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MINIREVIEW

Cryptosporidium Species, a Protean Protozoan

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INTRODUCTION

Cryptosporidium species is now considered to be an important enteric protozoan pathogen, but recognition of this role evolved slowly in the 80 years since its discovery. Initially described by Tyzzer as an obscure protozoan isolated from the gastric mucosa of asymptomatic mice in 1907 (78), *Cryptosporidium* species received little attention until it was suspected of causing diarrhea in turkeys in 1955 (72). More widespread disease in animals, particularly calves and lambs, was documented with the use of improved diagnostic techniques (67, 79). The first reported human cases in 1976 were associated with exposure to farm animals (59, 64), and subsequent cases confirmed the classification of cryptosporidiosis as a zoonotic infection (15, 24). In the early 1980s, most reported infections were in patients with the acquired immunodeficiency syndrome (AIDS) (34, 53, 63, 66, 75). Those individuals had protracted, often severe, watery diarrhea and wasting, and *Cryptosporidium* species was considered to be primarily an opportunistic pathogen. A larger role for *Cryptosporidium* species in human disease has emerged with an increased awareness of the organism and readily available simplified methods of stool diagnosis (33, 37, 53). *Cryptosporidium* species is now commonly identified in cases of acute, self-limited diarrheal illness in immunocompetent hosts in both developed (36, 40, 94) and developing (10, 56, 71; N. K. Hojlyng, K. Molbak, and S. Jepsen, Letter, Lancet i:734, 1984) nations. Travelers abroad have become infected (45, 52), and outbreaks have occurred among children in day-care centers (1, 18, 77). Fecal-oral spread among humans and animals and ingestion of contaminated water (26) appear to be the principal modes of transmission.

Thus, from incidental finding, veterinary disease, zoonosis, and opportunistic infection, *Cryptosporidium* species is now acknowledged as a cause of human diarrheal disease worldwide (30). The intriguing discoveries of the epidemiology, pathogenesis, immunology, clinical features, and microbiology of this protozoal pathogen provide an exciting story and constitute the basis of this review.

CLASSIFICATION AND LIFE CYCLE

Taxonomy. The taxonomic classification of the genus *Cryptosporidium* denotes its structure and basic biology (49). Like other sporozoan parasites in the phylum Apicomplexa, *Cryptosporidium* species has an apical complex but no cilia or flagella. Because *Cryptosporidium* species has both sexual and asexual reproduction, it is placed in the class Sporozoasida. As is characteristic of other members of the subclass Coccidiasina, *Cryptosporidium* species has small intracellular gametes. The development of merozoites within meronts (both type I and II) in the process of merogony places *Cryptosporidium* species in the order Eucoccidiorida. *Cryptosporidium*, *Isopora*, *Sarcocystis*, and *Toxoplasma* species are members of the suborder Eimeriorina, because macrogametocytes, with a single macrogamete, and microgametocytes, which produce multiple microgametes, form independently. As a member of the family Cryptosporidiidae, the genus *Cryptosporidium* develops in a special anatomic niche, just under the surface membrane of the host cell, as an intracellular yet extracytoplasmic parasite (30, 63, 79). Only a single host (monoxenous) is required for development of the complete life cycle of *Cryptosporidium* species.

Species differentiation, an area of major controversy, has been evaluated by cross-inoculation studies with organisms recovered from different hosts (80). *Cryptosporidium* species has been identified in a broad range of vertebrates, including mammals, fish, birds, and reptiles. On the basis of a review of cross-transmission experiments, Tzipori et al. (80) suggested that *Cryptosporidium* may be a single-species genus, whereas Levine (50) has proposed four species within the genus based on host preference. Most recently, Upton and Current (20, 89) classified the species as follows: (i) *C. parvum* Tyzzer, 1912, which has smaller (5.3- to 6.5-μm) oocysts, found primarily in the small intestine and responsible for most cryptosporidial diarrheal disease in humans and cattle; (ii) *C. muris* Tyzzer, 1907, which has larger (6.5- to 7.9-μm) oocysts, found in gastric glands of mice and associated with a milder clinical syndrome in cattle; and (iii) *C. baileyi* sp. nov., with 6.2- by 4.6-μm oocysts, recently isolated from the ileum and large intestine of commercial broiler chickens (25).

