Cryptosporidium spp., a Frequent Cause of Diarrhea in Liberian Children

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This report presents results from a sample survey designed to investigate the possible role of Cryptosporidium spp. in childhood diarrhea in a developing country, Liberia, West Africa. During the four months of January to April 1983, a house-to-house study was carried out in two geographically and socially different communities—an urban slum and three rural villages. Stool samples from 374 children, aged 6 to 59 months, were tested for Cryptosporidium spp. Among the children with diarrhea 8.4% were Cryptosporidium spp. positive compared with a prevalence rate of 5.9% in asymptomatic children. Of the children living in a household with a Cryptosporidium spp.-positive index child, 8.6% had a positive stool sample. Of all children attending a clinic because of diarrhea, 14.6% were Cryptosporidium spp. positive. Cryptosporidiosis was more frequent in younger children; 24 of the total of 29 positive cases (83%) were below 2.5 years old. Actual or previous bottle feeding (formula) was a risk factor, particularly in children below 18 months old. Of the bottle-fed children, 28% were Cryptosporidium spp. positive versus 9.1% of children never bottle fed. Crowding is another possible risk factor. The prevalence of cryptosporidiosis was 13.5% in big urban households with more than 10 children, whereas the prevalence in the small urban households was 6.1%. Ethnic and religious differences were particularly evident in the rural area. No Muslim households had cryptosporidiosis, whereas the prevalence in non-Muslim tribes was 9%. The general belief that cryptosporidiosis is primarily a zoonosis is questioned in this study, partly because many carriers and asymptomatic household contacts were found.

In many developing countries gastroenteritis is the single biggest cause of early childhood mortality (19). In industrialized countries gastroenteritis is rarely fatal, but it is an important health and socioeconomic burden. Despite modern diagnostic techniques, approximately half of the known cases of gastroenteritis remain without etiological diagnosis, making causal therapy difficult.

Enteric cryptosporidiosis, caused by the coccidian parasite Cryptosporidium sp., has become of increasing interest since the first case of human cryptosporidiosis was reported in 1976 (15). Much of this interest is due to its opportunistic behavior in immunocompromized patients, particularly in those with acquired immune deficiency syndrome (6, 12, 14, 16; Editorial, Lancet, i:492-494, 1984). In these immunocompromized patients, cryptosporidiosis often occurs as a severe, persisting watery diarrhea, with fluid losses up to 15 liters per day. A variety of therapies have been tried unsuccessfully (4), but according to recent reports from the Centers for Disease Control, Atlanta, Ga., spiramycin has shown some effect (5). In well-nourished individuals with normal immune functions, the disease often occurs as a short, self-limiting gastrointestinal illness (14, 16).

Before 1982, diagnosis of human cryptosporidiosis usually required intestinal biopsies. Better and faster methods for detecting the infective oocyst in stool samples now facilitate large-scale screening (8, 10, 11). At the time of this survey no information had been published on cryptosporidiosis in developing countries, and the study was therefore undertaken to elucidate epidemiological and clinical aspects of human cryptosporidiosis in children aged 6 to 59 months from a developing country, Liberia, West Africa.

MATERIALS AND METHODS

The study was carried out during the dry and late dry season, January to April 1983.

Study population. Two communities, an urban slum and three rural villages, were selected and geographically and socially stratified to enable comparison. Social stratification was based on the income and property level of a household and the educational level and social status in the community of all members of a household.

The urban slum, West Point, was a shanty town situated on a 0.25-km² peninsula extending from the capital city, Monrovia, into the Atlantic Ocean. The estimated population was 30,000, living primarily in one-storey tin shacks.
with few or no facilities. The community was served by one public health clinic without a permanent doctor and one school. In the rural area, Bong county, situated 200 km inland in a forest area, three typical villages with a total population of approximately 3,500 were selected. People lived in adobe houses with no facilities, but a small public health clinic and a school were functional in two of the villages. A household was defined as an extended family living together and sharing one cooking pot. Only children aged 6 to 59 months were included. Households were visited repeatedly until all children living for more than 4 weeks in the household were seen. The refusal and dropout rates were less than 4% in both areas.

