*Am. J. Trop. Med. Hyg.*, 59(6), 1998, pp. 1008–1014


# ETIOLOGIES OF ACUTE, PERSISTENT, AND DYSENTERIC DIARRHEAS IN ADULTS IN BANGUI, CENTRAL AFRICAN REPUBLIC, IN RELATION TO HUMAN IMMUNODEFICIENCY VIRUS SEROSTATUS

YVES GERMANI, PIERRE MINSSART, MICHEL VOHITO, SIMON YASSIBANDA, PHILIPPE GLAZIOU, DIDIER HOCQUET, PIERRE BERTHÉLÉMY, AND JACQUES MORVAN

*Institut Pasteur de Bangui, Bangui, Central African Republic; Hôpital Communautaire, Bangui, Central African Republic; Hôpital de l'Amitié, Bangui, Central African Republic*

*Abstract.* A study of the etiologies of diarrhea in adults in relation to their human immunodeficiency virus (HIV) serostatus and number of CD4+ cells was carried out in the Central African Republic. In cases and controls, multiparasitism was observed. *Salmonella* spp. were identified mainly during acute diarrhea, with 50% of the *S. enteritidis* isolated during the study being responsible for septicemia and/or urinary tract infection in immunodeficient patients. Enteroaggregative *Escherichia coli* (EAggEC) were the most frequently identified agent in HIV+ patients with persistent diarrhea; 42.8% of the patients with EAggEC as sole pathogens had bloody diarrhea, and these strains were negative for the presence of a virulence plasmid. Coccidia were found in those with acute and persistent diarrhea. Blood was observed in 53.3% of infections involving coccidia as the sole pathogen. *Microsporidium* spp. and *Blastocystis hominis* were found only in HIV+ patients with persistent diarrhea. *Shigella* spp., *Campylobacter* spp., and *Entamoeba histolytica* were found in HIV+ and HIV− dysenteric patients; bacteria resembling spirochetes that could not be cultivated were identified only in HIV+ cases with dysentery. Shiga-like toxin-producing *E. coli* O157:H− was isolated from two cases with hemolytic-uremic syndrome. Fungi were identified as the sole pathogen in 6.4% of the HIV+ patients with persistent diarrhea. Most of enteropathogenic bacteria identified were resistant to ampicillin and trimethoprim-sulfamethoxazole, remained susceptible to ampicillin plus clavulanic acid, and were susceptible to amikacin, gentamicin, and ciprofloxacin.

Diarrhea is one of the hallmarks of advanced human immunodeficiency virus (HIV) disease, and in Africa, diarrhea occurs in 60–90 % of those with HIV infection.[1] The Central African Republic has been strongly affected by the HIV epidemic.[2] In this country, nearly 72% of the adults with acquired immunodeficiency syndrome (AIDS) present initially with diarrhea. This condition is a serious public health problem affecting the quality of life and contributing to the HIV disease outcome. Among the wide array of microorganisms that may produce enteric infections in HIV-positive persons,[3] only bacteria, parasites, and fungi are relevant to antimicrobial treatments. In The Central African Republic, the etiologies and epidemiology of diarrheal diseases in adults remain undefined. Antibiotics recommended by the World Health Organization (United Nations–AIDS) and the Central African Republic Ministry of Health for the empiric treatment of diarrhea are ineffective and suggest that monitoring sensitivity to antibiotics in this country is necessary for optimum selection of effective antibiotics and elimination of those with little therapeutic value. Consequently we conducted a survey in Bangui among HIV-seropositive (HIV+) and -seronegative (HIV-) subjects to identify major types and prevalences of enteric pathogens in adult patients, and patterns of resistance to antibiotics of enteropathogenic bacteria, with the aim to provide guidance to physicians for case management.

## POPULATION, MATERIALS, AND METHODS

**Study population.** Stool specimens were collected from patients > 18 years old who presented with diarrhea to the Hôpital Communautaire, Hôpital de l'Amitié in Bangui or the Institut Pasteur de Bangui from October 1995 to December 1996. Chronic diarrhea was defined as an average of three or more loose or watery bowel movements per day for at least two weeks before the study. Acute diarrhea was defined as an episode that lasted less than 14 days. Dysentery was defined as diarrhea with the presence of blood and pus in stools regardless of duration. Bloody diarrhea was defined as liquid stools with the presence of blood visible either by the naked eye or microscopically. A control group was recruited among HIV− and HIV+ adults selected from individuals who had no history of a diarrheal illness during the preceding month and were hospitalized during the study period. Controls were not matched to cases. All subjects underwent a complete physical examination. Patient details and histories were recorded by a physician on a standardized code sheet. This covered civil status, address, sexual practices, contacts with animals, diet and description of meals previous to acute diarrhea or dysentery, and the source of the household's drinking water. Criteria for a definition of AIDS were those defined by the Centers for Disease Control and Prevention (Atlanta, GA).[4] A clinical score for AIDS was defined by the Central African Republic AIDS Program (Bangui, Central African Republic), with values ranging from 0 to 38. A score of 14 or more is the threshold for clinical AIDS definition in the Central African Republic. All aspects of the study were approved by the *ad hoc* Ethical Committee of the Central African Republic AIDS Program and prior consent to participate in the study was obtained from the subjects.