Life cycle. Upon release from ingested thick-walled oocysts, sporozoites adhere to the epithelial surface and begin development into trophozoites below the surface membrane (Fig. 1). All other developmental stages appear to take place in the resultant parasitophorous vacuole. A characteristic dense zone of attachment to the host cell is noted by electron microscopy. During the merogony (asexual multiplication) stage of development, type I meronts produce six or eight merozoites that recycle as invasive forms after their release into the lumen of the gut. Based on structural observations in naturally infected guinea pigs, it appears that the method of reentry of merozoites into host cells is by membrane invagination (55). In contrast to the type I meronts that enable augmented infection directly, type II meronts produce four merozoites that differentiate separately into gametocytes. Macrogametocytes produce a single macrogamete, but microgametocytes produce about 16 microgametes. Release of microgametes enables fertiliza-

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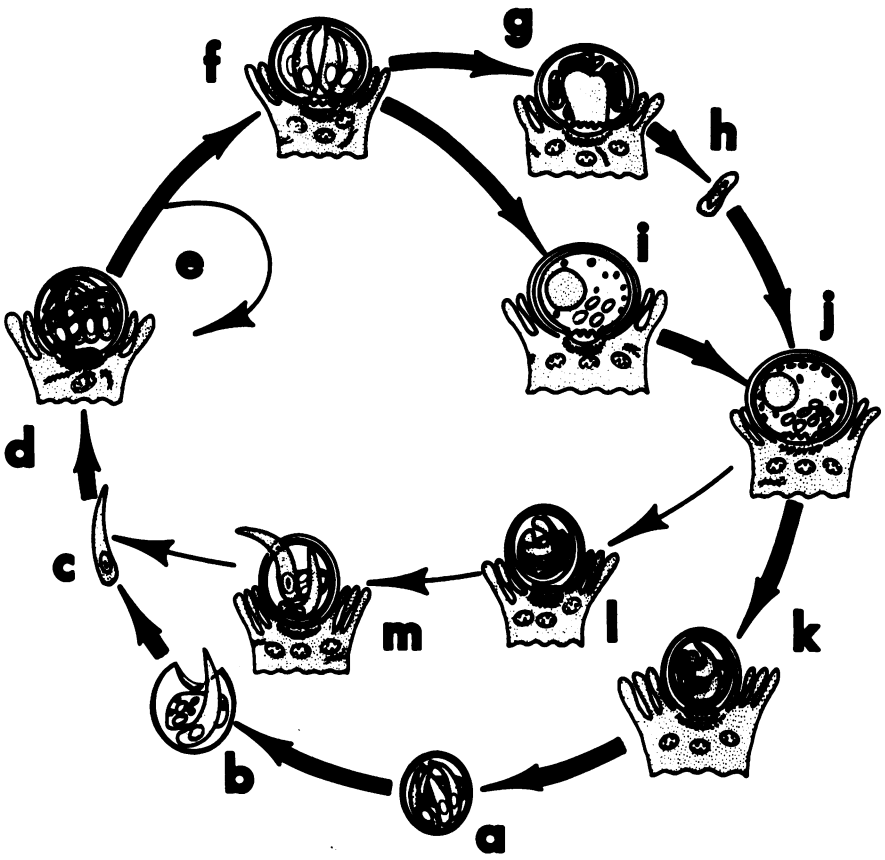

FIG. 1. Proposed life cycle of *Cryptosporidium* species. Infective oocysts (a) release sporozoites (b, c) which penetrate beneath the intestinal epithelial cell membrane and develop into type I meronts within the parasitophorous vacuole (d). These may recycle at this point (e) or develop into type II meronts with four merozoites (f). Microgametocytes (g) release microgametes (h) which fertilize macrogametes (i) to form zygotes (j). A minority of these zygotes form thin-walled oocysts (l, m) which augment the infection within the host (c). Most pass outside the host as readily infective, environmentally hardy thick-walled oocysts (k, a) (figure reproduced with permission; Current, Clin. Microbiol. Newsl.).

tion of the single macrogametes and formation of zygotes. Subsequently, development in this sexual cycle leads to either a fragile thin-walled oocyst or, more commonly, an environmentally hardy thick-walled oocyst. The former excysts to release internally autoinfective sporozoites within the host, whereas the latter is passed outside the host. Sporozoite formation (sporogony) occurs within the original host cell in both oocyst forms before their release. This life cycle has been most consistently demonstrated in mammalian intestinal tissue, in culture in chicken chorioallantoic membranes (22), and in several cultured cell lines (21). The life cycle of *Cryptosporidium* species has been reproduced at 35, 37, and 41°C, which reflects the spectrum of temperatures found in its fish and snake, mammalian, and avian hosts (22). *Cryptosporidium* species is the only coccidian species that can complete its entire life cycle in cell culture from sporozoite to merogony (asexual multiplication) or to sporogony (sexual multiplication with resultant infective oocysts) (21; W. L. Current, Clin. Microbiol. Newsl. 7:167–170, 1985).

