Cryptosporidiosis in Children from the Sultanate of Oman

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البوغ الخفي في سلطنة عمان
ج. ج. ويندسر، س. شافي، س. ويلتش وف. الأتسكي

خلاصة: لقد تم خلال عام 8.7% من الأطفال الذين تتراوح أعمارهم بين 0-12 سنة في السلطنة عمان تواجد البول في فحص اليوتوشيمي (Cryptosporidium) وحيضات جراثيم في البروبيكل (Zielh-Neelsen). قد تم فحص العينات بعد مسحها بالبروبيكل (Zielh-Neelsen) وتحضير مواد الحمضية (Phenol-auramine) في عينة نسبة 28% من حالات البول الخفي. وجدنا أيضاً أن هناك نسبة عالية من حالات البول الخفي في الأطفال الذين يعانون من العدوى البولية، وكان هذا نتيجة لانعدام الشفافية في البول الخفي ووجود كمية كبيرة من البول الخفي.

ABSTRACT: Over a one year period, 807 faecal samples from children (0-12 years) were submitted for routine microbiological examination and screening for Cryptosporidium oocysts. A Phenol-auramine stain was used and putative oocysts were measured after confirmatory staining with the modified Zielh Neelsen method. Cryptosporidium were identified in 16 (1.9%) of the samples, and was the third most common enteropathogen after Giardia lamblia (6.0%) and Entamoeba histolytica/dispar (2.3%). With the exception of two patients with underlying immunodeficiency disorders, all were immunocompetent. No cases of Cryptosporidium were diagnosed during the hottest months of May and June.

Cryptosporidium is recognised as an important aetiological agent associated with diarrhoeal diseases of humans and animals. It was first described in humans in 1976 (Meisel et al., 1976; Nime et al., 1976). Up until 1982 few cases had been reported, most of which were in patients with underlying immuno-deficiency disorders. The number of cryptosporidiosis cases rose dramatically with the spread of HIV, the causative agent of Acquired Immunodeficiency Syndrome (AIDS). In 1985, 3.6% of AIDS cases reported to the Centers for Disease Control in the United States were positive for Cryptosporidium (Navin, 1985). Since 1980, numerous studies have been performed on immunocompetent patients in Europe (Casemore and Jackson, 1983; Hunt et al., 1984; Baxby and Hart, 1986; Corbett-Feeney, 1987), North America (Current et al., 1983; Ratnam et al., 1985; Watson et al., 1985), Africa (Bogaerts et al., 1984; Holley et al., 1986; Smith and Van den Ende, 1986; Molbak et al., 1997), Asia (Matran et al., 1985; Shahid et al., 1985), and Australia (Biggs et al., 1987). Many of these studies have shown children to be more commonly infected than adults (Navin, 1985). Some authors have reported that children under two years of age are more susceptible to infection (Baxby and Hart, 1986). Cryptosporidium has also been associated with traveller's diarrhoea (Sterling et al., 1986), outbreaks in day-care centres (Alpert et al., 1984), and large outbreaks associated with contamination of water supplies (Hayes et al., 1989; and MacKenzie et al., 1995). Cryptosporidiosis was initially thought to be primarily a zoonosis, but it has been demonstrated that person-to-person transmission is common (Current and Garcia, 1991).

Although a few reports have been published on the incidence of cryptosporidiosis in the Middle East (Khan et al., 1998; Daoud et al., 1990; Bolbol, 1992), no definitive study has been reported from the Sultanate of Oman. The purpose of the present study was to determine the occurrence of Cryptosporidium among children in the Sultanate of Oman.

Materials and Methods

During a one year period starting on 1st January 1994, all faecal samples collected from children (0-12 years) presenting to Sultan Qaboos University Hospital (SQUH) were examined for Cryptosporidium oocysts.

Air-dried faecal smears were fixed in methanol and stained by the Phenol-auramine method (Fleck and Moody, 1988). Smears were scanned for oocysts by
fluorescent microscopy at 200X and 400X. Samples showing yellow fluorescence with a pale halo were further subjected to the modified Ziehl-Neelsen staining method (Casemore et al., 1984). Oocysts were then measured using an eyepiece graticule (Olympus Optical Co Ltd, Tokyo, Japan). It is essential to measure putative oocysts to distinguish them from *Cyclospora*, which is associated with traveller's diarrhoea (Editorial, 1994). *Cryptosporidium* oocysts measure 4-5 μm whereas *Cyclospora* oocysts are much larger (8-9 μm). The staining characteristics are similar, therefore failure to measure oocysts could result in misdiagnosis. A positive control was used for each batch of smears. All specimens were examined for the presence of *Salmonella* spp, *Shigella* spp and *Campylobacter* spp using standard bacteriological techniques. In addition faecal samples from children under 3 years were examined for enteropathogenic *Escherichia coli*. Saline and iodine wet mounts were examined microscopically for the presence of parasitic cysts and ova.