**Laboratory techniques.** Serum antibodies to HIV were detected by two enzyme immunoassays (Genelavia Mixt; Sanofi-Diagnostics Pasteur, Marnes la Coquette, France and Vironostika HIV Uni-form II; Organon Teknika, Fresnes, France) and confirmed by Western blotting (New Lav Blot; Sanofi Diagnostics Pasteur). CD4 lymphocyte counts were undertaken using Dynabeads T4 Quant technology (Dynal, Oslo, Norway).

All cases and controls had stool specimens collected and

TABLE 1
Oligonucleotide primers used to differentiate pathogenic enteric *Escherichia coli**

| Target gene (enteropathogen) | Primer sequences | Location (bp) from 5′ end | Amplicon size (bp) |
|---|---|---|---|
| BFP gene (EPEC) | 5′CAATGGTGCTTGCGCTTGCT3′<br>5′GCCGCTTTATCCAACCTGGT3′ | 119–138<br>443–422 | 325 |
| *eaeA* (EPEC) | 5′GCAAATTTAGGTGCGGGTCAGCGTT3′<br>5′GGCTCAATTTGCTGAGACCACGGTT3′ | 2412–2436<br>2905–2881 | 494 |
| LT gene (ETEC) | 5′GCGACAAATTATACCGTGCT3′<br>5′CCGAATTCTGTTATATATGT3′ | 59–76<br>765–746 | 707 |
| STa gene (ETEC) | 5′CTGTATTGTCTTTTTCACCT3′<br>5′GCACCCGGTACAAGCAGGAT3′ | 79–98<br>260–241 | 182 |
| *ipaH* (EIEC) | 5′GCTGGAAAAACTCAGTGCCT3′<br>5′CCAGTCCGTAAATTCATTCT3′ | 1061–1080<br>1484–1465 | 424 |
| EAggEC | 5′CTGGCGAAAGACTGTATCAT3′<br>5′CAATGTATAGAAATCCGCTGTT3′ | 1–64<br>765–693 | 630 |
| SLT1 | 5′GAAGAGTCCGTGGGATTACG3′<br>5′AGCGATGCAGCTATTAATAA3′ | 1191–1210<br>1301–1320 | 130 |
| SLT2 | 5′TTAACCACACCCACGGCAGT3′<br>5′GCTCTGCATGCATCTCTGGT3′ | 426–445<br>752–771 | 346 |
| AFA (DAEC) | 5′GCTGGGCAGCAAACTGATAACTCTC3′<br>5′CATCAAGCTGTTTGTTCGTCCGCCG3′ | 889–914<br>122–146 | 750 |

* bp = basepairs; BFP = bundle-forming pili; EPEC = enteropathogenic *E. coli*; *eaeA* = *E. coli* attaching and effacing gene A; LT = heat-labile enterotoxin; ETEC = enterotoxigenic *E. coli*; STa = heat-stable enterotoxin; *ipaH* = invasion plasmid H; EIEC = enteroinvasive *E. coli*; EAggEC = enteroaggregative *E. coli*; SLT1 = Shiga-like toxin 1; SLT2 = Shaga-like toxin 2; AFA = afimbrial adhesin; DAEC = diffuse adherent *E. coli.*

processed in the same way. No transport media were used; specimens were quickly examined in the laboratory after collection. Specimens were used for cultures of bacteria and fungi with appropriate media and culture conditions.[5] Wet mount preparations of fresh stool samples were examined immediately for motile spirochetes, trophozoites, red blood cells, and leukocytes by dark-field microscopy. Formalin-ether concentrates and smears stained with merthiolate-iodine-formaldehyde solution were prepared from each of the specimens and examined microscopically. Examination of the fecal specimen by the Baermann procedure was performed. *Microsporidia* were detected by a modified trichrome stain with a high concentration of chromotrope 2 R.[6] Stool specimens were also used in the three-step detection method for coccidia.[7] The modified Kinyoun acid fast stain was used for detection of mycobacteria.