EPIDEMIOLOGY

Incidence and prevalence. *Cryptosporidium* species has been found in association with diarrheal disease in most parts of the world, often at rates rivaling those of *Campylobacter*, *Salmonella*, and *Shigella* species, *Escherichia coli*, and *Giardia lamblia* (10, 35, 39, 40, 56, 69). In large prospective studies in which consecutive stool samples were examined by reliable methods, *Cryptosporidium* species was identified in 3 to 13% of patients with diarrhea in developing countries (Table 1), whereas the isolation rate from matched asymptomatic controls was virtually zero. The sensitivity of different staining methods varies (33, 53), however, and light infections may be difficult to detect. In one study from Vellore, India, where exposure begins at birth and close human contact with cattle is commonplace, *Cryptosporidium* species was isolated in 13.1% of individuals with diarrhea and in 9.8% of controls (57). This observation suggests that very early and persistent exposure may be associated with asymptomatic excretion and immunity to illness.

Cryptosporidium species is rarely found in the stools of asymptomatic controls in developed countries; the prevalence was 0.1% (1 of 867) in three combined studies (6, 41, 86). In contrast, *Cryptosporidium* species was found in 0.6 to 7.3% of stools from patients with diarrhea (Table 2). The lowest rate was noted in Denver, Colo., in serial specimens from patients of all ages submitted to three local hospitals and to the State health department. The highest rate was noted in symptomatic children under 5 years of age in Great

TABLE 1. Isolation of *Cryptosporidium* species from stool specimens from persons in developing countries, 1978 to 1985

Location (reference)	Population	Patients with diarrhea		Healthy controls		% of infected persons with other pathogens	Diagnostic method	
		No. studied	% with *Cryptosporidium* species	No. studied	% with *Cryptosporidium* species		Concn[a]	Stain[b] (reference)
Bangladesh[c]	Calf handlers and families	165	8.5	155	0	ND	No	Giemsa
Rwanda (10)	Children	193	10.4	94	0	21.7	Yes	Mod. Z-N (37) or safranin–MB[d] or both
	Adults	100	3.0					
Liberia[e]	6 mo–5 yr	278	7.9	ND[f]		54.5	No	Mod. Z-N (37)
Costa Rica (56)	Preschool children	613	3.3	90	0	25	No	Giemsa
Bangladesh (71)	All ages	578	4.3	ND		20	No	Giemsa and mod. A-F (33)
Venezuela (65)	<2 yr	120	10.8	ND		ND	No	Giemsa or mod. Z-N (37) or both
India (57)	≤3 yr	682	13.1	418	9.8	51.3	Yes	Safranin–MB[d]
Brazil (91)	All ages	117	8.0	22	0	55.6	No	Mod. Kinyoun A-F and mod. aur-rhod (53)
Thailand[g]	<10 yr	410	3.2	410	0.2	46.2	No	DMSO-mod. A-F (12)
Ghana[h]	2 mo–5 yr	474	12.9	ND		ND	No	Mod. Z-N (37)
South Africa (74)	Hospitalized children	259	11.9	103	0	38.7	No	Mod. Z-N[i]

[a] Concn, Concentration procedure performed.
[b] Mod. Z-N, Modified Ziehl-Neelsen; safranin-MB, safranin-methylene blue; mod. A-F, modified acid-fast; mod. Kinyoun A-F, modified Kinyoun acid-fast; mod. aur-rhod, modified auramine-rhodamine; DMSO-mod. A-F, dimethyl sulfoxide acid-fast.
[c] Rahaman et al., Letter.
[d] D. Baxby and N. Blundell, Letter, Lancet ii:1149, 1983.
[e] Hojlyng et al., Letter.
[f] ND, Not done.
[g] Taylor and Echeverria, Letter.
[h] P. A.-K. Addy and P. Aikens-Bekoe, Letter, Lancet i:735, 1986.
[i] Casemore et al., Letter, 1984.

Britain (40). Worldwide, this age group comprises most cases in immunocompetent persons (6, 10, 40, 74, 94). Background rates in children are low; only 2 of 220 (0.9%) day-care-center toddlers, both symptomatic, and none of 78 non-day-care-center toddlers excreted the organism in a recent prevalence survey in Denver (T. Novotny, R. Hopkins, P. Shillam, and E. N. Janoff, submitted for publication). In contrast, *Cryptosporidium* species was found in up to 65% of symptomatic day-care-center children during an outbreak of diarrhea (1).