In all households the medical history was recorded, and the children were physically examined by a physician. A freshly voided stool and a blood sample were collected from each child. To consider a child as having diarrhea, two criteria had to be fulfilled. The child should have diarrhea, according to its parents, and the fecal sample should take the shape of the stool container used.

Group A comprised 41 children with diarrhea (mean age, 20.5 months; range, 6 to 58 months) seen at the public health clinic, West Point, and 20 household contacts (11 with diarrhea) of Cryptosporidium sp.-positive children. All stool samples were examined for cryptosporidiae.

Group B included 284 West Point children with or without diarrhea living in 144 households randomly sampled from a house-to-house map. Stools were examined for cryptosporidiae in 80 children with diarrhea (mean age, 33.2 months; range, 6 to 59 months) and in a further 65 randomly picked children without diarrhea with similar age distribution as groups A and B. Another 13 children without diarrhea were examined as household contacts of Cryptosporidium sp.-positive children.

Group C comprised all of the children in the three villages in Bong county. From 260 households, 518 children were examined. Stools were examined for cryptosporidiae in 105 children with diarrhea (mean age, 30.6 months; range, 6 to 59 months) and in a further 37 randomly picked children without diarrhea with a similar age distribution. Another 13 children without diarrhea were household contacts of positive children. Children \( n = 191 \) from groups A, B, and C were reexamined after 1 month.
Microbiological methods. Stools were collected in sterile containers and kept in an insulated box with ice packs (temperature, 5 to 10°C) until processing within 24 h.

Two slides with unconcentrated stool, one unstained and one iodine stained ( Lugols), were examined for identification of Entamoeba histolytica and Giardia lambia cysts; Trichuris trichiura, Ascaris lumbricoides, and hookworm ova; and Strongyloides stercoralis larvae and ova. Specimens were preserved in 10% Formalin and later concentrated by the method of Ritchie as improved by Allen and Ridley (1). A thick smear was made from the sediment, which was then air dried and fixed in a flame and with methanol. The smears were stained by a modified Ziehl-Neelsen technique, as described by Henriksen and Pohlenz (10). Because a large amount of material took up carbol fuchsin, a long staining and decolorizing period was used. Known Cryptosporidium sp.-positive slides were stained with each batch as a control. Stained preparations were examined under oil immersion at a magnification of ×1,000. A preparation was considered negative only after screening of more than 200 fields.

The bacteriological examination included culturing within 24 h after sampling for Shigella sp., Salmonella sp., Yersinia enterocolitica, and Escherichia coli by routine methods and media from the Department of Diagnostic Bacteriology, Statens Seruminstitut, Copenhagen, Denmark. All isolated colonies were kept in beef extract agar, and within 1 month they were sent to the laboratory at the Statens Seruminstitut for verification and further identification.

Strains isolated from diarrheic children aged less than 30 months were, if identified biochemically as E. coli, serotyped and tested for production of heat-labile enterotoxin by the Y-1 adenalin cell assay and heat-stable enterotoxin by the suckling mouse assay.

For identification of Campylobacter sp., culturing was done on Skirrow medium (18). The plates were incubated at 35°C in an anaerobic jar with an activated GasPak and catalyst (BBL Microbiology Systems, Cockeysville, Md.) for 48 h. Suggestive campylobacterlike colonies were examined by phase-contrast microscopy and categorized as Campylobacter sp. when spiral or curved rods exhibiting typical darting motility were observed.

Swabs were taken from all of the fresh fecal samples, kept in Stuart transport medium at room temperature (20 to 25°C), and cultured in and seeded from selenite enrichment medium within 1 month for identification of Salmonella sp. and Y. enterocolitica. A suspension from the swabs was used to examine for rotavirus by enzyme-linked immunosorbent assay (Dakopatts, Copenhagen, Denmark).

The chi-square test with Yates correction and the Mantel-Haenszel test were used when appropriate for statistical comparison. A P value less than 0.05 was considered significant.

RESULTS

Cryptosporidium oocysts were isolated from 29 of 374 children examined. Data on these children are given in Table 1.

