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CRYPTOSPORIDIOSIS IN IMMUNOCOMPETENT PATIENTS

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Abstract The intestinal protozoan cryptosporidium is known to cause diarrhea in immunocompromised patients, but few cases have been reported in detail in immunocompetent persons. During a 12-month period, we identified cryptosporidium in the stools of 43 immunocompetent patients. The numbers of cases were increased in those under 4 years old and in those from 30 to 39 years old. Of 30 index cases, 23 (77 per cent) were diagnosed in the late summer or the fall. Fifteen of the 43 patients (35 per cent) had other gastrointestinal pathogens, of which only *Giardia lamblia* was statistically associated with cryptosporidium. In the 28 patients in whom other gastrointestinal pathogens were not identified, the clinical manifestations were predominantly watery, nonbloody diarrhea and, less commonly, abdominal discomfort, anorexia, fever, nausea, and weight loss. The infection was self-limited in all 43 patients. Clustering of cases occurred in a day-care center and in two families.

These clinical observations confirm worldwide findings and suggest that cryptosporidium is a relatively common nonviral cause of self-limited diarrhea in immunocompetent persons in the northeastern United States. (N Engl J Med 1985; 312:1278-82.)

THE intestinal protozoan cryptosporidium is a well-known cause of gastroenteritis in animals and has recently been shown to cause a similar illness in human beings.[1-4] The organism was initially described on the gastric mucosa of asymptomatic mice in 1907,[5] but was not associated with disease in animals until 1955, when Slavin[6] reported diarrheal illness in turkeys. Subsequently, cryptosporidium was found to cause disease in calves, lambs, pigs, and other domestic and wild animals.[1-3] The first case of human infection was reported in 1976,[7] and only seven additional cases were documented before 1982.[1] Since then, the

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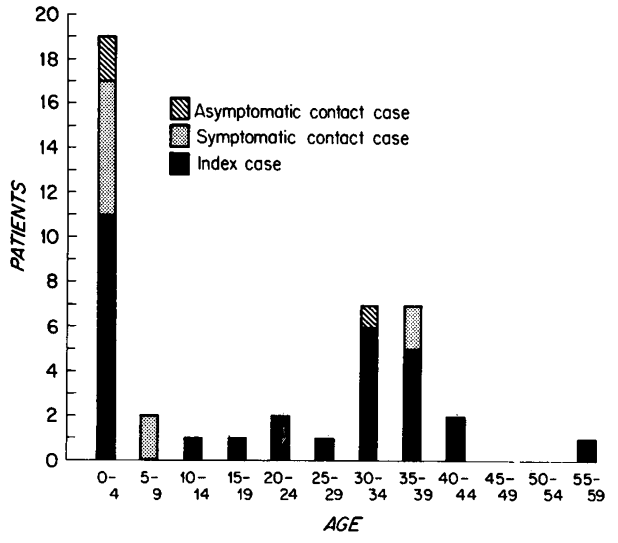

Figure 1. Ages (in Years) of Patients with Cryptosporidium.

number of cases identified has increased substantially, largely because of the recognition of a severe form of infection in patients with the acquired immunodeficiency syndrome (AIDS) and because of the development of rapid and convenient screening methods.[1-4,8]

The clinical manifestations of cryptosporidial infection depend in part on the immune status of the patient. In immunocompromised persons, such as patients with AIDS, the organism may cause the loss of liters of fluid daily for many months.[1-4] In immunocompetent persons, cryptosporidium may cause self-limited gastroenteritis lasting from days to weeks.[1-4] For immunocompetent persons, studies of stool specimens[9-15] or serologic studies of blood[16] suggest that cryptosporidial infection may not be uncommon, but relatively few cases have been reported in detail.[1-4]

Over a 12-month period, all stool samples submitted for ova and parasite examination in our parasitology laboratory were evaluated for cryptosporidium. Forty-three immunocompetent patients with cryptosporidiosis were identified and are the subject of this report.