Among homosexual men, *Cryptosporidium* species was found in 28% (5 of 18) of symptomatic patients with AIDS but in none of 240 asymptomatic homosexual men in the United States (B. E. Laughon, D. J. Shirazian, D. A. Druckman, A. A. Vernon, R. Hashmi, and B. F. Polk, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C-355, p. 387). Almost half of 131 patients with AIDS seen in Haiti with opportunistic infections were infected with *Cryptosporidium* species. Most of these patients presented with chronic diarrhea. Sexual preference was not a risk factor,

TABLE 2. Isolation of *Cryptosporidium* species from stool specimens from patients with diarrhea in developed countries, 1982 to 1986

Location (reference)	Population age	No. studied	% with *Cryptosporidium* species	% of infected persons with other pathogens	Diagnostic method	
					Concn[a]	Stain[b] (reference)
North Wales[c]	<10 yr	196	2.5	ND[d]	No	Giemsa and mod. Z-N (33)
	≥10 yr	304	0.7			
Denmark[e]	All ages	800	1.2	ND	No	Mod. Z-N (37)
Australia (86)	<15 yr	697	4.7	13.8	No	Giemsa
	≥15 yr	187	1.6			
Great Britain (40)	<5 yr	329	7.3	ND	No	Mod. Z-N[f]
	≥5 yr	528	3.5			
United States (93)	All ages	1,703	2.8	34.9	Yes	Mod. A-F (53)
United States (39)	All ages	582	4.3	ND	Yes	Mod. Kinyoun A-F
Canada (69)	<10 yr	878	1.4	21.1	No	Auramine (89)
	≥10 yr	568	1.1			
Great Britain (6)	All ages	1,967	1.4	15.2	Yes	Safranin-MB[g]
United States[h]	All ages	1,556	0.6	ND	Yes	Mod. Kinyoun A-F (53) or auramine O (33) or both
Finland (45)	All ages	4,545	2.6	ND	Yes	Mod. Z-N (37)

[a,b] See footnotes *a* and *b* to Table 1.
[c] Casemore et al., Letter, 1983.
[d] ND, Not done.
[e] Holten-Anderson et al., Letter
[f] Casemore et al., Letter.
[g] D. Baxby and N. Blundell, Letter, Lancet ii:1149, 1983.
[h] This report.

and none of 68 control subjects, including 22 sexual partners, was concurrently infected (27).

Although reports from Costa Rica, Bangladesh, and India describe peak rates of isolation of *Cryptosporidium* species during the warm, rainy, humid months (56, 57, 71), seasonal patterns have not been consistent in Australia, Finland, and the United States (6, 39, 42, 86, 94).

Transmission. *Cryptosporidium* species appears to be transmitted by a variety of mechanisms. Zoonotic transmission was initially considered to be the principal source of human infection for several reasons. The organism is commonly associated with diarrhea in animals, particularly calves (2, 85), and interspecies transmission to lambs, pigs, birds, and other animals has been well documented (3, 79–82). The first two documented human cases in 1976 (59, 64) had farm animal exposure. In addition, symptomatic calf handlers and their families in Bangladesh (A. S. M. H. Rahaman, S. C. Sanyal, K. A. Al-Mahmud, A. Sobhan, K. S. Hossain, and B. C. Anderson, Letter, Lancet **ii**:221, 1984) and the United States (15) were found to have high rates of excretion of *Cryptosporidium* species. Although calves formerly seemed to be the source of human infection by fecal-oral contamination with oocysts, other later reports have described no significant contact with farm or domestic animals in most individuals with *Cryptosporidium* species (39, 40, 45, 94).

Person-to-person transmission is likely. A high prevalence in patient contacts, including exposed hospital personnel (46) and case clusters in families (19, 41, 94), have been described. Among immunocompromised patients, most cases are described in homosexual patients with AIDS (53, 66, 68). *Cryptosporidium* species was identified in 34 to 36% of children in two day-care-center outbreaks (1, 77). Fomites may also play a role in the spread of these resistant oocysts in day-care centers.

Contaminated water has been suggested as a vehicle of infection with *Cryptosporidium* species in travelers to Leningrad (45) and the Caribbean (52) and may represent the principal vehicle. A clear epidemiologic association was recently confirmed with clinical, microbiologic, and serologic data in a waterborne outbreak of gastroenteritis in a Texas community with a faulty sewage system (26). Although the organism was not recovered from water samples in that study, oocysts were found in the secondary sewage effluent in Arizona (62). The small size of this organism may limit the usefulness of commonly used filters. Food-borne disease with *Cryptosporidium* species has not been documented.

Airborne transmission may explain the colonization of the respiratory tract in animals with *Cryptosporidium* species, the presence of the organism on the respiratory epithelium and in the sputum of patients with AIDS (37, 54), and the infection of a research worker secondary to a coughing, infected rabbit (B. L. Blagburn and W. L. Current, Letter, J. Infect. Dis. **148**:772–773, 1983).

PATHOGENESIS

The exact mechanism by which *Cryptosporidium* species causes diarrhea is not known. Bloody, inflammatory diarrhea has not been described. A secretory mechanism is consistent with large stool volumes, persistence of diarrhea after the discontinuation of oral intake, and infrequent identification of fecal leukocytes and erythrocytes (93). Malabsorption of fat and carbohydrate may also play a role (73), particularly in patients with AIDS (68, 93).

