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Parasitic, Bacterial, and Viral Enteric Pathogens Associated with Diarrhea in the Central African Republic

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A total of 1,197 diarrheic children less than 15 years old were investigated for parasitic, bacterial, and viral enteropathogens from March 1981 through February 1982 in the Central African Republic. One or more pathogens were identified from 49.4% of the patients. Rotavirus was the most frequently identified pathogen among children less than 18 months old. Enteropathogenic *Escherichia coli* was the second most frequently isolated pathogen (12.1%) in children less than 2 years of age. *Campylobacter jejuni* was also isolated frequently from diarrheic children less than 5 years of age (10.9%). *Entamoeba histolytica* was identified in very young children and was found to be the most frequent enteropathogen associated with diarrhea in children over the age of 2 years. Enterotoxigenic *Escherichia coli* was rarely isolated (ca. 2%). There was a peak in the incidence of rotavirus during the dry season and in the incidence of *Campylobacter jejuni* during the rainy season.

Diarrhea is a major cause of mortality and morbidity in developing countries (27). Few studies have ben made in Central Africa to determine the incidence of the different enteric pathogens associated with diarrhea and particularly of the following recently recognized agents: rotavirus (5), *Campylobacter jejuni* (23), and enterotoxigenic *Escherichia coli* (ETEC) (22).

During the course of 1 year, we studied parasitic, bacterial, and viral agents present in the stools of diarrheic children of Bangui. Our goal was to inventory the different enteric pathogens associated with pediatric diarrhea in the Central African Republic (CAR) and to examine the fluctuations with age and seasons.

MATERIALS AND METHODS

Study population. Stools of 1,197 children with diarrhea were investigated from March 1981 to February 1982. All were children between the ages of newborn and 15 years who had diarrhea for less than 5 days and who had received no antibiotic therapy. Diarrhea was defined as the emission at least three times per day of stools taking the form of the container. The age distribution of the children is included in Table 1. All of the children were seen either at the National University Hospital of Bangui, where the majority of them were hospitalized, or at an urban dispensary (Foyer de Charite). A clinical history of each patient was collected by the physician. During this study period a group of 748 well children were cultured for the presence of rotavirus, *C. jejuni*, and ETEC, and these data, including age distribution, are presented in Table 2.

Parasitology. Each fresh stool was examined with a light microscope for trophozoites of *Entamoeba histolytica* and *Giardia lamblia* by using a saline and iodine preparation (19, 25); Kato and migration inhibition factor techniques were used for determinations of concentrations. Only the trophozoite form was considered pathogenic in the case of *Entamoeba histolytica*, whereas both cysts and trophozoites were considered pathogenic forms of *Giardia lamblia*.

Bacteriology. All the specimens were seeded within 3 h after admission and without the use of conservation medium. All were collected in sterile containers and plated on salmonella-shigella, brilliant green, eosine-methylene blue, and thiosulfate-citrate bile salts agar (Pasteur Institute Medium) and incubated at 37°C for 18 h. A portion of each sample was enriched in sodium selenite medium, incubated for 18 h at 37°C, and then plated on salmonella-shigella agar and held at 37°C. For the isolation of *Yersinia enterocolitica*, salmonella-shigella agar medium was plated and stored at room temperature for 48 h (28). *Salmonella* and *Shigella* spp. and enteropathogenic *Escherichia coli* (EPEC) were identified by standard methods (9).

Each sample was also plated on blood agar with a supplemented selective medium for *Campylobacter* spp. (Butzler supplement; Oxoid Ltd.) (3) and incubated at 42°C in an anaerobic jar with a gas pack (Oxoid) but without catalyst (13). The plates were read at 48 h; the colonies were Gram stained and tested for motility and oxidase and catalase production.

Ten colonies of *Escherichia coli* were selected from each plate of eosine-methylene blue agar and pooled (14). Of these pools, 314 were tested for the presence of heat-stable enterotoxin (ST) and heat-labile enterotoxin (LT) by DNA colony hybridization with specific probes both for LT and ST genes as described previously (10). The remaining pools were tested only for the presence of ST by the infant mouse assay (6).

Virology. A sample of each stool was frozen in a saline solution and tested by enzyme-linked immunosorbent assay for rotavirus antigen (29). The reagents were supplied by the World Health Organization Collaborating Center for Reference and Research on Rotaviruses, Birmingham, England. Of the 211 samples found positive by enzyme-linked immunosorbent assay, 120 were assayed with the blocking test by using World Health Organization reagents and techniques. Of these 120 samples, 96% were confirmed as positive.