Stools were cultured daily for *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Aeromonas* spp., *Plesiomonas* spp., *Vibrio* spp., *Campylobacter* spp., fungi and identified by standard methods.[5] Colonies resembling *Campylobacter* species growing on Skirrow modified plates were subjected only to a Gram's stain and catalase and oxidase tests for confirmation of the genus. In the case of motile, flexible, and spiral rods observed by dark-field microscopy, spirochetes were isolated on 5% sheep blood tryptic casein soy agar supplemented with 10% of fetal calf serum, spectinomycin (400 μg/ml), polymyxin B (5 μg/ml) and neomycin (30 μg/ml) under anaerobic conditions at 37°C for 21 days and examined for colonies or surface film. Diagnosis of infection with *Clostridium difficile* was performed by demonstration of cytotoxin in stool supernatants; specificity of the cytopathic effect on HEp-2 cells was shown by neutralization with antisera to *C. sordellii*.[8] Detection of *C. perfringens* enterotoxin in stool specimens was used for the diagnosis of *C. perfringens* enteritis.[9] Rotavirus were identified with a commercial latex agglutination test (Sanofi-Diagnostics Pasteur). *Escherichia coli* isolates were stored at 4°C on tryptic soy agar for later testing and processed within two months of the date of collection. *Escherichia coli* isolates were screened for virulence factors. Differentiation of pathogenic *E. coli* strains was performed using the polymerase chain reaction (PCR). For screening purposes, bacterial colonies from each patient were pooled for template DNA preparation.[10] Oligonucleotide primers used to detect enterotoxigenic (ETEC),[11–13] enteroinvasive (EIEC),[14] enterohemorrhagic (EHEC),[15] enteropathogenic (EPEC),[16,17] enteroaggregative (EAggEC),[18] and diffuse adherent (DAEC)[19] *E. coli* by PCRs are shown in Table 1. In the case of a positive result, colonies were individually tested. Toxin-producing strains as determined by PCR were then retested by the GM1-ELISA for heat-labile enterotoxin (LT),[20] the Gb3-ELISA for Shiga-like toxin (SLT),[21] and the biotin-ELISA for heat-stable enterotoxin (ST).[22] All *Escherichia coli* isolates were examined for HEp-2 cell adherence in eight-well chamber slides (Lab-Tek; Nunc, Inc., Naperville, IL);[23] HEp-2 cell adherence was characterized as localized (LA), aggregate (AA), or diffuse (DA) adherence. The antimicrobial susceptibility of bacterial enteropathogens was determined using the disk diffusion test.[24]

**Statistical analysis**. Patients were not paired according to the date of hospitalization due to difficulties of recruitment during several months of military rebellion or civil unrest in Bangui. For this reason, group sizes differed largely. Patients were classified according to their type of diarrhea (four groups: acute, persistent, dysenteric, and no diarrhea), and to their HIV serostatus. Distribution of each pathogen according to patient groups was not statistically compared due to the requirement of a high value of the threshold for chi-square statistics for a 0.05 type 1 error level when taking into account multiple comparisons to perform in many subgroups. This resulted in an extremely low power of the tests.

TABLE 2
Characteristics of studied populations*

| | Acute diarrheas | | Persistent diarrheas | | Dysenteries† | | Controls | |
|---|---|---|---|---|---|---|---|---|
| | HIV-positive | HIV-negative | HIV-positive | HIV-negative | HIV-positive | HIV-negative | HIV-positive | HIV-negative |
| Number | 51 | 79 | 110 | 27 | 9 | 14 | 73 | 67 |
| Mean age (years) | 33.6 | 31.3 | 27.8 | 50.3 | 33.8 | 37 | 29.4 | 30.2 |
| Sex ratio (male/female) | 1.2 | 1.2 | 1.2 | 0.8 | 5 | 1.8 | 1 | 0.8 |
| CD4 cell counts/ml (geometric mean) | 160 | 426 | 79 | 427 | 124 | 462 | 347 | 485 |
| Score for AIDS (geometric mean) | 8 | 0 | 17 | 0 | 24 | 0 | 0 | 0 |

* HIV = human immunodeficiency virus; AIDS = acquired immunodeficiency syndrome.
† The mean duration of dysenteric diarrhea in patients before inclusion in the study was 10.7 days (range = 9–13) in HIV-seropositive patients and 10.8 (range = 9–18) days in HIV-seronegative patients.

Means were compared using analysis of variance after log transformations when required.

## RESULTS

A total of 430 adults participated: 290 cases (67.4%) with diarrhea and 140 controls (32.6%). Distribution of patients according to the type of diarrhea and HIV serostatus is shown in Table 2. Mean age differed significantly between groups ($P < 10^{-5}$) but the sex distribution was not different. The geometric mean CD4 cell counts were significantly lower in the HIV+ groups ($P < 0.01$). AIDS-related symptoms were observed in all patients with CD4 cell counts < 283/ml. All subjects were heterosexual, none were intravenous drug users or received blood products. Overall, 53% of the cases had received ampicillin or trimethoprim-sulfamethoxazole before the stool sample was submitted. In each group of patients with diarrhea, the percentages of potential microbial enteropathogens identified in subjects who reported taking antibiotics were not significantly different from those in subjects who had not received antibiotics. Identified pathogens are shown in Table 3. No *Plesiomonas* spp., *Aeromonas* spp., *Vibrio* spp., *Yersinia* spp., EIEC, *C. perfringens*, or *Mycobacterium* spp. were found during the study.

*Cryptosporidium* spp. was the most frequently identified microorganism in controls (13.4 %), but was never observed in HIV+ asymptomatic subjects. Asymptomatic carriage with *S. bovis morbificans* was observed in two HIV+ and four HIV− controls, respectively; one *S. typhimurium* was isolated in an asymptomatic HIV− subject; four of the *S. bovis morbificans* (from one HIV+ and three HIV− subjects) and *S. typhimurium* were isolated after enrichment procedures. All of the HEp-2 cell-adherent *Escherichia coli* isolated in control groups were negative when tested by PCR.