Infection has been described in the intestinal and respiratory tracts of poultry (28, 38) and in the gastric (7) and biliary (9) tissue of humans, and pulmonary colonization and carriage have been reported in patients with AIDS (31, 54).

Various stages of the life cycle have been identified at multiple sites along the gastrointestinal tract, from esophagus to rectum, in humans, mice, and calves (24, 29). The histologic changes observed in small-bowel biopsies and postmortem sections are nonspecific. Mononuclear infiltrates of the lamina propria, increased crypt size, and some villous atrophy have been reported (63). The *Cryptosporidium*-containing parasitophorous vacuole described under Life Cycle above is found at the brush border surface of intestinal epthelial cells (23, 55). In animals, the histologic changes appear to be similar in kind but more severe in degree, especially in lambs and other young animals (82). In these animals, diarrhea is thought to be caused by loss of membrane-bound digestive enzymes in the upper small intestine and reduced capacity for absorption in the ileum.

A complete understanding of the pathogenesis of cryptosporidiosis in humans remains to be elucidated. Whether interference with absorption in the small bowel is caused by physical damage to the brush border of enterocytes, by parasitic exotoxins or metabolites, or by an immunological reaction to *Cryptosporidium* species is unclear (79).

IMMUNITY

Protective immunity to illness appears to develop with cryptosporidial infection in some chronically exposed populations (57). Cell-mediated immunity appears to be the primary mechanism of host defense. All reported immunocompetent persons infected with *Cryptosporidium* species have cleared the parasite spontaneously, whereas those on immunosuppressive therapy may experience persistent illness (19, 51, 59, 60, 68, 92). One such patient resolved his prolonged infection when steroid therapy was discontinued. In 7 of the 10 studies described in Table 2, the proportion of patients with symptomatic cryptosporidiosis who were immunocompromised ranged from 0% (86; D. P. Casemore and B. Jackson, Letter, Lancet **ii**:679, 1983; W. Holten-Anderson, J. Gerstoft, and S. A. Henriksen, Letter, N. Engl. J. Med. **309**:1325–1326, 1983) to 30% (this report). Of over 150 immunocompromised individuals reported with *Cryptosporidium* infection, the vast majority had AIDS. The T4 helper lymphocyte depletion described in patients with AIDS appears to also involve the small-bowel mucosa where the immunologic reaction to this intracellular protozoan occurs (70). Similarly, measles infection, another cause of cell-mediated immunodeficiency, has been implicated as a predisposing factor in developing countries (P. DeMol, S. Mukashema, J. Bogaerts, W. Hemelhof, and J. P. Butzler, Letter, Lancet **ii**:42–43, 1984), although malnutrition has not (10). In an animal model, athymic (nude) mice, with insufficient numbers of T lymphocytes, were unable to clear their *Cryptosporidium* infections, whereas euthymic white mice had only transient infections (36).

Both humoral and cellular immunity may be required to eliminate the infection. *Cryptosporidium* species has been identified in seven hypogammaglobulinemic patients (11, 14, 24, 47, 48, 73), at least four of whom were shown to have normal tests of T-cell function. Because the majority of both immunocompetent and AIDS patients infected with *Cryptosporidium* species appear to demonstrate systemic antibody to this pathogen (14, 88), the presence of organism-specific

antibody alone may not be protective. Delineation of the surface antigens of the organism and the utilization of inbred animal models will be important advances in understanding the development of protective immunity to this parasite.

CLINICAL FEATURES

Immunocompetent hosts. Now that over 600 cases of cryptosporidiosis in immunocompetent hosts have been reported, the breadth of clinical manifestations has been delineated. In most reports, the majority of cases are identified in children (40, 86). The incubation period has been reported to range from 1 day to 2 weeks (15, 42, 43; Blagburn and Current, Letter). The infective dose required to produce infection in humans is not known. Infant macaques developed a self-limited illness when inoculated with 10 oocysts, the number found in only 0.02 μl of stool (6×10^5 oocysts per ml of feces) (R. A. Miller, M. A. Bronsdon, and W. R. Morton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, B-148, p. 49). In humans, watery diarrhea is reported in 86 to 100% of index cases and 67 to 100% of infected contacts. Abdominal pain and cramping are found in over half the cases, and vomiting is also well described. Bloating and gas are less common symptoms than with *G. lamblia* infection, and 10 to 70% of patients experience a low-grade fever (35, 40, 43, 44, 94). Anorexia, malaise, and weight loss may also be found in this rather nonspecific intestinal syndrome. Symptoms last for 1 to 2 weeks on the average (35, 43, 94) but may continue for more than 1 to 2 months (40, 44). The clinical syndrome may be complicated by the presence of copathogens. Of 211 cases reported from eight developing countries (10, 56, 57, 71, 74, 91; Hojlyng et al., Letter; D. N. Taylor and P. Echeverria, Letter, Lancet i:320, 1986), other pathogens were identified in 41% (range, 20 to 56%), in comparison to 22% (range, 15 to 35%) of 131 cases reported from four developed countries (6, 69, 86, 94). In all reported cases to date in immunocompetent hosts, the symptoms and infection ultimately resolved spontaneously. A chronic carrier state is rarely described, although very few groups have been screened and identification of low numbers of organisms may be unreliable.