Of children with diarrhea, 8.4% (20 of 237) were Cryptosporidium sp. positive compared with 5.9% (6 of 102) of children without diarrhea and 8.6% (3 of 35) of children from a Cryptosporidium sp.-positive household. The differences were not significant. Of the nine Cryptosporidium sp.-positive children without diarrhea on the day of examination, six had a history of diarrhea during the preceding 2-week period.

Children attending West Point clinic (group A) complaining of diarrhea had a higher prevalence of cryptosporidiosis (14.6%; 6 of 41) than did children with diarrhea in groups B (7.5%; 6 of 80) and C (5.7%; 6 of 105). Among the youngest clinic children, aged 6 to 11 months, the prevalence was 25% (5 of 20). The observed differences between the prevalences in the urban groups, A versus B, and between the sample groups, B versus C, are not significant.

The peak prevalence was in the age group 6 to 11 months (14.9%), and 24 of the 29 positive children were below 2.5 years of age (X² = 9.4; P < 0.01) (Table 2). The mean age was 20 months.

The age-specific prevalences of other enteric parasites examined for are shown in Fig. 1. Prevalences of intestinal parasitoses increased with age and stayed high throughout childhood. In the older children, 42 to 59 months, approximately 90% had intestinal parasites.

Cryptosporidiosis was significantly more prevalent among children being or having been given any type of formula (bottle fed) than among children never bottle fed (16 versus 5.7%; X² = 7.43; P < 0.01). This pattern was even more striking in the younger age groups below 18 months (Table

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### TABLE 2. Age-specific prevalence for Cryptosporidium sp.

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
<th>Group C</th>
<th></th>
<th>Groups A, B, and C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive/total</td>
<td>%</td>
<td>Avg % in age groups</td>
<td>No. positive/total</td>
<td>%</td>
<td>Avg % in age groups</td>
<td>No. positive/total</td>
</tr>
<tr>
<td>6-11</td>
<td>5/22</td>
<td>22.7</td>
<td>18.4</td>
<td>1/21</td>
<td>4.8</td>
<td>7.6</td>
<td>4/23</td>
</tr>
<tr>
<td>12-17</td>
<td>0/5</td>
<td></td>
<td></td>
<td>1/25</td>
<td></td>
<td>40.0</td>
<td>1/19</td>
</tr>
<tr>
<td>18-23</td>
<td>0/5</td>
<td></td>
<td></td>
<td>2/13</td>
<td></td>
<td>15.4</td>
<td>1/17</td>
</tr>
<tr>
<td>24-29</td>
<td>2/6</td>
<td>33.3</td>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
<td>1/22</td>
</tr>
<tr>
<td>30-35</td>
<td>0/4</td>
<td></td>
<td>8.7</td>
<td>0/18</td>
<td></td>
<td>1.4</td>
<td>1/20</td>
</tr>
<tr>
<td>36-41</td>
<td>0/4</td>
<td></td>
<td></td>
<td>0/8</td>
<td></td>
<td></td>
<td>1/16</td>
</tr>
<tr>
<td>42-47</td>
<td>1/4</td>
<td></td>
<td>25.0</td>
<td>0/22</td>
<td></td>
<td></td>
<td>0/12</td>
</tr>
<tr>
<td>48-53</td>
<td>1/3</td>
<td>33.3</td>
<td>1/13</td>
<td>7.7</td>
<td></td>
<td></td>
<td>0/8</td>
</tr>
<tr>
<td>54-59</td>
<td>0/8</td>
<td></td>
<td></td>
<td>0/13</td>
<td></td>
<td></td>
<td>0/18</td>
</tr>
</tbody>
</table>

* The percentages of positive children in groups A, B, and C were 14.8, 4.4, and 8.4%, respectively, and the overall percentage was 7.8%.
3). For all children below 18 months of age, the difference was significant ($X^2 = 4.5; P < 0.05$). Of the total number of *Cryptosporidium* sp.-positive children, 43% (12 of 28) had been bottle fed, whereas only 19% (63 of 330) of the *Cryptosporidium* sp.-negative children had been bottle fed ($X^2 = 7.4; P < 0.01$). No infants exclusively breast fed were found to excrete *Cryptosporidium* oocysts.

