript{17} This observation and our description of a family outbreak of cryptosporidial infestation would suggest that cryptosporidia are at least moderately contagious. In view of the devastating clinical course in immunocompromised hosts and the absence of any effective treatment for this condition, it is vital that more is learned about the epidemiology of cryptosporidial infections.

Professor C B S Wood and Dr V F Larcher allowed us to report patients under their care. P Hindocha of the Department of Immunology performed the immunological investigations. The nursing staff kindly sent stool specimens from patients and controls.

References

Cryptosporidiosis in immunocompetent children


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