Immunocompromised hosts. The clinical syndrome described in immunocompromised individuals, most of whom have AIDS, is far more virulent and persistent than that noted above. Water diarrhea of many months' duration with anorexia, abdominal pain, and weight loss is common (66, 75, 93). Vomiting, weakness, malaise, and low-grade fever often are noted. Malabsorption (68, 93) and stool volumes of up to 8 liters/day are well documented, as are fatalities caused by dehydration and cachexia. Cough is occasionally seen in association with *Cryptosporidium* infection, and the organism has been identified in six immunocompromised patients with progressive pulmonary disease (31, 47, 54, 61). Other major pathogens were found, however, in tissue sections from five of these patients. Although the data are limited, *Cryptosporidium* species appears to be able to colonize the lung and may be responsible for bronchitic symptoms. It appears unlikely to be a common cause of debilitating respiratory disease.

Spontaneous resolution of the infection in one child with AIDS (8), a report of asymptomatic carriage in one adult patient with AIDS (95), and our clinical experience with *Cryptosporidium*-infected patients with AIDS in Denver suggest that some of these individuals may tolerate their infection relatively well, either with or without drug therapy. In those with a deteriorating clinical course, attention should be focused on fluid and nutritional support.

LABORATORY DIAGNOSIS

Microscopy. Rapid and reliable techniques for the detection of *Cryptosporidium* species in stool provided the single most useful advance in documenting the worldwide prevalence of this infection. Before 1978, the organism was diagnosed histologically only in tissue sections and could be seen at the intestinal epithelial surface by electron microscopy or by staining with hematoxylin and eosin, Giemsa, periodic acid-Schiff, or toluidine blue. Methods for identification of *Cryptosporidium* species in stool samples have shown that patients with diarrhea most often excrete high numbers of oocysts, whereas asymptomatic persons with formed stools pass fewer organisms (32). The most sensitive screening methods reported employ a murine monoclonal antibody to the oocyst wall (32, 76). The indirect immunofluorescence method, which is not yet available commercially and which requires fluorescence microscopy, showed 100% sensitivity and specificity, even with low numbers of oocysts in the sample and without concentration (32).

Concentration methods are recommended for identifying oocysts in the asymptomatic host and during epidemiologic or outbreak investigations (58) and may increase the diagnostic yield in the routine clinical laboratory. The Sheather sucrose flotation technique (24) has been shown to be equal to (58) or better than (53) the Formalin-ethyl acetate (ether) technique for concentrating oocysts. Aerosolization of infected material with the flotation method can be limited by the use of screw-top tubes (24). We recommend the Formalin-ethyl acetate concentration method, because it is efficient, easier and faster to perform, and better than the Sheather sucrose method for identifying *G. lamblia* and other protozoans (58). The auramine O fluorescent stain may be the fastest screening procedure available (90). Oocysts have a beaten or cratered appearance, but no internal structures are seen and morphology may be variable.

A comparison of 13 currently used staining techniques found the modified Ziehl-Neelsen hot acid-fast stain to be the best overall for sensitivity and morphology when used in conjunction with a 10% KOH initial digestion (33). Slides can be screened under low or high dry power. With the modified acid-fast stain, the 4- to 6-μm oocysts stain bright pink-red against a green background (Fig. 2) (33). A dimethyl sulfoxide-carbolfuchsin stain (12) and the modified Kinyoun acid-fast stain (52) eliminate the need for heat or steam and show similar staining and sensitivity. Yeasts, which are often confused with *Cryptosporidium* oocysts because of their size and morphology, stain green with the acid-fast procedures and brown with iodine, whereas oocysts do not stain with iodine. A Giemsa stain may show clear oocysts with pale blue internal structures but does not differentiate yeasts from oocysts. The safranin-methylene blue stain, reported to be both sensitive and rapid (10), may be visually taxing when large numbers of samples are screened. Negative stains, such as iodine, modified periodic acid-Schiff, and methenamine silver, may be less reliable than the others listed (33). Familiarity with two staining techniques and use of control slides are advisable to assure diagnostic accuracy.

