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Occurrence of *Cryptosporidium* Oocysts in Fecal Samples Submitted for Routine Microbiological Examination

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During a 7-month period, 2,252 fecal samples submitted for routine microbiological examination from 1,621 patients were screened for *Cryptosporidium* oocysts by the auramine staining method with Kinyoun acid-fast stain as the confirmatory stain. *Cryptosporidium* oocysts were detected in fecal samples from 19 (1.2%) patients, 18 of whom had gastroenteritis. Diarrheic stools from 14 of these 18 patients were negative for the usual enteropathogens but contained the oocysts in moderate to large numbers. Although *Cryptosporidium* oocysts were found in patients of all ages, they occurred slightly more frequently in infants and children than in the rest. *Cryptosporidium* species was one of the common enteropathogens identified in fecal samples submitted for routine parasitological examination during the period of the survey and was second only to *Giardia* species in terms of frequency. Considering cryptosporidiosis in the differential diagnosis of gastroenteritis in immunocompetent persons and including a search for *Cryptosporidium* oocysts in routine parasitological examinations of fecal samples appear warranted.

Although well known in veterinary medicine, until recently *Cryptosporidium* species was considered to be a rare opportunistic human pathogen causing disease primarily in persons with various immunologic disorders, particularly those with acquired immune deficiency syndrome (4, 13, 14). Recent reports have indicated, however, that *Cryptosporidium* species can cause diarrhea not only in immunosuppressed or immunocompromised patients but also in immunocompetent persons (1, 5, 6, 13). Because the symptoms of cryptosporidiosis are not different from those of many other gastrointestinal infections (10) and because diarrheic stools are not routinely tested for *Cryptosporidium* species, the sudden awareness of this newly emerging pathogen has raised the question of whether *Cryptosporidium* species is an unrecognized cause of diarrhea in an otherwise healthy population. Recent studies in Australia and Finland have indicated that cryptosporidial infections could indeed be an important and common cause of mild to severe transient diarrhea in immunologically normal patients (10, 15). Similar studies carried out subsequently in Costa Rica and England have confirmed the occurrence of *Cryptosporidium* species in routinely processed diarrheic stools (2, 8, 9, 12). Nevertheless, the prevalence of cryptosporidiosis in humans, particularly in North America, is largely unknown (13, 14). Recent reports have also indicated geographical variation in the prevalence of cryptosporidiosis (8, 9). Moreover, to our knowledge, the value of routine testing of diarrheic stools for *Cryptosporidium* species has not been adequately assessed in North America. This report describes a survey which was carried out to determine the prevalence of *Cryptosporidium* species in stool specimens submitted for routine microbiological examination.

MATERIALS AND METHODS

Fecal samples submitted to the Newfoundland Public Health Laboratory and a few hospitals across the province of Newfoundland and Labrador for routine bacteriological and parasitological examinations were included in the survey. These were from patients of all ages with gastrointestinal symptoms such as abdominal cramps, vomiting, and diarrhea; most of these patients were otherwise healthy. Additional sequential fecal specimens were sought from those patients found positive for *Cryptosporidium* species to determine the shedding pattern of the oocysts and the duration of excretion. Clinical details of the patients tested were obtained from attending physicians.

Fecal samples were received in buffered glycerol, in sodium acetate-acetic acid-Formalin fixative (17), or in Amies transport medium and were processed for *Cryptosporidium* species within a day or two of collection. Fecal smears were prepared directly from specimens, air dried, fixed with methanol, and stained by the auramine method (16). Smears were scanned for *Cryptosporidium* oocysts by fluorescence microscopy at 250× and 400×. All positive findings were confirmed with additional direct smears prepared directly as described above and stained by the Kinyoun acid-fast method (16) with 10% H_2SO_4 for decolorization. Stool specimens were processed for the usual enteric bacterial and parasitic pathogens in accordance with standard procedures (11). The parasitology protocol included concentration of all specimens by the Formalin-ether method and examination of saline and iodine wet mounts as well as trichrome-stained smears of the sediment. Fecal samples from children below 5 years old were routinely tested for rotavirus with the Rotazyme kit (Abbott Laboratories, Diagnostics Div., North Chicago, Ill.).