**Results and Discussion**

A total of 807 faecal samples, from 768 different patients, were screened over the one year period. *Cryptosporidium* oocysts were found in 17 stools (2.1%) from 16 patients (1.9%). The children ranged in age from one month to 12 years. Twelve (75%) of the infected patients were less than two years old. Of the 16 patients, 9 were male and 7 were female. In addition to diarrhoea, three patients presented with fever and one with vomiting. Two of these patients were co-infected with a bacterial pathogen, namely *Campylobacter* spp, and *Shigella flexneri* type 6. Two patients were immunocompromised, one with AIDS, the other with hyper IgE syndrome. Most cases presented in the cooler months of December and January, and no cases at all were recorded during the hottest months of May and June (Table 1).

*Giardia lamblia* was found in 6.0% of the faecal samples examined. Other enteropathogens found are shown in Table 2.

Our results show that cryptosporidiosis is an important cause of gastroenteritis in Oman, and is a commoner pathogen than *Salmonella* spp (1.7%), *Shigella* spp (1.8%), and *Campylobacter* spp (1.1%). It was second in frequency only to *Giardia lamblia* (6.0%) and *Entamoeba histolytica/dispar* (2.3%). Our incidence of 1.9% was slightly higher than that previously reported in Saudi Arabia (1.0 and 1.1%) (Khan et al., 1988; Bolbol, 1992), and Kuwait (1.6%) (Daoud et al., 1990). Some studies in the less developed countries of Africa and Asia have reported an incidence as high as 20% (Current and Garcia, 1991).

Seventy-five percent of the patients in the present study were less than two years of age. Baxby and Hart (1986) in the UK have reported similar findings. Although

<table>
<thead>
<tr>
<th>MONTH</th>
<th>NUMBER OF SAMPLES</th>
<th>POSITIVE SAMPLES</th>
<th>% POSITIVE SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>60</td>
<td>4</td>
<td>6.6</td>
</tr>
<tr>
<td>February</td>
<td>66</td>
<td>2</td>
<td>3.0</td>
</tr>
<tr>
<td>March</td>
<td>47</td>
<td>2</td>
<td>4.2</td>
</tr>
<tr>
<td>April</td>
<td>72</td>
<td>2</td>
<td>2.7</td>
</tr>
<tr>
<td>May</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>June</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>July</td>
<td>74</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>August</td>
<td>61</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>September</td>
<td>104</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>October</td>
<td>82</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>November</td>
<td>79</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>December</td>
<td>67</td>
<td>3</td>
<td>4.4</td>
</tr>
</tbody>
</table>

**Enteropathogens Recovered.**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Giardia lamblia</em></td>
<td>49</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Entamoeba histolytica/dispar</em></td>
<td>19</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Cryptosporidium</em> dispar</td>
<td>16</td>
<td>1.9</td>
</tr>
<tr>
<td><em>Salmonella</em> spp</td>
<td>14</td>
<td>1.7</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>8</td>
<td>0.9</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>5</td>
<td>0.6</td>
</tr>
<tr>
<td><em>Shigella boydii</em></td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>Enteropathogenic <em>Escherichia coli</em></td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp</td>
<td>9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

breast-feeding is encouraged in Oman, many mothers alternate between breast and bottle feeding. This exposure to food and water may be a contributing factor for the high incidence found in this age group. Unfortunately, however, it was not possible to examine the water.
supplies from the various regions of the country represented in this study.

No cases of cryptosporidiosis were found during the hottest months of May and June. This absence of cases during the summer months has also been reported in Kuwait (Daoud et al., 1990). Indeed, seasonal variations in the incidence of cryptosporidiosis have been reported in other studies, but the reasons for this phenomenon is still unclear (Baxby and Hart, 1986; Corbett-Feeney, 1987).

The sources of infection in the present study are unknown. It is well established, however, that horizontal transmission among humans is not uncommon and explains the large numbers of cases in urban areas (Casemore, 1986). Cryptosporidia can also be transmitted to humans from domestic animals, such as cattle and sheep (Hayes et al., 1989). It is presently unknown whether the principle livestock animals in Oman, namely goats and camels, are important reservoirs of infection. Cryptosporidia have been described in goats, and an organism described as C. muris-like has been reported in a camel (Fayer et al., 1991). We are presently investigating the incidence of cryptosporidiosis in these animal species. There are some reports that indicate that there are antigenic differences between Cryptosporidia found in animals and man (Awad-El-Kareem et al., 1995).

Cryptosporidium oocysts are very resistant in the external environment and can persist in water supplies even after treatment (Hayes et al., 1989). Two large waterborne outbreaks have been reported in the United States, one involving 14,000 people (Hayes et al., 1989), the other involving >400,000 people (MacKenzie et al., 1995). Many residences in Oman are supplied with water from tankers that may be a potential source of infection.

Conclusions

The results of the present study indicate that Cryptosporidium is an important pathogen associated with diarrhoea of children in Oman. Indeed, it was second in importance only to Giardia lamblia and Entamoeba histolytica/dispar. To ascertain the source of infection, future studies will incorporate the investigation of faecal samples of family members of the infected children, livestock and screening of water supplies.

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References


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