Serology. There is no commerically available assay for the serodiagnosis of *Cryptosporidium* infections. By indirect immunofluorescence of infected lamb mucosa, Tzipori and Campbell demonstrated antibody to *Cryptosporidium* species in 86% (18 of 21) of random human serum samples in Ireland and in 80 to 100% of sera from nine animal species (83). These data suggest a high rate of human and animal exposure to this organism, but the specificity of the assay

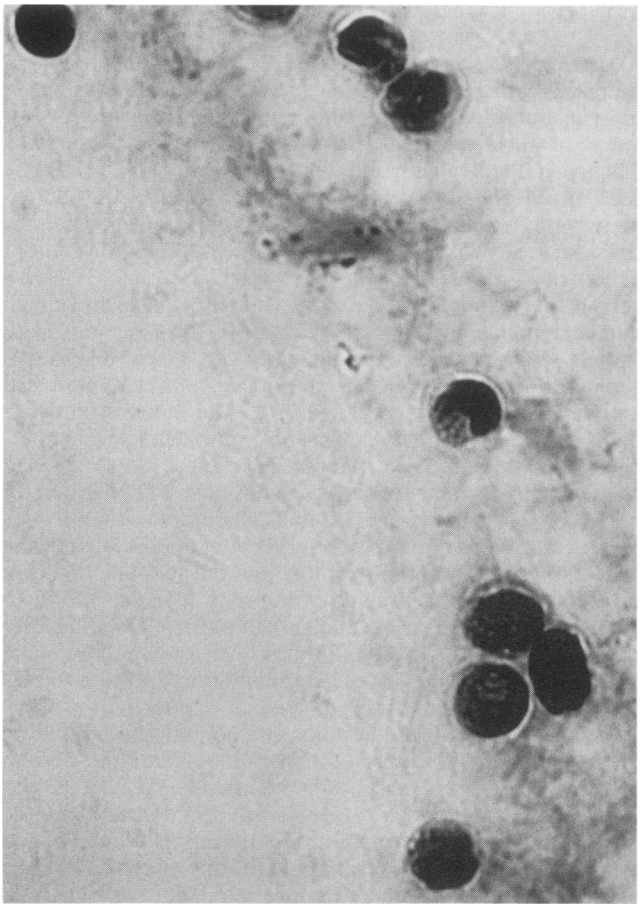

FIG. 2. Modified Kinyoun stain of *Cryptosporidium* species of a stool specimen from a patient with AIDS.

was not well defined. Little cross-reactivity with four other coccidian species was shown using a similar indirect immunofluorescence assay with *Cryptosporidium*-infected mouse tissue (14). In this study, low titers were found in 10 persons with no known exposure. Elevated antibody titers followed infection and persisted for over a year. Each of five infected patients with AIDS also had detectable titers. Most recently, Ungar et al. (87) described an enzyme-linked immunosorbent assay for the detection of *Cryptosporidium*-specific immunoglobulin G and immunoglobulin M serum antibodies with purified calf oocysts. Of 15 infected immunocompetent persons, 13 (87%) showed organism-specific antibody, as did each of 26 infected patients with AIDS. The immunoglobulin M response appeared to reflect recent infection in the non-AIDS group. Only 3 of 60 (5%) people with no known exposure had positive results, although high rates of seropositivity were noted in persons with other parasitic infection. A 23,000-dalton antigen from a cyst preparation was commonly recognized by immune human sera and may be organism specific. A 25,000-dalton sporozoite antigen was also recognized by immune cattle sera, but recognition of this and other antigens by sera from *Cryptosporidium*-infected persons was variable (J. R. Mead, M. J. Arrowood, and C. R. Sterling, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, E-27, p. 101). These methods will provide useful tools in establishing the diagnosis and defining the epidemiology and immunology of cryptosporidial infections.

THERAPY

Immunocompetent persons have self-limited infections which may require hospitalization (35) for rehydration and symptomatic relief. Immunocompromised patients, who rarely improve spontaneously (16), may require additional therapy. Most pharmacological trials have been performed in animals, in which *Cryptosporidium* species is a common cause of morbidity (4, 84). In humans, multiple-drug regimens have been tried with limited success. Of 21 patients with AIDS initially reported, 1 had a microbiologic cure when treated with furazolidone for 2 months, as did a second patient who received diloxanide furoate while simultaneously discontinuing steroid therapy (16). Several of these patients, and others since, have shown inconsistent and infrequent symptomatic improvement, with persistent oocyst shedding, when treated with tetracycline, furazolidone, or a combination of quinine and clindamycin (17). Only one of three patients treated with amprolium, a toxic quaternary organic base, showed any clinical response (66; D. P. Casemore, M. Armstrong, and B. Jackson, Letter, Lancet **i**:734–735, 1984; S. J. O. Veldhuyzen Van Zanten, J. M. A. Lange, H. P. Saverwein, A. C. Rijpstra, J. J. Laarman, P. J. G. M. Reitra, and S. A. Danner, Letter, Lancet **ii**:345–346, 1984).