Observation for leukocytes. For each sample the presence of leukocytes was determined by microscopic examination after coloration with May Grunwald Giemsa stain.

Climatology. Maximum and minimum temperatures and

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TABLE 1. Enteropathogens associated with diarrhea in children according to age group

Pathogens	% of diarrheic children in following age group from whom enteropathogen was isolated:						
	0–5 mo (n = 274)	6–11 mo (n = 298)	12–17 mo (n = 231)	18–23 mo (n = 124)	2–5 yr (n = 203)	6–15 yr (n = 67)	Total (n = 1,197)
Shigella spp.	1	3	3.4	4	3.4	1.4	2.7
Salmonella spp.	2.5	2.3	4.7	7.2	1.9	4.4	3.4
Salmonella typhi	0	0.3	0	0.8	3.9	10.4	1.4
EPEC	14.5	12.7	8.2	12.9	—[a]	—[a]	12.1
Campylobacter jejuni	9.1	14	13.4	8	9.3	2.9	10.7
ETEC							
ST	0.8	1.6	1.1	0	1.2	0	1
LT	1.9	1.6	1.5	2.1	0	0	1.5
ST and LT	0	3.2	0	0	0	4	0.9
Entamoeba histolytica	1	2	2.5	8	12.8	22.3	5.5
Giardia lamblia	1	0.6	5.1	4	6.4	2.9	3.9
Schistosoma mansoni	0	0	0	0	1.4	17.9	1.2
Tricharis trichura	0	0	0.4	3.2	0.9	4.4	0.8
Strongyloides stercoralis	0	0	1.2	2.4	3.4	7.4	1.5
Rotavirus	27.7	28.1	15.1	6.4	3.9	0	17.6
Unknown etiology	40.5	30.6	43.4	41	51.5	22	35.8

[a] —, Not investigated.

amounts of rainfall were recorded for each month. Bangui has a tropical, wet climate with a dry season from October to April and a rainy season from May to September. There are no large fluctuations in the seasonal temperatures, which have a mean of about 27°C.

RESULTS

One or more enteric pathogens were isolated from 49.4% of the diarrheal samples. The results of the examination of fecal samples are given in Table 1. Each sample was investigated for all enteric pathogens indicated above, with the previously noted exception of ETEC. We did not investigate EPEC strains in children over 2 years of age. The occurrence of recently recognized enteric pathogens isolated from well children is described in Table 2.

Rotavirus was the most frequently identified pathogen, occurring most frequently in the 11-months-and-under age group and decreasing to 0% in children over 5 years of age. Comparing these data with those from control children (Table 2), we found that the association of rotavirus and diarrhea is statistically significant (χ^2 analysis of 2 × 2 contingency table where $P < 0.001$ for children from 0 to 11 months and $P < 0.05$ for children from 12 to 17 months). EPEC strains were also found in this age group and were the second most frequently isolated pathogen in children under 2 years of age.

C. jejuni was the third most frequent enteric pathogen associated with diarrhea. Its incidence was highest in the children between 6 and 11 months old. Although the incidence was greater in diarrheic than in well children, this difference was not statistically significant. Asymptomatic carriers in developing countries have been described elsewhere (2).

Thirty-three *Shigella* spp. were identified as follows: 29 *S. flexneri* (serotypes 1, 2, 3, 4, and 6), 1 *S. boydii* (serotype 2), 1 *S. dysenteriae* (serotype 3), 1 *S. sonnei*, and 1 nonagglutinable (rough) strain. *Salmonella typhi* was isolated from 7 of the 67 stools of the children between 5 and 15 years of age. This high percentage is not surprising since *Salmonella typhi* is endemic in the CAR. Of the 17 diarrheic children who had *Salmonella typhi* in their feces, 2 had a typical syndrome of typhoid fever, 10 had febrile diarrhea, and 5 had diarrhea without fever. In 5 of the 17 cases, other enteric pathogens were isolated (three *Schistosoma mansoni*, one *Salmonella* sp., one *Entamoeba histolytica*).

The incidence of ETEC was found to be very low in patients (<2%) and in controls (<0.002%).

Neither *Vibrio cholerae* nor *Yersinia enterocolitica* was isolated. *Entamoeba histolytica* was identified in the stools of very young children (the youngest being 1 month old), and its frequency increased with the age of the patient. The incidence of other parasites, except for *Schistosoma mansoni* and *Strongyloides stercoralis*, appeared to be low in patients over the age of 5 years.