Methods

From February 1, 1983, through January 31, 1984, all stool samples submitted to the Parasitology Laboratory of Massachusetts General Hospital for ova and parasite examination were evaluated for the presence of cryptosporidium by a modified acid-fast method.[17] For four immunocompetent patients, the presence of cryptosporidium in stool was confirmed by Sheather's sugar flotation method[18] or by light and electron microscopy of tissue from the small intestine. For three immunocompromised persons, the diagnosis was confirmed by the sugar flotation method, light and electron microscopy of tissue, or both. Ova and parasite studies used saline and iodine direct examination of stool,[19] saline and iodine examination of formalin–ethyl acetate stool concentrates,[20] and examination of smears stained with chlorazol black E[19] and modified acid-fast-stained smears[17] of unconcentrated stool. The methods used for bacterial study of stool samples allow identification of shigella, salmonella, campylobacter, yersinia, or *Staphylococcus aureus*, but not enterotoxigenic or invasive *Escherichia coli*. Viral studies were not performed.

A case was defined as a patient in whose stool cryptosporidium was identified. An index case was defined as a patient with cryptosporidiosis who submitted a stool sample for ova and parasite examination that was unsolicited by the investigators. In families in which more than one patient submitted unsolicited samples, the family member submitting the first sample was defined as the index case. In the evaluation of index cases, clinical information was collected retrospectively by chart review and by contact with patients and their physicians. In the evaluation of secondary cases in day-care centers or in families, data were collected prospectively. Patients were categorized as immunocompetent when the history and follow-up information obtained from the patient, the patient's physician, chart review, and available laboratory studies all yielded no evidence of immunosuppressive disease or therapy, including neoplasia, AIDS, treatment with corticosteroids or cytotoxic agents, radiation therapy, a history of a wasting illness suggestive of occult neoplasia or chronic infection, or a history of recurrent infections. Diarrhea was defined as the occurrence of at least two successive watery stools or a substantial increase in the frequency of bowel movements in comparison to the normal pattern for a given patient.

Statistical analyses were performed with single-tailed Fisher's exact test and the chi-square test with Yates' correction.

Results

During a 12-month period, cryptosporidium was identified in 78 of 2821 stools (2.8 per cent) from 47 of 1703 patients (2.8 per cent). Of these 47 patients, 34 (2.0 per cent) were index cases, and 13 were secondary cases. Four index cases were men with AIDS. Cryptosporidiosis in these immunocompromised patients was manifested by large volumes of watery diarrhea and lasting for months. Cryptosporidial infection in patients with AIDS has been well described by others,[1-4] and our patients with AIDS will not be further characterized here.

Forty-three patients with cryptosporidium were immunocompetent. All 43 recovered from their infection. Twenty-three (53 per cent) were male. Patients were clustered in the under-5 and 30-to-39-year-old age ranges (Fig. 1). Of the patients under five years old, none were 11 months of age or younger, 11 were 12 to 23 months of age, 5 were 24 to 35 months of age, 3 were 36 to 47 months of age, and none were 48 to 60 months of age. Ten of the 19 children (53 per cent) under five years of age were enrolled in day-care centers. The 14 patients in the 30-to-39-year-old group included five hospital employees, two parents of infected children, two homosexual men, one children's day-care worker, and four persons with no known possible occupational exposure. Four of 43 patients (9 per cent) were hospitalized before identification of cryptosporidium. The infection was the reason for admission in one patient, a 59-year-old nursing instructor with diarrhea and dehydration; her diarrhea resolved slowly over four months. Admitting diagnoses for the other three hospitalized patients included one case each of diabetes mellitus, shigellosis, and 10 per cent body-surface-area burn; in all three, gastrointestinal symptoms antedated admission. Six of 43 patients (14 per cent) worked in a hospital; they included three members of the support staff, two nurses, and one physician.