Most promising has been the use of spiramycin, a macrolide antibiotic in a class with erythromycin and clindamycin. In uncontrolled trials, a number of patients have shown symptomatic improvement, and at least 12 sustained microbiologic cures have been reported (17, 19, 68, 92). The mechanism of action of spiramycin against *Cryptosporidium* species is unknown. Clinical improvement with therapy may be due to a direct effect on the protozoan, or it may result from effects on the bowel flora and copathogens. Side effects with the drug, primarily gastrointestinal, appear to be infrequent (68; G. M. Decaux and C. Deveroede, Letter, Lancet **ii**:993, 1978). Spiramycin is available with the approval of the U.S. Food and Drug Administration from Rhone-Poulenc Pharmaceuticals, Montreal, Quebec, Canada. Controlled trials are under way to evaluate the efficacy of the drug.

Treatment with α-difluoromethyl-ornithine, an inhibitor of polyamine synthesis with activity against a variety of protozoans, has also been associated with clinical improvement (R. Soave, A. Sjoerdsma, and M. J. Cawein, Abstr. Proc. 1st Int. Conf. AIDS 1985, abstr. no. 30, p. 77). Toxicity to the bone marrow and gastrointestinal tract are, however, significant impediments to the use of this drug. Other agents, such as oral bovine transfer factor (E. Louie, W. Borkowsky, P. H. Klesius, T. D. Haynes, S. Gordon, and H. S. Lawrence, Abstr. Proc. 2nd Int. Conf. AIDS 1986, abstr. no. 54B, p. 65) and trimexate, a folate antagonist, are under investigation (J. A. Kovacs, C. J. Allegra, J. C. Swan, J. D. Drake, B. A. Chabren, J. E. Parillo, and H. Masur, Clin. Res. **34**:522A, 1986).

A nontoxic effective therapeutic agent would be of value to the severely affected immunocompromised patient and may help to limit transmission of the relatively resistant oocysts (13). The development of an in vitro culture system by Current and Haynes and Current and Long (21, 22) may facilitate the identification of such agents. Rehydration and the reduction of other immunocompromising agents, such as steroids, are currently the mainstay of therapy.

Prevention of infection may be facilitated by improved methods for inactivation of infective oocysts. Viability may be maintained for over 9 to 12 months in vitro (21, 88), and the oocysts are resistant to several common disinfectants,

such as iodophores, cresylic acid, sodium hypochlorite, benzalkonium chloride, and sodium hydroxide (13) and 2-aldehyde-based compounds (5). Effective methods include exposure to temperatures below 0°C and above 65°C for 30 min (13; Current, Clin. Microbiol. Newsl.), as well as treatment with 10% formol saline and 5% ammonia or commercial bleach. Exposure to untreated water and uncooked vegetables should be avoided when traveling. Standard enteric precautions should be used when coming in contact with *Cryptosporidium*-infected persons or animals.

CONCLUSIONS

Cryptosporidium species is considered a protozoan cause of diarrheal disease worldwide. The life cycle is complex. Internal autoinfection within the intestine of mammalian hosts may explain the ability of the organism to establish a significant infection despite low inoculum size (23). The diagnosis should be considered in immunocompromised hosts with persistent diarrhea, in children, particularly those involved in day-care-center outbreaks, and in individuals with exposure to animals. Travelers with diarrhea and groups involved in waterborne outbreaks of diarrheal disease should also be suspected of infection with *Cryptosporidium* species. Enteric precautions should be taken when in contact with infected patients, because the incidence of disease in patient contacts appears to be high. Immunocompetent hosts may have an illness that lasts 5 to 14 days and is characterized by watery diarrhea, abdominal pain, and a low-grade fever.

Oocyst excretion may persist for 1 to 2 weeks and, rarely, up to 3 months after the resolution of symptoms, so the diagnosis may be made after routine tests show negative results. Fecal leukocytes or erythrocytes or both are infrequently found. Stool diagnosis has been enhanced by the use of special stains and, in part, by concentration methods. Acid-fast and auramine stains are widely used screening procedures, and familiarity with two different methods for screening and confirmation is recommended. Yeasts can be distinguished from *Cryptosporidium* oocysts with iodine wet-mount and acid-fast stains.

Therapeutic options are limited but should include fluid replacement. Spiramycin, an antibiotic not approved for use in the United States, may be beneficial for control or cure of severely infected immunocompromised persons.

Noninvasive diagnostic methods have greatly enhanced our concept of the epidemiology and clinical aspects of *Cryptosporidium* infections. The diverse host specificity, the range of mechanisms of transmission, the variety of organs affected, and the broad range of clinical manifestations suggest that *Cryptosporidium* species is indeed a protean protozoan. Challenges for the future include delineating the mechanism of illness and the immunologic response to infection, as well as development of effective therapeutic agents.

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