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Cryptosporidium spp., a Frequent Cause of Diarrhea in Central Africa

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***Cryptosporidium* oocysts were present in 20 (10.4%) of 193 Rwandese children and in 3 (3.0%) of 100 adults with diarrhea. In four of the children and in one adult, *Cryptosporidium* was associated with other enteric pathogens. The higher incidence of *Cryptosporidium* in diarrheic children was statistically significant. The parasite was not found in 94 formed stools submitted for parasitological examination. The mean age of the *Cryptosporidium*-positive children was 13.3 months. In four children, *Cryptosporidium* was associated with severe malnutrition. All of those required rehydration, and one child died as a direct consequence of severe diarrhea. The three adult patients showed no recognizable immunodeficiency, and their diarrhea resolved spontaneously. Staining with 1% safranin was not only more simple and rapid but also more sensitive than the modified Ziehl-Neelsen technique.**

Cryptosporidium is a protozoan which has recently emerged as a cause of diarrhea both in animals and in humans (24). Veterinary science especially has been interested in this new agent because of its serious economic implications.

The early literature on human cryptosporidiosis included only sporadic cases in immunocompetent or immunocompromised hosts, with death often being observed in the latter (3, 5, 16, 18, 19, 22, 23, 27, 28). Diagnosis of the earlier cases was most often based on intestinal biopsy. After the appearance of acquired immune deficiency syndrome (AIDS) in mid-1981, interest towards this parasite increased as *Cryptosporidium* appeared to be a frequent cause of severe diarrhea among subjects with this syndrome (13, 15). In the meantime, it was shown that the diagnosis could be simplified by demonstration of the oocysts in fecal smears (1, 4, 20, 25). Screening of larger populations revealed that this protozoan was also a significant cause of self-limiting diarrhea in normal persons in industrialized countries (6, 8, 12, 26).

Cryptosporidiosis is considered a zoonosis (24) because the disease is transmitted by contact with infected animals. Since the oocysts are infective when discharged in the stool, human-to-human transmission is not excluded.

In this study we describe our 3-month experience with cryptosporidiosis in an urban general hospital in Central Africa.

MATERIALS AND METHODS

This study was done at the Centre Hospitalier of Kigali, the capital of Rwanda, a rapidly expanding town with 150,000 inhabitants. It is situated 1,500 m above sea level and has a climate with two rainy and two dry seasons. This is a general hospital and serves the rural and urban population. The microbiological laboratory of this hospital is the only one in the prefecture of Kigali, a region with 700,000 inhabitants.

Between 24 October and 24 January 1984, we looked for *Cryptosporidium* in 293 liquid stool specimens sent for routine stool culturing. These specimens were immediately concentrated by the method of Ritchie (21). One drop of the sediment was spread on a microscope slide and air dried. The unfixed smears were stained either by a modified Ziehl-Neelsen technique (11) or with 1% safranin and 0.5% methylene blue as a counterstain (4). Stained preparations were examined under oil immersion by using a ×50 objective lens for screening and a ×100 objective lens for identification of oocysts. A specimen was only considered negative after screening the entire microscope slide, corresponding to ca. 120 fields.

Of the 293 specimens, 148 were stained by both methods to compare their sensitivity and specificity. For each positive slide, the number of oocysts was counted in 40 fields (or in 120 when needed), and the average per field was calculated for all the positive slides in each technique. All 293 stools were examined for intestinal parasites and cultured for *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter* isolates. Culturing for enterotoxigenic *Escherichia coli* and demonstration of rotavirus could not be performed. All isolates were identified according to the *Manual of Clinical Microbiology* (14). As soon as cryptosporidia were found, the clinician was requested to closely observe the progress of the patient and to complete his medical history. The nutritional status of each child was assessed by anthropometric measures, based on the Wellcome classification (2). Those whose weight after rehydration was less than 60% of the expected value for their ages were diagnosed as having marasmus. No case of nutritional edema (kwashiorkor) was observed among the *Cryptosporidium*-infected children. The control group consisted of 94 patients with formed stools submitted for parasitological examination. These stool specimens were suspended in 10% Formalin and examined for *Cryptosporidium* after concentration by the method of Ritchie and staining with safranin as described above. Culturing was not performed. The composition of this group by age and social status was comparable to that of the diarrheal patients.

RESULTS

Cryptosporidium was the second most frequent pathogen in the stools of diarrheal patients, after *Salmonella* spp. and

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TABLE 1. Enteric pathogens in diarrheal stools

Pathogens found	No. (%) of patients per group (*n*):					
	Children (193)		Adults (100)		Total (293)	
Nontyphoid *Salmonella* spp.	16 (8.3)		9 (9.0)		25 (8.5)	
Cryptosporidium spp.						
Alone	16 (8.3)		2 (2.0)		18 (6.1)	
With *Salmonella* spp.	2				2	
With *Shigella* spp.		(10.4)	1	(3.0)	1	(7.8)
With *Campylobacter* spp.	2				2	
Shigella spp.	10 (5.2)		3 (3.0)		13 (4.4)	
Campylobacter jejuni	8 (4.1)		3 (3.0)		11 (3.8)	
Giardia lamblia	7 (3.6)		1 (1.0)		8 (2.7)	
Entamoeba histolytica	2 (1.0)		2 (2.0)		4 (1.4)	
Mixed infections[a]	5 (2.6)		1 (1.0)		6 (2.0)	
No pathogens found	125 (64.8)		78 (78.0)		203 (69.3)	

[a] Pathogens in mixed infections (number of patients): *Salmonella* and *Shigella* spp. (1), *Salmonella typhimurium* and *E. histolytica* (2), *Shigella* sp. and *E. histolytica* (1), *Giardia lamblia* + *E. histolytica* (2).

before *Shigella* and *Campylobacter* spp. (Table 1). It was found in 7.8% of the liquid specimens. Oocysts were more frequent in children than in adults (10.4% versus 3.0% [χ^2, 4.9; $P < 0.05$]). The difference between children and adults remained significant even when only specimens containing *Cryptosporidium* as the sole pathogen were considered (8.3% versus 2%; χ^2, 4.5; $P < 0.05$). Moreover, no *Cryptosporidium* could be detected in the control group.