TABLE 3
Etiologies of acute, dysenteric, and persistent diarrheas among adults in relation with their human immunodeficiency virus (HIV) serostatus

| | Acute diarrhea n = 130 (%) | | Dysenteries n = 23 (%) | | Persistent diarrhea n = 137 (%) | | Controls n = 140 (%) | |
|---|---|---|---|---|---|---|---|---|
| Enteropathogen | HIV-positive n = 51 (39.2) | HIV-negative n = 79 (60.7) | HIV-positive n = 10 (43.4) | HIV-negative n = 13 (56.5) | HIV-positive n = 110 (80.3) | HIV-negative n = 27 (19.7) | HIV-positive n = 73 (52.1) | HIV-negative n = 67 (47.9) |
| *Salmonella* spp. | 14 (27.4) | 20 (25.3) | 0 | 0 | 0 | 1 (3.7) | 2 (2.7) | 5 (7.4) |
| *Shigella* spp. | 0 | 0 | 2 (20)* | 5 (38.4)† | 0 | 0 | 0 | 0 |
| *Campylobacter* spp. | 0 | 0 | 1 (10)‡ | 2 (15.3)‡ | 0 | 2 (7.4) | 0 | 0 |
| *Clostridium difficile* | 1 (1.9) | 0 | 0 | 0 | 1 (0.9) | 1 (3.7) | 0 | 0 |
| Spirochetaceae | 0 | 0 | 4 (40) | 0 | 0 | 0 | 0 | 0 |
| HEp2-adherent *Escherichia coli* | | | | | | | | |
| Localized | 0 | 0 | 0 | 0 | 2 (1.8) | 0 | 0 | 0 |
| Diffuse | 0 | 0 | 0 | 0 | 9 (8.2) | 0 | 1 (1.4) | 3 (4.5) |
| Aggregative | 1 (1.9) | 0 | 0 | 0 | 14 (12.7) | 0 | 0 | 0 |
| Enterotoxigenic *E. coli* | 2 (3.8)§ | 1 (1.3)¶ | 0 | 0 | 0 | 0 | 0 | 0 |
| Enterohemorragic *E. coli* | 0 | 2 (2.5) | 0 | 0 | 0 | 0 | 0 | 0 |
| *Entamoeba histolytica* | 1 (1.9) | 3 (3.8) | 2 (20) | 2 (15.3) | 0 | 0 | 0 | 0 |
| *Giardia lamblia* | 0 | 3 (3.8) | 0 | 0 | 1 (0.9) | 0 | 0 | 0 |
| *Schistosoma mansonii* | 0 | 0 | 0 | 0 | 0 | 3 (11.1) | 0 | 0 |
| *Cryptosporidium* spp. | 0 | 2 (2.5) | 0 | 0 | 7 (6.4) | 0 | 0 | 9 (13.4) |
| *Isospora belli* | 0 | 1 (1.3) | 0 | 0 | 3 (2.7) | 0 | 0 | 0 |
| *Cyclospora cayetanensis* | 0 | 2 (2.5) | 0 | 0 | 5 (4.5) | 0 | 0 | 0 |
| *Microsporidium* spp. | 0 | 0 | 0 | 0 | 6 (5.4) | 0 | 0 | 0 |
| *Blastocystis hominis* | 0 | 0 | 0 | 0 | 4 (3.6) | 0 | 0 | 0 |
| *Strongyloides stercoralis* | 0 | 0 | 0 | 0 | 2 (1.8) | 0 | 0 | 0 |
| Fungi | 0 | 0 | 0 | 0 | 7 (6.4) | 0 | 0 | 0 |
| Multiple pathogens | 9 (17.6)# | 1 (1.3)# | 1 (10)# | 4 (30.8)# | 7 (6.4)# | 0 | 28 (38.4) | 38 (56.7) |
| Total | 28 (54.9) | 35 (44.3) | 10 (100) | 13 (100) | 68 (61.8) | 7 (25.9) | 31 (42.5) | 55 (82.1) |

* One *S. dysenteriae* and one *S. flexneri* were also involved in septicemia.
† Five *S. dysenteriae*.
‡ All strains were resistant to cefalotin but not to nalidixic acid.
§ One heat-stable enterotoxin (ST)– and one heat-labile enterotoxin–producing strain.
¶ One ST-producing strain.
# Mixed infectious diarrhea always involved parasites observed in the corresponding control groups.

TABLE 4

Etiologies of 66 (47.1%) asymptomatic infections with more than one pathogen in control stools*

| Parasites | HIV seropositive n = 38 (%) | HIV seronegative n = 28 (%) |
|---|---|---|
| Flagellates | | |
| *Dientamoeba fragilis* (trophozoites) | 11 (28.9) | 6 (21.4) |
| *Trichomonas intestinalis* (trophozoites) | 15 (39.5) | 4 (14.3) |
| *Chilomastix mesnili* (cysts) | 15 (39.5) | 2 (7.1) |
| Amebae | | |
| *Entamoeba histolytica* (cysts) | 12 (31.6) | 14 (50) |
| *Entamoeba hartmani* (cysts) | 3 (7.9) | 4 (14.3) |
| *Entamoeba coli* (cysts) | 6 (15.8) | 5 (17.8) |
| *Endolimax nana* (cysts) | 8 (21.1) | 8 (28.6) |
| *Iodamoeba bütschlii* (cysts) | 4 (10.5) | 9 (32.1) |
| Helminths | | |
| *Ascaris lumbricoides* (eggs) | 26 (68.4) | 11 (39.3) |
| *Trichuris trichura* (eggs) | 21 (55.3) | 14 (50) |
| *Enterobius vermicularis* (eggs) | 29 (76.3) | 15 (53.4) |
| *Taenia saginata* (eggs) | 6 (15.8) | 3 (10.7) |
| *Strongyloïdes stercoralis* | 0 | 3 (10.7) |
| Coccidia | | |
| *Cryptosporidium* spp. | 0 | 16 (57.1) |

* HIV = human immunodeficiency virus.

