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Detection of *Cryptosporidium* amongst diarrhoeic and asymptomatic children in Jeddah, Saudi Arabia

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Between the March and May of 2000, stool specimens were collected from children aged <5 in six pre-school crèches and clinics in the Jeddah area of Saudi Arabia. Sixty-three (25%) of the children had diarrhoeal disease but the other 190 were asymptomatic. When the stool samples were stained and examined for the oocysts of *Cryptosporidium* spp. and other enteric protozoa, 20 (32%) of the symptomatic children but only nine (4.7%) of the asymptomatic were found to be excreting *Cryptosporidium* oocysts. Similarly, *Cyclospora cayetanensis* was found, always as a co-infection with *Cryptosporidium* sp., in seven (11%) of the children with diarrhoeal disease but only eight (4.2%) of the asymptomatics. One asymptomatic child was found to be co-infected with *Isospora belli* and *Cryptosporidium*. *Giardia intestinalis* and cysts of *Entamoeba histolytica/dispar* were detected in 18 (29%) and nine (14%) of the children with diarrhoeal disease and in eight (4.2%) and 13 (6.8%) of the asymptomatic children, respectively. This appears to be the first report of *Cy. cayetanensis* from Saudi Arabia and the highest prevalence of *Cryptosporidium* infection ever described.

Coccidian parasites are been increasingly recognised as important causes of diarrhoea in children and immuno-compromised patients world-wide (Cegielski *et al.*, 1999). Species of *Cryptosporidium* are ubiquitous in their distribution, and various genotypes of *Cr. parvum* and anthropophilic and zoonotic species such as *Cr. meleagridis*, *Cr. muris* and *Cr. felis* have all been implicated in human illness (Morgan *et al.*, 1998; Morgan-Ryan *et al.*, 2002; Tiangtip and Jongwutiwes, 2002; Gatei *et al.*, 2003).

In temperate climates, cryptosporidiosis accounts for only 1%–4% of the cases of childhood diarrhoea and is usually self-limiting (although it can produce devastating diarrhoea in HIV-positive individuals who have fewer than 200 $CD4^+$ lymphocytes/μl). In tropical environments, however, cryptosporidiosis is much more prevalent, accounts for 4%–20% of the cases of childhood diarrhoea, and is associated with high morbidity (Mathan *et al.*, 1985; Sallon *et al.*, 1991; Hart, 1999; Iqbal *et al.*, 1999) and mortality in children (Molbak *et al.*, 1993).

There appear to be few published reports of studies on cryptosporidiosis in the Middle East. Between 6.7% and 19% of the symptomatic children investigated in Gaza (Sallon *et al.*, 1991, 1994) and Jordan (Nimri and Hijazi, 1994) were found to be infected with *Cryptosporidium*. Cross-sectional studies in Kuwait (Daoud *et al.*, 1990; Iqbal *et al.*, 2001), Egypt (Khashba *et al.*, 1989; Mikhail *et al.*, 1989; Stazzone *et al.*, 1996) and the Sudan (Robinson *et al.*, 1986) and among Bedouin children in Israel (Dagan *et al.*, 1991)

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revealed infection prevalences of 1.6%–10%. In Saudi Arabia, Khan *et al.* (1988) found *Cryptosporidium* oocysts in two (1%) of 209, routinely processed, faecal samples from children with diarrhoea attending clinics at the Maternity and Children's Hospital in Dammam. In a similar study at King Abdul Aziz University Hospital in Riyadh, *Cryptosporidium* oocysts were also found in stool samples from 1% (two of 174) of the children investigated (Bolbol, 1992). In none of these reports from the Middle East was infection with other enteropathic protozoa, such as *Isospora belli* and *Cyclospora cayatensis*, reported, although a case of isosporiasis in an HIV-infected patient has been described in Dakar, Senegal (Dieng *et al.*, 1994). Isosporiasis causes a similar spectrum of illness to cryptosporidiosis in children and immunodeficient patients (Curry and Smith, 1998). The geographical distribution of *Cyclospora* infection appears more patchy than those of *Cryptosporidium* or *Isopora*, and links between *Cyclospora* and severe illness in immuno-deficiency have not been definitively established (Cegielski *et al.*, 1999).

In Saudi Arabia, diarrhoeal disease (DD) is an important cause of morbidity in children but the contribution made to it by coccidian parasites is unknown. The primary aim of the present, pilot study, based in the Jeddah area, was to determine and compare the prevalences of *Cryptosporidium* in young children with and without DD.

