Cryptosporidium, chronic diarrhoea and the proximal small intestinal mucosa

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Abstract

The association between *Cryptosporidium*, chronic diarrhoea and a proximal small intestinal mucosal enteropathy was reviewed over a six and a half year period. One hundred and twenty three children with cryptosporidiosis and no clinical evidence of immune deficiency were identified. 50% of children excreting only *Cryptosporidium* had chronic diarrhoea. Most cases (63%) of chronic diarrhoea occurred in the first two years of life. A mild to moderate enteropathy was present in all nine children undergoing a small intestinal biopsy and seven showed the presence of *Cryptosporidium* adhering to villous epithelium. All patients eventually recovered spontaneously. *Cryptosporidium* is a cause of chronic diarrhoea and a proximal small intestinal mucosal enteropathy in children without immune deficiency. Screening for the parasite should be part of the investigative procedures in children with chronic diarrhoea.

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Cryptosporidium is a coccidian protozoan parasite found in birds, fish, reptiles, and mammals. It is an obligate parasite with a complex life cycle.¹ There are at least four species of which *C parvum* seems to be responsible for most human infection. The parasite was originally described in 1907² but the first human case was not recognised until 1976³ and only eight cases were described in the literature before 1982.⁴ Between 1982 and 1986 it changed from being described as an uncommon parasite⁴ to the most common

parasite identified⁵ and over 11 000 cases were reported between 1985 and 1988.⁶ There is good evidence that *Cryptosporidium* causes acute gastroenteritis as it has been found more frequently in the stools of children with diarrhoeal disease than controls⁷⁻⁹ and has been detected in diarrhoeal outbreaks.^{10 11} It has been accidentally transferred from animals to man¹² and has been experimentally transmitted from man to calves and mice.¹⁰ *Cryptosporidium* has been detected in the proximal small intestine,⁷ the large intestine and the rectum in man.^{3 13} Seroconversion has been shown¹⁴ but there are no human volunteer studies. Cases have been reported from Europe, Africa, India, the West Indies, North and South America indicating world wide prevalence.¹

Two main forms of clinical presentation have been described; a chronic, life threatening illness in the immunocompromised and an acute self limiting gastroenteritis in immunocompetent patients.¹⁵ Indeed, chronic diarrhoea has been an infrequently reported finding in studies of immunocompetent children with *Cryptosporidium*. We have previously reported two cases associated with chronic diarrhoea, failure to thrive and a proximal small intestinal enteropathy.¹⁶ In order to further study the relationship with chronic diarrhoea we have reviewed patients presenting at Queen Elizabeth Hospital for Children with cryptosporidiosis since 1983 when routine screening of the stools was introduced.

Methods

PATIENTS

Patients presenting at Queen Elizabeth Hospital for Children between October 1983 and April 1990 found to be excreting *Cryptosporidium* in the stool were reviewed retrospectively to assess the presenting symptoms, duration of diarrhoea and patient characteristics. Chronic diarrhoea was defined as diarrhoea persisting for at least 14 days. The routine microbiological examination of the stools included: negative staining electron microscopy for gastrointestinal viruses and light microscopy for ova, cysts, and parasites. Bacterial culture was performed in order to identify pathogens including *Salmonella*, enteropathogenic *E coli*, *Aeromonas*, *Campylobacter*, and *Shigella*. Cryptosporidial oocysts were identified by a modified Ziehl-Nielsen stain as described previously.⁷ The total number of patients who had stool samples analysed could only be accurately assessed in the one year period (1989) when records were computerised.

In nine patients with chronic diarrhoea and severe failure to thrive a proximal small intestinal biopsy was done using a pediatric twin port

Figure 1: Cases of cryptosporidiosis per year.

Figure 2: Accumulated cases of cryptosporidiosis per three month period.

biopsy capsule¹⁷ after obtaining informed consent. The small intestinal mucosa was studied by histology, morphometry,¹⁸ biochemistry,¹⁹ and electron microscopy.²⁰ The immune competence of patients with the most severe symptoms was screened by studying serum immunoglobulins, lymphocyte transformation in response to phytohaemagglutinin stimulation, the formation of T cell rosettes with sheep erythrocytes, yeast opsonisation, and neutrophil chemotaxis and nitro blue tetrazolium chloride reduction.

Results

In the 6.5 year study period 123 children had *Cryptosporidium* identified in the stool and the notes of 109 of these were found and reviewed. In the single one year period (1989) when computer records were available a total of 1662 children provided stools for microbiological examination and 27 (1.6%) had cryptosporidiosis. The annual figures (Fig 1) showed marked variation from

Figure 3: Duration of diarrhoea in patients with cryptosporidiosis.

year to year: on average 18 cases were identified per year, with fewer cases in 1984 and 1988, and more cases in 1986 and 1989. Significantly more cases of cryptosporidiosis occurred between April and June and less cases were seen between October and December (Fig 2) over the whole period of the study (χ^2 test, both $p < 0.005$).