Asymptomatic infections with more than one pathogen occurred only with parasites and are shown in Table 4. Of 73 HIV+ controls, nine (12.3 %) were infected with six parasites and 39.7% with 3–5 parasites. Except for *Strongyloides stercoralis* and *Cryptosporidium* spp., flagellates, amebae (cysts) and helminths (eggs) shown in Table 4 were also observed in patients. Multiparasitism with microorganisms shown in Table 4 was diagnosed in 8.5% (five HIV+ and six HIV− cases), 21.7% (two HIV+ and three HIV− cases), and 20.4% (12 HIV+ and 16 HIV− cases) of patients with acute, dysenteric, and persistent diarrhea, respectively.

*Salmonella* spp. were the most frequently identified microorganism during acute diarrhea (i.e., in 34 patients [25.2%] as the sole pathogen and in five patients [3.7%] with multiple infections); among patients with persistent diarrhea, *S. typhimurium* was isolated from one HIV− patient with diarrhea lasting 16 days. In HIV+ patients with acute diarrhea, *S. enteritidis* was found in eight fecal specimens as the sole pathogen and from three fecal specimens in association either with Rotavirus (two cases) or *Giardia intestinalis* (one case). *Salmonella enteritidis* was also a cause of urinary tract infection, septicemia, or both in two, five, and one HIV+ individuals with acute diarrhea, respectively. These eight infections were observed in patients with CD4 cell counts ranging from 44 to 195 (median = 68)/ml. In HIV− patients with acute diarrhea, *S. typhimurium* was found in seven fecal specimens as the sole pathogen; *S. enteritidis* was identified from patients in 13 cases as the sole pathogen and in one case in association with Rotavirus. In this group, four septicemia to *S. enteritidis* were observed only in patients with CD4 cell counts ranging from 381 to 481 (median = 387)/ml. Septicemia with *S. enteritidis* was also diagnosed in a patients with dysentery with an *S. enteritidis* and *Cryptosporidium* spp. in stools and a CD4 cell count of 81/ml.

*Shigella* spp. were the major bacterial cause of dysentery. However, motile, flexible, and spiral rods resembling spirochetes were observed in four HIV+ patients with dysentery (fever, gross bloody diarrhea, abdominal pain) and CD4 cell counts ranging from 24 to 127/ml; in all cases, 50–100 motile elements per field were observed microscopically; no culture was obtained after 21 days of incubation. *Campylobacter* spp. and *Entamoeba histolytica* were also found in dysenteric stools of HIV+ patients, either as the sole pathogen or in mixed infectious diarrhea (*Campylobacter* spp. on one occasion and *Entamoeba histolytica* on three occasions).

Non-motile EHEC strains that belong to serogroup O157 were isolated from two patients with acute bloody diarrhea and fatal hemolytic-uremic syndrome. These patients did not live in the same household. A DNA fragment corresponding to the amplified VT1 toxin gene fragment was obtained in both cases and the Gb3-ELISA confirmed the production of toxin at high levels. Excluding multiple infections (two HIV+ patients were infected with EAggEC and two others with *E. coli* expressing LA, all with parasites observed in controls), HEp-2 cell-adherent *E. coli* were isolated from 18.2% (25 of 137) patients with chronic diarrhea compared with 0.8% (1 of 130) of patients with acute diarrhea and with 2.8% (4 of 140) of asymptomatic subjects. Among the HEp-2 cell-adherent *E. coli*, mainly aggregative and diffuse adherent patterns were represented. In HIV+ patients with persistent diarrhea, the isolation rates of EAggEC and DAEC were higher (14 of 110, 12.7% and 9 of 110, 8.2%, respectively) than in the corresponding asymptomatic groups (0 of 73, and 1 of 73, 1.4%, respectively). Among patients with EAggEC in their stools as the sole potential pathogen, 42.8 % (6 of 14) had bloody diarrhea. In the HIV+ case with acute diarrhea, EAggEC identified by the HEp-2 cell test were isolated in pure culture from an episode of bloody diarrhea lasting 12 days. EAggEC isolates from four episodes of bloody diarrhea and two of non-bloody diarrhea caused the detachment of the cell monolayer from the glass when tested in the HEp-2 cell adhesion assay; none of these strains produced hemolysin. All the patients with EAggEC, locally adhering *E. coli*, or DAEC in their stools, either with single or multiple infections, had low CD4 cell counts ranging from 25 to 70 (median = 32)/ml. All the DAEC isolated in patients and controls were afimbrial adhesin–negative when tested by the PCR. All *E. coli* strains with localized adherence patterns were (bundle-forming pilus) *bfp* negative, indicating these were not classic EPEC strains. All EAggEC strains were negative when tested by the PCR.