SUBJECTS AND METHODS

The subjects were children, aged <5 years, from the Jeddah area of Saudi Arabia who, between the March and May of 2000, attended six (randomly selected) crèches or, because of diarrhoea, presented at four paediatric clinics. The purpose of the study was explained to the principal of each crèche and to the parents or guardians of all the children. A child was only investigated if one of his or her parents or guardians gave his or her informed consent. In the crèches, teachers and nursery attendants were asked to record the age and sex of each child and the presence or absence of DD. The attendants were provided with disposable gloves and were instructed in the safe collection of a single faecal sample from each child. Similar data and stool samples were collected, routinely, in the clinics.

Before it could be examined, each stool sample was stored cool, in 10% formalin, for <24 h. Each sample was strained through a fine (0.6-mm-pore) sieve, mixed with 3 ml of ether or ethyl acetate for 1 min and then centrifuged at $1000 \times g$ for 1 min. The sediment left after the supernatant fatty plug, debris and liquid had been discarded was mixed well and then used to prepare two smears on clean slides. One smear was examined wet, unfixed and unstained. The other was allowed to air dry before being fixed in methanol (3 min), stained with unheated carbol fuchsin (15 min), washed with tap water and then counterstained with 0.4% methylene blue (30 s). Each smear was checked carefully for parasites, under a light microscope, by a microscopist in Jeddah who was unaware whether the sample came from a symptomatic or asymptomatic child. For quality control, all of the parasite-positive smears and a proportion of the negative were rechecked by an experienced parasitologist in Liverpool.

The prevalence data were collected and analysed using version 6.03 of the Epi Info software package (Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS

All six crèches approached agreed to participate in the study and together provided stool samples from 190 children, all of whom were considered asymptomatic by their nursery attendants. A further 63 stool samples, all from children with DD, were obtained from the four paediatric clinics. There were similar numbers of male and female subjects, both overall (55.7% female)

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and in each age-group considered in the data analysis (see below). Forty-three of the children investigated were aged <1 year. At least one species of protozoan parasite was detected in 120 (47.4%) of the 253 stool samples (Table 1). Of the 120 samples positive for protozoan parasites, 61 came from girls and 59 from boys. *Cryptosporidium* oocysts were detected in the stools of 20 (32%) of the children with DD but only nine (4.7%) of the asymptomatic children ($P < 0.001$; Table 2). Similarly, *Giardia intestinalis* was found much more frequently among the diarrhoeic children than among the asymptomatic (29% *v.* 4.2%; $P < 0.001$). *Entamoeba histolytica* and *E. dispar* could not be distinguished with the techniques available. Infection with *E. histolytica/dispar*, however, was also more frequently detected in the subjects with diarrhoea than those without (14% *v.* 6.8%; $P = 0.03$; Table 2). The individual role of *Cyclospora cayetanensis* could not be assessed as this parasite was only detected as a co-infection with *Cryptosporidium*. However, the co-infection appeared significantly associated with DD, being seen in 11% of the diarrhoeic children but only 4.2% of the asymptomatic ($P = 0.05$). Only one child was found to excreting *I. belli* and he was asymptomatic though co-infected with *Cryptosporidium*. Among the children aged <2 years or 3–5 years, those with DD were significantly more likely to be excreting *Cryptosporidium* occysts that those who appeared asymptomatic (Table 3).

TABLE 1. *Parasite distribution among the 141 girls and 112 boys investigated*

Parasite	No. and (%) of: Males infected	Females infected
Cryptosporidium alone	17 (15)	12 (9)
Cyclospora cayetanensis and *Cryptosporidium*	4 (4)	11 (8)
Isospora belli and *Cryptosporidium*	1 (1)	0 (0)
Giardia intestinalis	11 (10)	15 (11)
Entamoeba histolytica/dispar	13 (12)	9 (6)
Other*	15 (13)	11 (8)

**Ascaris lumbricoides*, hookworm and/or *Enterobius vermicularis*.

DISCUSSION

It is estimated that DD is responsible for more than 2 million deaths in children under 5 years of age, most of those deaths being in developing countries (Hart *et al.*, 2002). Rotavirus is the commonest cause of acute watery diarrhoea in children throughout the world (Hart and Cunliffe, 1999). *Cryptosporidium* spp., however, are usually the third or fourth commonest cause of DD, and the prevalence of *Cryptosporidium* infection is much higher in developing countries than elsewhere (Fayer and Ungar, 1986; Morgan *et al.*, 1998; Hart, 1999). Previous hospital-based studies in Saudi Arabia have revealed a low prevalence (1%) of *Cryptosporidium* infection among children with diarrhoea

TABLE 2. *Parasite distribution among the 63 symptomatic and 190 asymptomatic children*