The most common presenting symptom was diarrhoea in 100/109 (92%) patients. This was usually watery and offensive with occasional mucus or blood staining. Fifty six patients (51%) had vomiting and 23 (21%) had significant abdominal pain. Nine patients without diarrhoea had unexplained pyrexia (three), nausea (one), abdominal pain (four), and/or vomiting (four). There was no clinical evidence of immunodeficiency in any of the 109 patients. Duration of diarrhoea was documented in 90/109 cases and is shown in Figure 3. Fifty two of 90 children (58%) had chronic diarrhoea. In 35 cases (39%) the diarrhoea persisted for at least 21 days and in seven cases (8%) for more than six months. In all cases the diarrhoea eventually resolved spontaneously.

Sixty four of 90 children had *Cryptosporidium* alone, 32 of these (50%) had diarrhoea persisting for 14 days or more, and 21 (33%) had diarrhoea persisting for over 21 days. Half of the cases (32/64) occurred in children under two years of age and 20 (63%) of these had chronic diarrhoea compared with only 38% (12/32) of children aged greater than two years ($p < 0.05$). Details concerning the length of excretion of *Cryptosporidium* in relation to symptoms is not available in the majority of cases. Chronic diarrhoea was more frequent in those with mixed infections (20/26 (77%)) compared with those with *Cryptosporidium* alone ($p < 0.025$); 14 had diarrhoea for 21 days or more. The other organisms detected in the children with mixed infections included *Giardia lamblia*, *Aeromonas*, enteropathogenic *E. coli*, *Shigella*, *Salmonella*, *Campylobacter*, rotavirus, adenovirus, calicivirus, and astrovirus. Nineteen children had one other organism, five children had two, and two children had three.

Weight was documented in 82/90 and 19 (23%) were below the third percentile. This was not related to the duration of diarrhoea (χ^2 test, $p > 0.05$). Seven children who were above the third percentile had recently lost weight or were failing to thrive. Although a greater proportion of patients with mixed infections had weight below the third percentile (eight of 21) this was not significantly different to those with *Cryptosporidium* alone (11/61).

Two patient subgroups were identified: these were children who had recently travelled abroad (18), and children who had an itinerant existence within the United Kingdom, generally living in caravan sites (17). Mixed infections were common in both (seven cases in the former subgroup, and six in the latter). Considering the children who were only excreting *Cryptosporidium*, chronic diarrhoea occurred in nine of 11 children (82%) who had returned from travel abroad (Indian subcontinent (eight), Europe (two), and Africa (one)) and in seven of 11 children (64%) from itinerant families (eight of nine and four of seven respectively having diarrhoea for greater than 21 days). In comparison

Figure 4: (a) Light microscopical appearance of small intestinal mucosa (Bar=200 µm). (b) Light microscopy of *Cryptosporidium* adhering to upper villous surface (arrows) (Bar=20 µm). (c) Transmission electron micrograph demonstrating *Cryptosporidium* adhering to the brush border with loss of microvilli and formation of attachment organelle (arrow) (Bar=500 nm). (d) Scanning electron microscopy showing *Cryptosporidium* adhering to the mucosal surface (arrows) (Bar=1 µm).

Figure 5: Measurement of villous height and crypt depth. Abbreviations: VH=villous height, CD=crypt depth, C=controls, Crypto=patients with cryptosporidiosis.

four of seven with the remaining cases, chronic diarrhoea was more frequent in those who had travelled abroad (χ^2 test, $p<0.005$), and was just significantly increased in patients from itinerant families ($p<0.05$).

Nine patients with *Cryptosporidium*, chronic diarrhoea and severe failure to thrive had a proximal small intestinal biopsy performed. The documented length of excretion of *Cryptosporidium* in these children was 60, 20, 19, 15, 8, 7, 7, 1, and 1 days. This information is incomplete however, as stool excretion was not regularly monitored and the latter two children only had single stools examined. One child had previous failure to thrive and developed diarrhoea which persisted for 14 days; the other eight failed to thrive after the onset of diarrhoea. In all eight cases the diarrhoea continued for over 21 days, in five it persisted for more than three months. Linear growth was impaired in four of the nine children.

Light microscopy of the small intestinal mucosa showed a mild to moderate enteropathy in all nine patients (Fig 4a). In seven of the nine patients *Cryptosporidium* was identified adhering to the mucosa. *Cryptosporidium* was found on villous, not crypt, epithelium (Fig 4b) and there was evidence of brush border effacement at sites of attachment (Fig 4c). Scanning electron microscopy, which affords a high resolution view of the mucosal surface, proved a very effective way of detecting the organism (Fig 4d). *Giardia lamblia* was identified in the mucosa in one patient but only *Cryptosporidium* was seen in the

Figure 6: Disaccharidase activities. Note log normal distribution. Abbreviations as for Figure 5.

other patients. Villous height was reduced in all patients compared with controls ($p < 0.001$) whereas crypt depth, although increased in individual cases, was not significantly increased in the group as a whole ($p > 0.05$) (Fig 5). Activity of the disaccharidases lactase, sucrase, and maltase was significantly reduced compared with controls ($p < 0.01$) (Fig 6) and the intraepithelial lymphocyte count was significantly increased ($p < 0.001$) (Fig 7).