We noted seasonal variations in the incidence of rotavirus and *C. jejuni* (Fig. 1 and Table 3). Comparing the occurrence of *C. jejuni* and rotavirus in rainy versus dry seasons, we found that the differences were statistically significant. Rotavirus was isolated more frequently in the dry season ($P < 0.001$), and *C. jejuni* was isolated more frequently in the rainy season ($P < 0.01$).

Of the 1,197 children investigated, 746 were hospitalized at the National University Hospital; of these, 7 children less than 12 months old died. No enteric pathogen was isolated from three of these seven children; one *Salmonella typhimurium*, one EPEC plus rotavirus, one *C. jejuni*, and one *C. jejuni* plus rotavirus were isolated from the remaining four fatal cases. The major clinical symptoms associated with the most frequently isolated enteric pathogens are presented in Table 4. Leukocytes were found in 240 stools. In 35% of

TABLE 2. Recently recognized enteropathogens isolated from 748 well children according to age group

Age group (no. of children)	% of well children from whom following pathogen was isolated:		
	Rotavirus	*C. jejuni*	ETEC
0–5 mo (111)	5.4	5.4	0
6–11 mo (189)	11.1	9.5	0
12–17 mo (144)	6.9	8.3	0
18–23 mo (102)	5.8	8.8	0
2–5 yr (113)	4.4	5.3	0.8
6–15 yr (89)	3.3	2.2	0

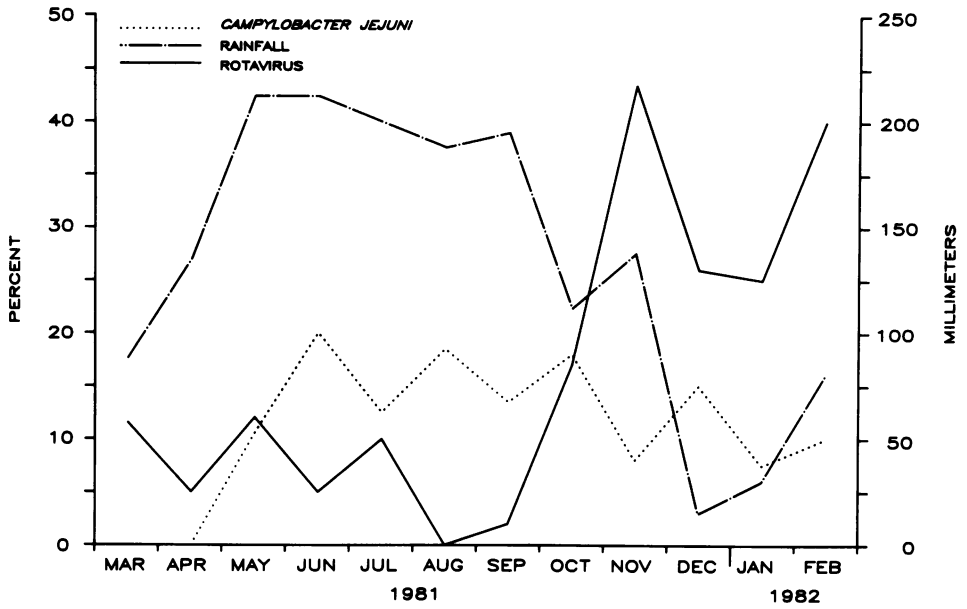

FIG. 1. Seasonal fluctuation in the incidence of *C. jejuni* and rotavirus in the CAR from March 1981 through February 1982. The monthly rainfall is given in millimeters, and the incidence for infection is given as a percentage of the total patients cultured.

them, no enteric pathogen was identified. Table 5 illustrates the most frequent pathogen or combination of pathogens associated with the occurrence of leukocytes in 131 stools. We cannot rule out the possibility that the finding of fecal leukocytes may be due to unidentified coinfection.

DISCUSSION

This study, which covered a 1-year period, is the first study in Central Africa of the agents associated with diarrhea in children.

Rotavirus was the pathogen isolated most frequently (17.6%). Other investigators in several developing countries have found the following incidences for these viruses: 0% in Gambia (20), 40% in Rhodesia (4), 15% in the Philippines (8), 61% in a small study in Nigeria (7), 27.8% in Addis Ababa (24), and 70% in a Southern Indian town (17). In our study, rotavirus was found most frequently among children less than 1 year old; this incidence decreased in children more than 1 year of age, and rotavirus was not identified in children older than 5 years. It should be noted that the group of children over 5 years old was the smallest in this study and that parents of these children were less likely to seek medical attention. These results are in agreement with those of previous studies in other countries (1, 5, 11, 17). However, we found leukocytes in 15.8% of rotavirus-infected stools, always in children less than 2 years of age. Few other observations have been made on this subject; Mutanda in Kenya found leukocytes in 73% of the stools of diarrheic rotavirus-infected children (16), although Ryder in Bangladesh found no leukocytes in similar cases (21).