Additional gastrointestinal pathogens were identified in 15 of the 43 immunocompetent patients with cryptosporidiosis (35 per cent). The stools of all 43

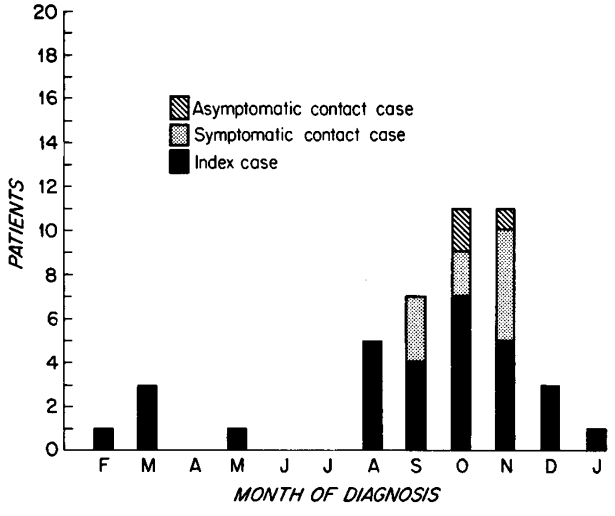

Figure 2. Date of Diagnosis of Cryptosporidiosis.
Except in one man, who began to have symptoms in October 1982 and was diagnosed in February 1983, diagnoses were made within three weeks of the onset of symptoms.

patients were examined for ova and parasites; *Giardia lamblia* was found in 10 patients and *Entamoeba histolytica* in three homosexual men. The stools of 23 of these 43 patients were also cultured for bacteria, and two grew pathogens: *Shigella sonnei* in one case and salmonella species in another. We have previously noted a statistically significant association between the identification of cryptosporidium and that of giardia in the stool samples of our patients over a nine-month period.[21] With three additional months of data, the statistical significance of the association remained essentially the same ($P<0.02$ by Fisher's exact test, analysis not shown).

Clinical manifestations in the 28 immunocompetent persons with cryptosporidiosis for whom other gastrointestinal pathogens were sought but not identified included the following. Twenty-four patients (86 per cent) had watery diarrhea that persisted for a median of 15 days (25th percentile, 5 days; 75th percentile, 32 days). Mucus was present in the stools of three patients. Gross blood was not observed in the stools of any of the patients, and Hemoccult tests were negative in all 17 patients evaluated. Other clinical manifestations included abdominal discomfort (five patients), anorexia (five), fever (three; temperatures, 37.8, 38.0, and 38.3°C), nausea (three), weight loss (three), failure to thrive (two), vomiting (one), and malaise (one). When clinical manifestations in patients with cryptosporidiosis with and without giardia were compared, no significant differences were apparent. Treatment was supportive, basically consisting of fluid replacement; no patients received antimicrobial agents directed toward cryptosporidium.

Twenty-three of the 30 index cases (77 per cent) were diagnosed during the late summer and the fall (Fig. 2). Cryptosporidium was identified in stool samples from 24 of 907 patients (2.6 per cent) from July through December, but from only 6 of 796 patients (0.8 per cent) from January through June ($P<0.0005$). Patients resided in Boston or surrounding suburban communities with different water supplies. Most patients had not traveled from the area. Attempts to link illness with exposure to animals (sick or well) revealed no convincing associations.

Apparent clustering of infection was seen in three instances. In a day-care center, 6 of 19 children (including the index case) and 1 parent whose stools were examined had cryptosporidium. In another day-care center that had children with a similar age distribution (one to four years) and was located in the same town, none of 23 children examined (all of whom were asymptomatic) had stools that contained cryptosporidium ($P = 0.014$). In a family of five, the mother (the index case), the father, and two children had cryptosporidium in their stools, and the third child had cryptosporidium and giardia. In a family of four, the mother (the index case), the father, and two children had cryptosporidium and giardia in their stools. Three of the 13 non-index cases were asymptomatic.