Immune function was screened in five of the nine patients requiring biopsy and all tests were normal.

Patients with diarrhoea were given oral rehydration therapy for 24 hours and then returned to their normal feeds. Five of the nine patients undergoing a small intestinal biopsy

Figure 7: Intraepithelial lymphocyte count (IEL). Other abbreviations as for Figure 5.

were subsequently placed on a diet free from cow's milk protein and lactose with no apparent clinical response. All patients eventually recovered spontaneously.

Discussion

The detection rate of cryptosporidiosis in the one year in which it was possible to be calculated was similar to the finding of 1.4% reported by Baxby and Hart.⁸ The Public Health Laboratory Service (UK), in a study of 9880 patients in England and Wales with presumed infective diarrhoea, found 5% of children aged between one and four years had *Cryptosporidium* in the stool.⁶ Although chronic diarrhoea (defined by them as greater than 21 days duration) occurred most frequently in this age group, this amounted to only 6% of cases – that is, 94% had diarrhoea which lasted for less than 21 days.

A surprising finding in the present study was that 32 of the 64 children – that is, 50% excreting only *Cryptosporidium* had chronic diarrhoea, and 33% (21/64) had diarrhoea for 21 days or more. In view of these findings and the world wide distribution of *Cryptosporidium* it is possible that the parasite may play an important role in chronic diarrhoea in developing countries, and in its associated morbidity and mortality,²¹ as indicated by others.^{22,23} It also appears to be a cause of traveller's diarrhoea in early childhood, as reported in older children and adults,²⁴ and to be associated with chronic diarrhoea in such circumstances. Indeed, the reason for the greater frequency of chronic diarrhoea in this study in comparison with others in the United Kingdom,^{6,25} may be partly explained by the subgroups of children who had recently travelled abroad and those who had an itinerant existence. This highlights the remaining 50% (16/32) of cases with chronic diarrhoea for whom there is no ready explanation for the persistence of their symptoms. It is possible that the natural history of cryptosporidiosis in the United Kingdom includes diarrhoea extending up to 21 days.^{6,25} Nine of these 16 remaining children, however, had diarrhoea for over 21 days, indicating that host factors may be important in the continuation of their symptoms. Twenty nine per cent of cases had a mixed infection, and chronic diarrhoea was more frequent in these patients. There was insufficient evidence to determine if the mixed infections were concurrent, intercurrent, or sequential. Indeed, more information is required concerning the pattern of excretion of *Cryptosporidium* and other organisms, in order to investigate whether the chronic diarrhoea in these cases is a result of persistent infection or is a postinfective phenomenon. In general, in immunocompetent children, it appears that *Cryptosporidium* and *Giardia lamblia*²⁶ infections may be associated with chronic diarrhoea, bacterial infections may extend into the chronic period,^{27,28} and viral infections are of short term duration.^{27,28}

Around one quarter of the children in this study were below the third percentile for weight suggesting an association between cryptosporidiosis and failure to thrive, as described in other centres.^{22,23,25} In developing countries it is

uncertain whether cryptosporidiosis leads to failure to thrive or whether malnutrition predisposes to cryptosporidiosis.^{22,23} These two postulates are not necessarily mutually exclusive. In this study infection with *Cryptosporidium* appears to be the primary event. Four of the nine patients requiring a small intestinal biopsy had impaired linear growth emphasising the potential serious effects of this illness. This also highlights the need to find an effective treatment, particularly as diagnosis is now relatively straightforward.

The association between a proximal small intestinal enteropathy and cryptosporidiosis in man has not been well established. In this study there was a remarkably consistent proximal small intestinal enteropathy of mild to moderate severity. Such an abnormality might be considered to be insufficient to explain the severity and chronicity of symptoms, however, involvement of widespread areas of the intestine including the colon have been described in cryptosporidiosis¹³ and the extent of the enteropathy may be an important factor in these cases.

The organism was only seen on villous epithelium. In immune deficient states *Cryptosporidium* is also found attached to crypt epithelium within the crypt lumen,^{29,30} indicating differences in the extent of colonisation of the mucosal surface in the two conditions. This observation is of interest in relation to the involvement of the immune system in influencing the topography of mucosal colonisation, and requires further investigation.

We conclude that *Cryptosporidium* is an important cause of chronic diarrhoea with failure to thrive in immunocompetent children and is associated with a proximal small intestinal enteropathy. Screening for *Cryptosporidium* should be routinely carried out in such children.

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