Fungi were considered as potential enteric pathogens when no other known enteropathogenic agent was identified. *Histoplasma* spp. and *Candida* spp. were identified in two (1.8%) and five (4.5%) HIV+ patients with chronic diarrhea, respectively, all seven had CD4 cell counts ranging from 31 to 40 (median = 36)/ml. Both *Histoplasma* spp. and *Candida* spp. represented 20–60% of the microorganisms observed per microscopic field. Coccidia were identified as the sole pathogens in five HIV− and immunocompetent patients with acute watery diarrhea; their symptoms stopped after a mean of 18 days of symptomatic therapy. Coccidia were also identified with the same symptoms in HIV+ but immunocompetent patients with mixed infections (two *Cryptosporidium* spp. and three *Cyclospora cayetanensis*) involving flagellates and helminths also observed in corresponding controls. In persistent diarrheas, coccidia were isolated as the sole patho-

TABLE 5
Antibiotic susceptibility patterns of bacterial enteropathogens isolated in Bangui*

| Isolates and characteristics (no. tested) | % Resistant to antibiotics† | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AN | AM | AMC | ATM | CRO | CF | C | TE | GM | NA | PEF | SXT |
| *Salmonella* spp. (49) | 0 | 94 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 77.5 |
| *Shigella* spp. (7) | 0 | 71 | 0 | 14 | 0 | 57 | 86 | 100 | 0 | 0 | 0 | 86 |
| *Campylobacter* spp. (6) | 0 | 83 | 17 | 100 | 83 | 100 | 0 | 0 | 0 | 0 | 0 | 100 |
| Enterotoxigenic *Escherichia coli* (3) | 0 | 67 | 0 | 0 | 0 | 67 | 33 | 100 | 0 | 33 | 0 | 100 |
| HEp2-adherent *E. coli* | | | | | | | | | | | | |
| Localized (4) | 0 | 50 | 0 | 25 | 0 | 50 | 25 | 75 | 0 | 0 | 0 | 100 |
| Diffuse (13) | 0 | 54 | 0 | 38 | 0 | 80 | 30 | 85 | 0 | 0 | 0 | 80 |
| Aggregative (18) | 0 | 89 | 0 | 5 | 0 | 83 | 28 | 83 | 0 | 0 | 0 | 83 |
| Enterohemorragic *E. coli* (2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Isolates from both diarrhea patients and controls are included.
† AN = amikacin; AM = ampicillin; AMC = amoxicillin plus clavulanic acid; ATM = aztreonam; CRO = ceftriaxone; CF = cefalotin; C = chloramphenicol; TE = tetracycline; GM = gentamicin; NA = nalidixic acid; PEF = pefloxacin; SXT = trimethoprim-sulfamethoxazole.

gen from 13.6% (15 of 110) of HIV+ patients and from 2.7% (3 of 110; i.e., one *Cryptosporidium* spp. and two *Cyclospora cayetanensis*) of HIV+ patients with 42, 70, and 61 CD4 cells/ml and mixed infections. Among patients with persistent diarrhea, excluding mixed infectious diarrhea, coccidia, *Microsporidium* spp. and *B. hominis* represented 96.1% (25 of 26) of parasitic etiologies in HIV+ patients that were never observed in HIV− patients with chronic diarrhea or in the corresponding control group; blood was observed in 53.3% (8 of 15, i.e., five *Cryptosporidium* spp. and three *Cyclospora cayatenensis*) of infections involving coccidia as the sole pathogens. These parasites were identified from subjects with CD4 cell counts ranging from 28 to 55 (median = 42)/ml whereas the other seven patients with nonbloody diarrhea had CD4 counts ranging from 281 to 298 (median = 292)/ml.

Table 5 shows the antibiotic susceptibility of the 102 bacterial enteropathogens recovered from both cases and asymptomatic subjects. Essentially, all isolates were susceptible to amikacin, gentamicin, and ciprofloxacin. With the exception of *Campylobacter* strains, all isolates were also susceptible to broad-spectrum cephalosporins. *Salmonella* spp., *Shigella* spp., HEp-2 cell adherent *E. coli*, and ETEC were highly resistant to ampicillin and trimethoprim-sulfamethoxazole; interestingly, all these species remained susceptible to ampicillin plus clavulanic acid (β-lactamase inhibitor).

## DISCUSSION

In Bangui, where 20% (i.e., approximately 500 000 inhabitants) of the population of the Central African Republic lives, persistent diarrhea is a major component of diarrhea morbidity in HIV+ adults. No reason was found for the much older age for the HIV group with persistent diarrhea. An interesting finding is that the majority of enteropathogenic bacteria occurred at higher frequencies in patients than in controls. However, the prevalence of infection in controls for *Salmonella* spp. and *E. coli* with a DA phenotype suggests that subjects are frequently exposed to these pathogens. Another interesting finding is that the majority of parasites occurred at similar frequencies in both patients and controls with mixed infections; except for *Cryptosporidium* spp. and *Strongyloides stercoralis* in HIV+ patients, caution is indicated for interpreting the pathogenic role of flagellates, amebae, and helminths shown in Table 4. Nonetheless, *Cryptosporidium* spp. and *Strongyloides stercoralis* must be considered as potential causes of diarrhea in immunocompromised hosts.