RESULTS

During the 7-month period between May and November 1984, a total of 2,252 fecal samples from 1,621 patients were screened for *Cryptosporidium* oocysts. Two or occasionally three fecal specimens collected on consecutive days were available for testing from ca. 40% of the patients; the rest had submitted only a single stool specimen. Fecal samples

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from 19 of the 1,621 (1.2%) patients contained *Cryptosporidium* oocysts. Additional consecutive submissions of one or two fecal specimens were available from 8 of these 19 patients; all of these contained oocysts. The remaining 11 positive patients were recognized through examination of the only available single fecal specimen from each of them. Most fecal samples positive for *Cryptosporidium* oocysts contained the oocysts in moderate to large numbers. *Cryptosporidium* species was found in patients of all age groups but occurred slightly more frequently in infants and children than in the rest (Table 1).

With the exception of one patient, all of the patients who were positive for *Cryptosporidium* oocysts had fever, vomiting, and watery, nonbloody diarrhea two to nine times a day, and the duration of the diarrhea ranged from 3 days to a maximum of 2 months in one patient, with a median of 11 days. Generally, diarrhea was severe in infants and children; the largest numbers of *Cryptosporidium* oocysts were also observed in this age group. Salmonellae were simultaneously isolated from two children aged 8 and 11 years and from two adults. The remaining 15 patients were negative for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and *Giardia* species, *Entamoeba histolytica*, *Dientamoeba fragilis*, hookworm, *Trichuris trichiura*, and rotavirus. One of these 15 patients was an 11-year-old girl who had a 2-week history of frequent vague abdominal cramps, especially after eating or drinking, without diarrhea and vomiting. Another patient was an 80-year-old woman; she initially had cardiac failure as a result of ischemic heart disease and subsequently developed profuse nonbloody, watery diarrhea with frequencies up to nine times a day which persisted for 2 months. No specific treatment was given, and recovery was spontaneous in all 19 patients.

Follow-up stool examinations could be carried out for 6 of the 19 patients who were positive for *Cryptosporidium* oocysts. The duration of diarrheal illness in these six patients ranged from 6 to 14 days. Fecal samples were obtained from them at 2- to 4-day intervals for up to 3 weeks and examined for *Cryptosporidium* oocysts. Nearly all the fecal specimens collected during the period of acute illness contained the oocysts in moderate to large numbers. Four patients became negative within days after the cessation of symptoms, whereas the remaining two excreted the oocysts in small numbers for nearly 2 weeks after complete clinical recovery. These two patients were adults who, incidentally, were also positive for *Salmonella* species.

The frequency of *Cryptosporidium* species in fecal samples routinely processed for parasitic agents was determined with 685 consecutive fecal specimens obtained from a subpopulation of 251 patients of all ages who had gastrointestinal symptoms. A parasitological examination of fecal specimens was specifically requested for all these patients. For the great majority of the patients, two or three fecal samples collected on consecutive days were available for testing, and all specimens were submitted in sodium acetate-acetic acid-Formalin fixative. *Cryptosporidium* oocysts were detected in 9 specimens from 5 patients, and the other recognized parasitic pathogens were found in 14 specimens from 9 other patients. In terms of frequency, *Cryptosporidium* species was found to be the second most common parasitic agent of known pathogenicity after *Giardia* species (Table 2).

TABLE 1. Age-specific prevalence of *Cryptosporidium* species in diarrheic stools

Age (yr)	No. of patients tested	No. (%) of patients positive
0–9	878	12 (1.4)
10–19	197	2 (1.0)
>20	371	4 (1.1)
Unknown	175	1 (0.6)
Total	1,621	19 (1.2)

TABLE 2. Frequency of *Cryptosporidium* species and other parasitic pathogens in diarrheic stools[a]

Parasite	% Positive
Giardia lamblia	1.5
Cryptosporidium species	1.3
Ascaris lumbricoides	0.3
Trichuris trichiura	0.3
Entamoeba histolytica	0
Dientamoeba fragilis	0
Hookworm	0

[a] Based on 685 fecal samples.