Crowding was found to be associated with cryptosporidiosis. In the condensed urban slum with many big households, the prevalence of cryptosporidiosis in big households with more than 10 children below 5 years of age was 20% in group A (2 of 10) and 11.1% in group B (3 of 27). The prevalence in households with fewer than 10 children below 5 years of age was 14.3% (7 of 49) in group A and 3.1% (4 of 131) in group B. In the rural villages, the critical size of a household was found to be smaller. The prevalence of cryptosporidiosis in all of the villages was 11.5% (3 of 26) in households with five or more children and 7.8% (10 of 129) in smaller households.

In Bong county, 21 children who belonged to a Muslim tribe were examined (mean age, 33.1 months). None of these children had cryptosporidiosis, whereas the prevalence was 9% in children from other tribes (mean age, 30.3 months).

No socioeconomic parameters other than crowding and bottle-feeding practices were found to correlate with cryptosporidiosis.

A total of 45 children had their stools reexamined for *Cryptosporidium* 1 month after the first examination, and 6 (13.3%) were positive. Of the 45 children, 6 were positive at the first examination, and 2 of these were still excreting *Cryptosporidium* oocysts 1 month later.

**DISCUSSION**

Although human cryptosporidiosis was first reported in 1976 (15), it is only in the last few years, following the epidemic outbreak of acquired immune deficiency syndrome, that an increase in studies on cryptosporidiosis has been reported.

Very little information is available on the prevalence, significance, and prognosis of *Cryptosporidium* infection in children (9, 21), particularly in developing countries, where childhood diarrhea constitutes a major problem (2, 13).

In this sample survey there was no significant difference in the prevalences of cryptosporidiosis among children with diarrhea, asymptomatic children, and household contacts of positive cases (8.4, 5.9, and 8.6%, respectively). However, two-thirds of the asymptomatic excretors (carriers) had a history of diarrhea in the 2 weeks prior to examination. High carrier rates were also found with other well-known pathogens like *Shigella* spp. (3.7% in the group of children with diarrhea; 1.6% in the control group) and *Campylobacter* sp. (39.6% in the group of children with diarrhea; 31.0% in the control group). It is well documented in other studies from developing countries, though not well explained, that many asymptomatic infections occur when diarrhea is prevalent and fecal transmission and point prevalence of known pathogens are high (7).

Among all of the children attending the clinic in the slum area and complaining of diarrhea, thus perhaps having more severe diarrhea, the prevalence of cryptosporidiosis was much higher (14.6%) than among the sample group children with diarrhea (6.5%). This pattern was not seen for any of the other intestinal parasites looked for and could indicate that *Cryptosporidium* infection causes a more severe type of diarrhea but might also simply be explained by the fact that younger children are overrepresented among children attending the clinic. Larger studies would be needed to confirm this.

The age distribution of cryptosporidiosis was very different from the age distribution of the other intestinal parasites examined for in this study. Whereas these other intestinal parasites were characterized by prevalences increasing with age to a high and stable level in older children, *Cryptosporidium* prevalence peaked in the age group 6 to 12 months and declined to a stable low level in older children (Fig. 1). These results agree with the few other reports from developing countries where similar age distributions of cryptosporidiosis and relations to other intestinal parasites have been reported. Thus, in a hospital study from Rwanda (2) the mean age of *Cryptosporidium* sp.-positive children was 13.3 months, and only 4 of 20 positive children had other enteric parasites. In Costa Rica (13) the mean age varied between rural (22.7 months) and urban (9.7 months) children, and only 3 of 12 *Cryptosporidium* sp.-positive children had other enteric parasites. This reverse pattern in age-specific prevalence might reflect a different way of transmission or more likely the induction of protective immunity against the *Cryptosporidium* parasite. If the latter, this would facilitate the development of a vaccine for risk groups. More knowledge, particularly on the immune response in cryptosporidiosis, is needed to elucidate this.