Parasite	No. and (%) infected: Symptomatic children	Asymptomatic children	*P*
Cryptosporidium alone	20 (32)	9 (4.7)	<0.001
Giardia intestinalis	18 (29)	8 (4.2)	<0.001
Entamoeba histolytica/dispar	9 (14)	13 (6.8)	0.03
Isospora belli and *Cryptosporidium*	0 (0)	1 (0.5)	–
Cyclospora cayetanensis and *Cryptosporidium*	7 (11)	8 (4.2)	0.05
Other*	11 (17)	15 (7.8)	0.03

**Ascaris lumbricoides*, hookworm and/or *Enterobius vermicularis*.

TABLE 3. *Detection of* Cryptosporidium *by age-group*

	No. and (%) of symptomatic children:		No. and (%) of asymptomatic children:		
Age-group (months)	Investigated	Infected	Investigated	Infected	*P*
0–12	5	2 (40)	38	0 (0)	0.01
13–24	14	5 (36)	43	2 (5)	0.00
25–36	10	3 (30)	23	4 (17)	0.35
37–48	14	7 (50)	35	5 (14)	0.01
49–60	20	10 (50)	51	7 (14)	0.00

(Khan *et al.*, 1988; Bolbol, 1992). In the present study, the prevalence of mono-infection with *Cryptosporidium* among the children with diarrhoea (who were presenting to paediatric out-patient clinics) was much higher (32%) and appears to be the highest ever reported (Hart, 1999). This indicates that there is a particularly high level of exposure to *Cryptosporidium* in this setting, an observation further emphasised by the discovery that 4.7% of the asymptomatic children were excreting cryptosporidial oocysts. In Vellore, India, Mathan *et al.* (1985) also found oocyst excretion to be quite frequent (9.8%) among asymptomatic children. The results of a large cohort study among Peruvian children indicated that, although the likelihood of *Cyclospora*-attributable diarrhoea decreased significantly following an initial episode of cyclosporiasis, the corresponding trend for *Cryptosporidium* was less consistent and did not achieve statistical significance (Bern *et al.*, 2002). If an initial episode of symptomatic *Cryptosporidium* infection does give some immunity against disease on rechallenge with *Cryptosporidium*, then the prevelance of asymptomatic cryptosporidiosis should increase with age. This is clearly not the case in the present study population (Table 3). It must be remembered, however, that there is a large number of different species and genotypes of *Cryptosporidium* capable of infecting humans (Gatei *et al.*, 2003), with no good evidence, as yet, of cross-protection.

Cyclospora cayetanensis can cause DD in both immuno-competent and immuno-compromised individuals. Infection with this parasite has been detected in a number of different geographical regions, including North, Central and South America, the Indian sub-continent and South–east Asia (Herwaldt, 2000). In Europe, infection with *Cy. cayetanensis* is most often associated with travellers returning from visits overseas (Crowley *et al.*, 1996; Jelinek *et al.*, 1997). Although Osman *et al.* (1999) observed *Cy. cayetanensis* in Egypt, albeit at low prevalence (1.3%), there appear to be no previous reports of this species in Saudi Arabia. In the present study, interestingly, all of the 15 children found to be infected with *Cy. cayetanensis* were co-infected with *Cryptosporidium*. Co-infection with these two coccidia was significantly associated with DD. Although there are superficial similarities between *Cy. cayetanensis* and *Cr. parvum*, there are also considerable differences (Herwaldt, 2000; Bern *et al.*, 2002). In Peru, human infections with either parasite are more common during the warm season than during the cooler part of the year (Bern *et al.*, 2002). Oocysts of *Cryptosporidium*, however, are fully infective when excreted whereas those of *Cy. cayetanensis* are unsporulated on excretion and require several days outside the host to sporulate (Herwaldt, 2000). Co-infection with *Cy. parvum* and *Cr. cayetanensis* has been reported before, but only in an adult with AIDS (Scaglia *et al.*, 1994).

Only one case of *I. belli* infection was detected in the present study and that in an asymptomatic child. *Isospora* is known to be a rare cause of childhood diarrhoea. In India, for example, Mirdha *et al.* (2002) only

detected seven cases of symptomatic isosporiosis among 4112 children with DD. No *I. belli* infections were observed in the diarrhoeic Egyptian children studied by Osman *et al*. (1999).

In conclusion, the present results reveal a very high prevalence (32%) of *Cryptosporidium* mono-infection and a high prevalence (11%) of *Cyclospora*/*Cryptosporidium* co-infection among children with DD attending paediatric outpatient clinics in Jeddah, Saudi Arabia. It is clear that *Cryptosporidium* infection is an important cause of DD but one that does not always necessitate hospital management.

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