*Salmonella enteritidis* or *S. typhimurium* most often cause acute diarrheas; individuals with AIDS have been shown to be at higher risk than the general population for *Salmonella* spp. infections. An almost equivalent number *of S. enteritidis* and *S. typhimurium* isolates was found in fecal specimens from HIV+ or HIV− individuals, but all septicemia and urinary tract infection cases in both HIV+ and HIV− patients were caused by *S. enteritidis*. Interestingly, it has also been observed that certain strains of *S. enteritidis* were able to cause septicemia in HIV+ individuals in New York.[25,26] This serotype was never found in control groups and the question as to why this serotype, which is a strict pathogen in our study, causes diarrhea, septicemia, and urinary tract infections is of interest. These strains should be examined to identify virulence factors that overcome the defense mechanisms of individuals with significant or moderate immunodepression.

Motile, flexible, and spiral rods observed in HIV+ and immunocompromised patients with dysentery closely resembled spirochetes; these are presumably opportunistic pathogens because 1) colonization of the intestine was heavy regarding the number of bacteria observed per field in each patient, 2) symptoms were always severe and one infected patient died of the consequences of the dysentery, 3) these microorganisms were never observed in control groups or immunocompetent patients, and 4) no other enteric pathogen was found. Cultures were negative presumably because the optimal conditions were not available, but potential agents resembling spirochetes that could not be cultivated could not be excluded.

Enterohemorrhagic *E. coli* producing SLT toxin has never been identified before in the Central African Republic. Considering that bloody diarrhea outbreaks due to *E. coli* O157 were recently observed in eastern and southern parts of Africa,[27,28] this bacteria should be considered to be a putative causative agent of hemorrhagic colitis epidemics in the Central African Republic. Our report presents the first description in this country of non-EPEC strains with different patterns of adherence to HEp-2 cells in adults with diarrhea. The HEp-2 cell adherent *E. coli* were previously identified in adults with HIV-associated diarrhea in Zambia.[29] This

study revealed an association between the isolation of EAggEC and persistent diarrhea in HIV-infected patients, an association previously suspected in an HIV-infected adult in the United States.[30] An aggregative adherence pattern is associated with the presence of large plasmids closely linked to the virulence mechanism (fimbriae and toxin).[23] The PCR targeting the plasmid was not successful in screening for the presence of EAggEC. These EAggEC strains should be further investigated. The AA phenotype may be pertinent to their capacity to cause illness or may be a marker for a yet unknown virulence factor in these strains. The EAggEC have been recognized as a cause of persistent diarrhea in HIV-infected infants in countries that have common borders with the Central African Republic[31] and in several other countries.[32–34] In the Central African Republic, EAggEC strains were found only in HIV+ immunodeficient adults who had no other enteric pathogen and should be considered an opportunistic agent whenever isolated from diarrhea stools of AIDS patients. Conversely, more caution seems indicated for interpreting the pathogenic role of *E. coli* with localized or diffuse adherence phenotype encountered in this study. Nearly one-third of the EAggEC were isolated from bloody diarrheas; this was also observed in an HIV-noninfected infant in Mexico.[33] Six strains harbored a phenotype (cell-detaching *E. coli*) representing a putative new enteric virulent category of *E. coli* previously identified in Australia.[34] According to other studies, these observations point to the existence of different mechanisms by which EAggEC strains may cause disease exist.[29,33,35] These isolates are under investigation for their epidemiologic features.

The importance of coccidia as a widespread cause of diarrhea is now increasing.[36] Our findings suggest that coccidia should be considered etiologic agents for immunocompromised adults with persistent diarrhea and potential etiologic agents for immunocompetent adults with acute diarrhea. The prevalence of coccidia is similar to that observed in other developing African countries.[31–37] The prevalence of *Cryptosporidium* spp. in control patients is high. An asymptomatic carrier state has now been clearly demonstrated in numerous studies.[36] Among patients with coccidia, eight had bloody diarrhea and one had dysenteric symptoms. Fecal blood and leukocytes are rarely found in coccidial diarrhea, but some studies have reported their presence in a significant number of HIV-seropositive patients.[36–38] In the case of mixed infections with *S. enteritidis*, the role of coccidia in symptoms is difficult to interpret. In the other cases, bloody diarrheas occurred only in patients with low CD4 counts. Previous studies on the pathology and pathogenesis associated with coccidia found inflammatory changes, villous atrophy, and crypt hyperplasia in patients with diarrhea and coccidia-positive stool specimens.[39,40] These findings were not present in asymptomatic controls. The clinical manifestations of *Cryptosporidium* spp. infection seem to be influenced by the immune status of the HIV+ host and correlate with the CD4 counts.[36] Further investigations are needed, however, because symptoms may result from a combination of interactions between host, virulence factors, cofactors, and environment. According to surveys performed in Africa, microsporidia are exclusively reported in HIV-infected patients and associated with chronic, watery, and non-bloody but severe diarrhea.[41] No other potential enteric pathogen was observed in the patients studied and most died a few days after diagnosis, with significant weight loss.[41] Gastrointestinal symptoms were present in all patients with fungal infection and likely reflect disseminated histoplasmosis and candidosis in patients with severe immunodepression.