The aim of this study was to pinpoint possible risk factors of acquiring cryptosporidiosis. The prevalence of *Cryptosporidium* excretors was significantly higher in those chil-

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**TABLE 3. Prevalence of *Cryptosporidium* excretors in relation to history of bottle feeding**

<table>
<thead>
<tr>
<th>Location and age range</th>
<th>Bottle fed Total no.</th>
<th>Total no. No. <em>Cryptosporidium</em> sp. positive</th>
<th>% Positive</th>
<th>Never bottle fed Total no.</th>
<th>No. <em>Cryptosporidium</em> sp. positive</th>
<th>% Positive</th>
<th>Frequency of bottle feeding (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Point All ages</td>
<td>68</td>
<td>8</td>
<td>11.8</td>
<td>136</td>
<td>7</td>
<td>5.1</td>
<td>33.3</td>
</tr>
<tr>
<td>Below 18 months</td>
<td>21</td>
<td>4</td>
<td>19.1</td>
<td>48</td>
<td>3</td>
<td>6.3</td>
<td>30.4</td>
</tr>
<tr>
<td>Bong</td>
<td>7</td>
<td>4</td>
<td>57.1</td>
<td>147</td>
<td>9</td>
<td>6.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Below 18 months</td>
<td>4</td>
<td>3</td>
<td>75.0</td>
<td>40</td>
<td>5</td>
<td>12.5</td>
<td>9.1</td>
</tr>
<tr>
<td>West Point and Bong</td>
<td>75</td>
<td>12</td>
<td>16.0</td>
<td>283</td>
<td>16</td>
<td>5.7</td>
<td>20.9</td>
</tr>
<tr>
<td>Below 18 months</td>
<td>25</td>
<td>7</td>
<td>28.0</td>
<td>88</td>
<td>8</td>
<td>9.1</td>
<td>22.1</td>
</tr>
</tbody>
</table>
dren being or having been bottle fed for some period (P < 0.01). This was particularly true for children less than 18 months old. Interestingly, the two children who shed oocysts at the reexamination 4 weeks later were both bottle fed and below 18 months of age. These results support a study from Costa Rica, where a strong negative correlation was found between cryptosporidiosis and breast feeding (13). In the present study no infants exclusively breast fed were found to excrete Cryptosporidium oocysts.

In the children being bottle fed at the time of the survey, the higher rate of infection was probably best explained by the use of often contaminated food and bottles.

The reason why this association persisted even in older children not being further bottle fed is not clear. A possible explanation is the earlier acquisition of infections added to the often very poor dietary bottle food, leading to malnutrition and possibly impaired immune function (17, 20).

Crowding was difficult to define in the urban slum, where conditions were so bad that virtually everyone lived under similar crowded, unhygienic conditions. Crowding, expressed as the number of residents (or children) per the number of rooms, was more than twice as high as in the rural area. Moreover, the average room was smaller in the urban slum. In this area the prevalence of cryptosporidiosis was highest in very large families with more than 10 children. In rural villages with better-spaced housing conditions, the critical family size seemed to be smaller. We believe that this association with crowding is indicative of the importance of person-to-person transmission in these areas.

The observation that oocysts were not found in a single child from the Muslim tribe living in Bong county is probably explained by differences in hygienic practices based on a religious background. Many Muslim households (47.1 compared with 17.7% in the non-Muslim tribes) had private latrines. Better access to water, no domestic pigs, and traditions of hand washing may also play a part in the explanation.

In conclusion, this study suggests widespread existence of Cryptosporidium sp. in children in Liberia. Cryptosporidiosis was more prevalent in younger children and particularly in infants with severe diarrhea.

The concept of cryptosporidiosis as a zoonosis has recently been questioned (3). Our study demonstrates a human reservoir with carriers and household contacts; association between crowding, bottle-feeding, and cryptosporidiosis in regions where domestic animal are infrequent; and peak prevalence among infants still carried on their mothers' backs and thus not yet in intimate contact with the outside world and animals. These findings indicate that person-to-person transmission exists and might very well be the most important way of transmitting Cryptosporidium oocysts.

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LITERATURE CITED