Discussion

During a 12-month period, cryptosporidium was identified by a modified acid-fast method in stool samples from 34 of 1690 patients who submitted specimens to our Parasitology Laboratory, and evaluation of their contacts revealed 13 additional cases in a total of 1703 subjects. Forty-three of the 47 patients were immunocompetent, suggesting that cryptosporidiosis in immunocompetent persons is common in the northeastern United States. In prior reports, the prevalence of cryptosporidium in the stools of immunocompetent patients was 4.1 per cent for patients with diarrhea in a hospital in Australia,[11] 4.3 per cent for Costa Rican children with diarrhea,[9] 7.9 per cent for Liberian children with diarrhea,[10] and 1.4 per cent for patients with diarrhea studied in the United Kingdom.[13]

Our series of immunocompetent patients with cryptosporidium is the largest reported to date. The clinical characteristics of our patients were similar to those of previously described immunocompetent persons with cryptosporidium.[1-4] The numbers of cases were increased in the groups under 4 and 30 to 39 years old. In Australia,[11] the United Kingdom,[13] Costa Rica,[9] and Liberia,[10] diarrhea in children, particularly those under five years old, was also more commonly associated with identification of cryptosporidium in the stool than it was in older patients. Our lack of patients under one year of age is unexplained but may reflect breast feeding[9] or other unknown factors. Of the 14 patients from 30 to 39 years old, 10 had possible occupational exposure (including parenting).

Other gastrointestinal pathogens were identified in

15 of the 43 immunocompetent patients (35 per cent). Studies by others have detected variable levels of mixed infections.[9-13] The previously noted association between the identification of cryptosporidium and that of giardia in the stool[21] was still present in data including an additional three months. A similar association was suggested by the data of Jokipii et al.[12] The reason for this association is not known, but the connection may represent a simultaneous infection from a common source or infection with one small-bowel parasite predisposing to infection or colonization with the other.[21] A significant association between cryptosporidial infection and virus-related enteritis in calves with diarrhea has also been reported.[3,22]

Because cryptosporidium may be found with other pathogens and because complete microbiologic studies have not been performed, the clinical characteristics of cryptosporidial infection are incompletely defined. However, in our patients in whom only cryptosporidium was identified, the clinical manifestations consisted of predominantly watery, nonbloody diarrhea and to a lesser extent abdominal discomfort, anorexia, fever, nausea, weight loss, failure to thrive, vomiting, and malaise. In addition, three patients were asymptomatic. The duration of diarrhea in our patients ranged from days to months, with a typical duration more prolonged than the 3 to 14 days described by others,[1-4,23] although symptoms lasting longer than three weeks have been documented previously.[11] The longer duration of diarrhea in our study may reflect a bias in patient identification, in that we identified our patients from the results of stool examinations for ova and parasites, and such examinations may be more often requested for patients with prolonged illness.

In our study and the studies of others,[9,11] infection was significantly more common in the summer and the fall than in the winter and the spring. The reason for this seasonal variation is not known.

We noted apparent clustering of cases of cryptosporidiosis in a day-care center and in two families. Clustering has been reported in other immunocompetent patients, including 12 who had contact with feces of infected calves,[23] a nurse caring for an infected infant,[24] two siblings and subsequently their mother,[25] two other siblings,[13] and 14 children in a day-care center.[26] Clustering of cases is not unexpected, considering the existing data for fecal–oral transmission of cryptosporidium and the accumulating evidence of person-to-person transmission.[1-3,23,27] Because of these data, we think precautions should be taken to avoid enteric transmission in the care of hospitalized patients with cryptosporidiosis.

In our study, a modified acid-fast method was used to identify cryptosporidium in the stool.[17] For several of our patients, the diagnosis was confirmed by other methods. A recent study suggested that various acid-fast stains were more useful than other methods for identification of cryptosporidium in the stools of immunocompromised patients.[8] However, it is important to note that neither the sensitivity nor the specificity of the modified acid-fast method or other methods for the identification of cryptosporidium in the stool has been determined.