Of the 20 children with oocysts in the stools, complete information was obtained on 15, 7 boys and 8 girls. The mean age was 13.8 months. Six were under 1 year, seven were between 1 and 2 years, and only two were older. Nine children were outpatients, and six were hospitalized. The clinical course was clearly different between the two groups. All of the ambulatory patients had a self-limited illness characterized by mild, watery diarrhea lasting from 4 to 90 days (median, 14 days) without more than 5% loss of the initial body weight and with vomiting only by one child. All of these children were well nourished.

On the other hand, the outcome was less favorable for the six hospitalized children, who all needed intravenous and oral rehydration. Four were marasmic with prolonged diarrhea for over 14 days. Severe vomiting and a loss of more than 10% of body weight was noted in all members of this group. In this group, one child had, simultaneously, a septicemia with *Salmonella typhimurium*, and another had a multiresistant *S. typhimurium* in the stool on admission. One child, who had only *Cryptosporidium* oocysts in the stools, died as a consequence of severe diarrhea. Another child was admitted, with a good nutritional status, for measles complicated by pneumonia and diarrhea. While in the hospital, this child developed a multiresistant *S. typhimurium* septicemia, treated with cefotaxime, but his general condition deteriorated. After 9 weeks of hospitalization and a 20-day period without diarrhea, he presented frequent watery stools for 21 days with severe dehydration. Only *Cryptosporidium* was found in the stools. He died later with extreme marasmus and *Salmonella*-positive stools but without *Cryptosporidium*. The only well nourished hospitalized child improved rapidly after 1 day of rehydration.

Since *Cryptosporidium*-enteritis is a potential marker of AIDS in adults (9) and since the syndrome appears to be endemic in Rwanda (unpublished data), we looked for other symptoms suggesting this syndrome. In the two female adult patients, *Cryptosporidium* was associated with a mild diarrhea which resolved after 4 to 5 days. They showed no evidence of decreased immunocompetency and follow-up stool specimens remained negative. The third adult patient, however, a 35-year-old man, showed symptoms suggestive of AIDS, including loss of weight for 4 months with fever and diarrhea. *Cryptosporidium* was present in his stools together with *Shigella flexneri*. Diarrhea resolved spontaneously after 2 weeks, and both pathogens were then absent. This patient showed neither lymphadenopathies nor lymphopenia, and there was no other evidence for opportunistic infections. However, a tuberculin skin test with 10 IU of purified protein derivative was negative, a rather uncommon finding among adults in tropical Africa. This patient was still well 2 months later, and no recurrence of fever or of cryptosporidia was observed in his stools.

In all of our patients, adults as well as children, cryptosporidia were found only in watery stools. One child, however, continued shedding oocysts at least 1 week after resolution of diarrhea.

Among the 148 specimens stained in duplicate, 1% safranin gave 19 positive results versus only 14 by the modified Ziehl-Neelsen method. All specimens that were positive by the Ziehl-Neelsen technique also were positive with safranin, but not the reverse. To exclude false negatives due to sampling problems, three entire slides of the Ziehl-negative, safranin-positive specimens were examined with constantly negative results. Three patients showed oocysts with safranin only in one stool specimen, but another specimen, examined at a 1-week interval, was positive by both methods. In the 14 stools that were found positive by both techniques, the average number of oocysts per microscopic field was 1.5 for safranin versus only 0.27 for the Ziehl-stained slides. This 5.5-times-lower yield by the latter technique could probably be explained by the occurrence on the Ziehl-stained smears of empty "ghosts" having the typical

size and shape of the oocysts. Such ghosts were also observed in one of the false-negative Ziehl-stained slides.

DISCUSSION

Cryptosporidiosis has been observed both in immunodeficient and in normal subjects, but only scant data are available on its prevalence in developing countries (8) and in pediatric populations (6). Our survey indicates that *Cryptosporidium* is a frequent cause of diarrheal disease in Rwanda, especially in children, in whom it was present as the sole pathogen (8.3% of those with diarrhea) during the period covered by our study. The clinical evolution was self-limited in the well nourished children, with mild, watery diarrhea being the major finding, without deterioration of their nutritional status. By contrast, in the four children with marasmus, the outcome was less favorable, with severe and prolonged diarrhea, vomiting, loss of weight, and, in one patient, death. The depression of cell-mediated immunity, observed in advanced malnutrition (7), might well offer an explanation for the more severe course in marasmic subjects.

Since one of our patients had no contact at all with animals (he developed diarrhea with *Cryptosporidium* after being in the hospital more than one month), we believe that people, food, and other sources may play a role in transmission of oocysts. Obviously, epidemiological and longitudinal clinical studies are needed to determine the seasonal distribution and the exact mode of transmission. A study on the prevalence and severity of cryptosporidiosis among a large group of malnourished children is under way.

Since diagnosis is possible with simple staining techniques, we believe that routine examination for *Cryptosporidium* must be part of the parasitological routine. In our opinion the safranin method combines the same specificity as the Ziehl-Neelsen method with greater sensitivity. Occasional fat drops may take the same color as the oocysts, but they are easily distinguished from the sporozoite-containing oocysts. Although acid-fast staining techniques have been recommended for the detection of fecal oocysts (10), there is evidence that oocysts do not always stain with carbol fuchsin (4, 17).

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