In temperate countries the highest incidence of rotavirus has been observed during winter (4, 11); however, in many developing countries, such as the CAR, the fluctuations of temperature are not significant (24). In Bangui we found a low incidence of rotavirus isolation during the rainy season from May to September. Other investigators have found this correlation (1, 18). Although temperatures are different from those of the CAR, Walker (A. C. Walker and W. C. Marshall, personal communication) found a peak incidence

TABLE 3. Distribution of 1,197 cases of diarrhea and number of rotavirus and *C. jejuni* isolations during the study period of March 1981 through February 1982

Yr and mo	Pathogen		
	Rotavirus	*C. jejuni*	Total
1981			
March	14	0	119
April	4	0	88
May	9	8	74
June	4	17	83
July	5	12	98
August	0	11	59
1982			
September	2	13	97
October	14	15	84
November	48	10	112
December	37	22	143
January	33	10	137
February	41	11	103

TABLE 4. Major clinical symptoms associated with the most frequently isolated enteric pathogens

Enteropathogen (no. of incidences)	% of diarrheic children with following Symptom:			
	Bloody stool	Vomiting	Fever	Dehydration
Rotavirus (211)	0.4	75.3	67.7	45.4
Campylobacter jejuni (129)	3.1	55.8	64.3	24.8
EPEC (99)	4.4	63.7	53.9	42.4
Entamoeba histolytica (66)	28.7	31.8	50	18.1
ETEC (41)	0	55.5	55.5	33.3
Salmonella spp. (41)	17	60.9	63.4	29.2
Shigella spp. (33)	30.3	48.4	63.6	30.3

TABLE 5. Pathogens most frequently associated with the presence of leukocytes

Enteropathogen	No. of positive stools	No. of samples with leukocytes (%)
Shigella spp.	22	18 (81.8)
Entamoeba histolytica	54	26 (48.1)
Salmonella spp.	26	12 (46.1)
Campylobacter jejuni	80	32 (40)
Rotavirus	179	27 (15.8)
EPEC	73	8 (10.9)
Campylobacter jejuni plus rotavirus	17	8 (47)

during the rainy season in Darwin, Australia, and Stintzing found two peaks in Addis Ababa, one in the rainy season and one in the dry season (24). Further studies need to be conducted over a period of several years to resolve this apparent discrepancy.

Although some controversy surrounds the role of EPEC strains in diarrheal disease, there is evidence that this pathogen is emerging as a problem in developing countries. Koornhof, in South Africa, has reported that EPEC strains were more frequently recovered from patients with diarrhea than from matched control subjects (12). These EPEC strains were the predominate enteropathogen in black infants in urban areas of South Africa (12). In a more recent study in Sao Paulo, Brazil, it was found that EPEC strains were the most frequent agents of diarrhea in children 0 to 5 months of age (26). Our data support these reports and suggest a need for more thorough studies on the epidemiology of these strains.

C. jejuni was the third most frequently isolated pathogen found in children with diarrhea in the CAR. It was found most often in patients between 6 and 17 months of age, and 40% of *C. jejuni*-infected stools contained leukocytes. In tropical countries, seasonal fluctuations of *C. jejuni* have not been well studied. De Mol has observed a higher incidence of this agent in Zaire during the wet season (P. De Mol, D. Brasseur, S. Lauwers, G. Zissis, and J. P. Butzler, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 696, 1980). We have confirmed this observation during our year of study; 14.8% of stools were found to be positive from May to September, whereas 8.6% were positive from October to May. In the dry months of March and April 1981, no *C. jejuni* was isolated.

In children over the age of 2 years, the enteric pathogens most frequently associated with diarrhea were the parasitic agents *Entamoeba histolytica* and *Schistosoma mansoni*.

Although all the samples were not tested for ETEC, many of the samples from each month were investigated; their incidence was very low. These results are quite different from those found in many developing countries, where ETEC is often the most frequent enteric pathogen identified, as follows: 28% in Bangladesh (1), 11% in the Philippines (8), and 12% in Addis Ababa (24). As in the South African study, diarrheal disease in the CAR appears to be a complex and multifaceted problem (12).

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