To date, the therapy of cryptosporidiosis is supportive. Many chemotherapeutic agents have been tried in this illness but have failed to prove effective in patients or animals.[1-4] Initial intriguing results were obtained when the antibiotic spiramycin was used to treat cryptosporidiosis in patients with AIDS[28,29] and in several recipients of bone marrow transplants,[25,28,29] and therapy with this drug seems to merit further investigation. We are not aware of published reports on the use of spiramycin to treat cryptosporidiosis in immunocompetent patients.

In conclusion, data are accumulating that strongly suggest that cryptosporidium is a relatively common nonviral cause of diarrhea in immunocompetent persons, particularly in children and particularly during the late summer and the fall. Routine laboratory studies to identify cryptosporidium in the stools of symptomatic patients seem justified, especially in the evaluation of immunocompetent persons during the summer and the fall and of immunocompromised persons during all seasons.

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STUDIES OF KIDNEY AND MUSCLE BIOPSY SPECIMENS FROM IDENTICAL TWINS DISCORDANT FOR TYPE I DIABETES MELLITUS

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Abstract To distinguish metabolic from genetic factors in the development of microangiopathy in diabetes, we evaluated biopsy specimens of kidney and quadriceps muscle from seven pairs of identical twins who were discordant for Type I (insulin-dependent) diabetes mellitus. Two of the diabetic patients had clinical diabetic nephropathy, including hypertension, marked albuminuria, and a substantially reduced creatinine clearance; the other five had normal renal function and only minor clinical indications of complications. All the twins of the diabetic patients had normal glomerular basement membrane widths and normal fractional volumes of the glomerular mesangium. Values for glomerular basement membrane width, tubular basement membrane width, and mesangial volume in each diabetic twin exceeded the values in the respective sibling ($P \leq 0.0035$), even if the value in the diabetic twin lay within established normal ranges. Values for muscle capillary basement membrane width in the diabetic twins did not differ from those in their siblings ($P = 0.5$).

Our observations suggest that the metabolic abnormalities of diabetes are necessary, if not sufficient, for the development of glomerular abnormalities. We also conclude that in diabetic patients, alterations in muscle capillary basement membrane width do not necessarily accompany pathologic lesions in the kidney. (N Engl J Med 1985; 312:1282-7.)

MICROANGIOPATHY can markedly reduce the life expectancy of the patient with diabetes, owing to the development of advanced nephropathy and renal failure, and can cause serious morbidity, especially blindness. Furthermore, diabetic angiopathy has continued to raise conflicting arguments about the role of metabolic abnormalities, as opposed to genetic predisposition, in the pathogenesis of the microvascular lesions.[1,2] The extended period over which renal or retinal disease develops in diabetic patients (10 to 20 years) challenges the investigator who attempts to elucidate the pathophysiologic process underlying the lesions. As a result, no technique or physical findings prospectively indicate which diabetic patients will have microangiopathy. Furthermore, one cannot discern whether renal or retinal disease indicates either an isolated or a generalized process. Subjects with diabetes have increased widths of the basement membranes of the muscle capillary[3,4] and glomerulus.[5] In one report on kidneys removed from diabetics at autopsy the width of the muscle capillary basement membrane correlated with the width of the glomerular basement membrane.[6] However, the relation between muscle capillary basement membrane width and lesions in the kidney has not been well defined. To address these issues we studied biopsy specimens of skeletal muscle and kidney from monozygotic twins who were discordant for Type I (insulin-dependent) diabetes mellitus.

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METHODS

Patients

Seven pairs of identical twins discordant for Type I diabetes mellitus underwent evaluation at the University of Minnesota Hospitals for consideration of renal or pancreatic transplantation. All observations reported here were completed before transplantation was performed. The subjects were admitted to the Clinical Research Center for metabolic, renal, ophthalmologic, neurologic, and psychiatric assessment. Detailed protocols were approved by the University of Minnesota Committee on the Use of Human Subjects in Research. Monozygosity was established by analyzing 15 to 20 blood groups (War Memorial Blood Bank, Minneapolis,