DISCUSSION

This survey indicated the occurrence of *Cryptosporidium* species in fecal specimens submitted for routine bacteriological and parasitological examinations from patients with gastrointestinal symptoms in our geographical region and confirmed recent reports from elsewhere that *Cryptosporidium* species may be an unrecognized cause of diarrhea and other gastrointestinal symptoms in patients who are otherwise healthy (8, 9, 12, 15). The overall prevalence rate of 1.2% that we found in eastern Canada closely correlates with those of 1.1% observed in the province of Manitoba in central Canada (L. Sekla, personal communication) and 1 and 1.4% reported in Finland and England, respectively (8, 10) but is lower than the prevalence rate of 4.1% found in Australia (15). Whether cryptosporidial infections occur primarily as sporadic cases or in outbreaks is not known (9), nor can we speculate on whether our prevalence data represent endemic or epidemic rates. However, it would be interesting to know how these figures compare with those for the rest of Canada and the United States. We also found *Cryptosporidium* species to be one of the common intestinal parasitic pathogens in the patient population screened during this survey. This is in agreement with recent studies which have shown *Cryptosporidium* species to be one of the leading enteric pathogens currently recognized (10, 12). Cryptosporidiosis has been reported to be seasonal, the infection being more common during the summer months (12, 15). Although our survey was carried out during the summer and fall seasons, the numbers of our positive cases were too small to determine this difference, and we do not know if there is a seasonal variation in the prevalence of cryptosporidiosis in our region.

In this survey, *Cryptosporidium*-positive cases included nine young children (seven were <1 year old and two were 2 years old) and an 80-year-old person. The opportunistic behavior of *Cryptosporidium* species so clearly established in patients with acquired immune deficiency syndrome may well be applicable to patients at extremes of age. Cryptosporidial infections have been reported to be more common in children than in adults (9, 15). Although there was an indication of this trend, *Cryptosporidium* oocysts were equally detected in older children and adults in our

study. It cannot be said with certainty that finding *Cryptosporidium* oocysts in fecal specimens implied an etiologic role in every case that was observed, but a cryptosporidial etiology was strongly suggested in most instances; fecal samples from 14 of 18 patients with gastroenteritis were negative for the commonly recognized enteric pathogens but contained *Cryptosporidium* oocysts in moderate to large numbers. Also, as with several other microbial infections, an asymptomatic carrier state of *Cryptosporidium* oocysts, especially among healthy adults, cannot be excluded at this time. Cryptosporidiosis is a zoonosis, and domesticated animals are considered to be a potential source of infection (3, 5). Contact with pet animals appeared to be common for most of our patients, but this could not be definitively established as the source of infection.

The excretion of *Cryptosporidium* oocysts during the period of diarrhea has been reported to be not uniform (8). Nearly all follow-up fecal samples obtained from our patients during the acute stage of the illness uniformly contained the oocysts in moderate to large numbers. Although this would appear to suggest that a reliable diagnosis of cryptosporidiosis can be made by testing a single fecal sample collected during the acute stage, the above data are based on a small number of patients studied, and further investigations are required to determine this parameter. It was also interesting that two of our patients excreted *Cryptosporidium* oocysts for up to 2 weeks after becoming asymptomatic. Asymptomatic shedding of oocysts may be important in the transmission and spread of cryptosporidiosis.

There are several simple and reliable methods now available for the detection of *Cryptosporidium* oocysts in fecal specimens (7, 13). We found the auramine method to be a rapid technique; all positive fecal samples were detected in less than 1 min by this method. However, the disadvantages of this method are occasional nonspecific staining and a difficulty at times in visualizing definitive morphology, but these could be easily overcome by the use of the Kinyoun or the Ziehl-Neelsen acid-fast stain as a confirmatory stain; we found such a combination reliable and rapid. The auramine method may be particularly useful for screening large numbers of fecal samples for *Cryptosporidium* oocysts. The examination of fecal smears prepared from KOH-treated Formalin concentrate or fecal smears obtained through the sugar flotation technique has been reported to be more effective than direct fecal smear examination for the detection of *Cryptosporidium* oocysts (7, 13). We think that these methods may be more applicable for specimens containing few oocysts, as may be the case in low-level asymptomatic carriers, than for diarrheic stools that are from patients with acute cryptosporidiosis and that appear to contain large numbers of oocysts. However, the use of such techniques is certain to yield higher positive rates. Because we examined only direct fecal smears throughout this survey, the data from our study may underestimate the prevalence of cryptosporidiosis in the community.

Cryptosporidiosis is a newly emerging syndrome and appears to be an important disease from clinical, epidemiological, and public health points of view (13, 14). Our results indicate the need to consider cryptosporidiosis in the differential diagnosis of gastroenteritis in immunocompetent persons of all ages. It also points out the value of including a search for *Cryptosporidium* oocysts in routine parasitological examinations of fecal specimens.

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