The enteric bacteria prevalent in Bangui displayed high levels of *in vitro* resistance to the antimicrobial drugs commonly used to treat infectious diarrhea and opportunistic infections in the Central African Republic, mainly ampicillin and trimethoprim-sulfamethoxazole. The *C. difficile* infections observed are presumably a consequence of the use of ampicillin in the three cases observed. The reasons for the resistance patterns observed are presumably the absence of antimicrobial susceptibility testing before antibiotherapy and the extensive use of antibiotics without controls. With the exception of chloramphenicol, in the case of salmonellosis, extended-spectrum cephalosporins, quinolones, aminoglycosides, and amoxicillin plus clavulanic acid were the agents tested to which most bacterial isolates were susceptible to *in vitro*. None of these antimicrobial agents are easily available in the Central African Republic, limiting the therapeutic options. The failure to find pathogens in 44.5% of the patients in this study may be due, in part, to the relative insensitivity for techniques used. Furthermore, several enteric pathogens were not searched for, repeated stool cultures were not performed, and diarrhea due to alternative mechanisms has not been explored.

Acknowledgments: We thank D. Azoumy, P. M. Binguima, J. P. Damboguina, X. Konamna, J. R. Mbecko, and R. Zenguela for excellent technical assistance. We are indebted to B. Garin, G. Baranton, and E. Fournie for laboratory assistance. We also thank the personnel of the Service de Gastro-entérologie (Hôpital de l'Amitié, Bangui), the Service de Médecine, and the Service de Chirurgie Orthopédique (Hôpital Communautaire) for help, and R. Ferrero for reading the manuscript.

Financial support: The work was partly supported by the Agence Nationale de Recherche sur le Syndrome d'Immunodéficience Acquise (SIDA) (grant no. 97085) and the Action Concertée Inter Instituts Pasteur (ACIP 1996).

Authors' addresses: Yves Germani, Institut Pasteur de Bangui, Laboratoire des Maladies Infectieuses Opportunistes, Boîte Postale 923, Bangui, Central African Republic. Pierre Minssart, Hôpital Communautaire, Service de Médecine, Boîte Postale 1377, Bangui, Central African Republic. Michel Vohito and Simon Yassibenda, Hôpital de l'Amitié, Service de Gastro-entérologie, Boîte Postale 2166, Bangui, Central African Republic. Philippe Glaziou, Institut Pasteur de Bangui, Unité d'Epidémiologie, Boîte Postale 923, Bangui, Central African Republic. Didier Hocquet, Institut Pasteur de Bangui, Laboratoire d'Analyses Médicales, Boîte Postale 923, Bangui, Central African Republic. Pierre Berthélémy, Hôpital Communautaire, Service de Chirurgie, Boîte Postale 1377, Bangui, Central African Republic. Jacques Morvan, Institut Pasteur de Bangui, Direction, Boîte Postale 923, Bangui, Central African Republic.

Reprint requests: Yves Germani, Institut Pasteur de Bangui, Laboratoire des Maladies Infectieuses Opportunistes, Boîte Postale 923, Bangui, Central African Republic.

REFERENCES

1. Bartlett JG, Belitsos PC, Sears CL, 1992. AIDS enteropathy. *Clin Infect Dis 15:* 979–982.
2. Massanga M, Ndoyo J, Hu DJ, Pau CP, 1996. A highly heter-

ogenous HIV-1 epidemic in the Central African Republic. *Emerg Infect Dis 2:* 222–224.
3. Guerrant RL, Hughes JM, Lima NL, Crane J, 1990. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. *Rev Infect Dis 12:* 41–50.
4. Center for Disease Control, 1993. Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Morb Mortal Wkly Rep 41:* 1–19.
5. Murray PR, Barron EJ, Pfaller MA, Tenover FC, Yolken RH, 1995. *Manual of Clinical Microbiology.* Sixth edition. Washington, DC: American Society for Microbiology.
6. Weber R, Bryan RT, Aven RL, 1992. Improved light-microsporidial detection of *Microsporidia* spores in tool and duodenal aspirates. *N Engl J Med 326:* 161–166.
7. Ma P, Soave R, 1983. Three-step stool examination for cryptosporidiosis in 10 homosexual men with protected watery diarrhea. *J Infect Dis 147:* 824–828.
8. Murray PR, Webber C, 1983. Detection of *Clostridium difficile* cytotoxin in HEp-2 and CHO cell line diagnosis. *Microbiol Infect Dis 1:* 316–333.
9. McLane B, Strouse RJ, 1984. Rapid detection of *Clostridium perfringens* type A. enterotoxin. *J Clin Microbiol 19:* 112–115.
10. Tornieporth NG, John J, Salgado K, De Jesus P, Latham E, Melo MLN, Gunzburg ST, Riley LW, 1995. Differentiation of pathogenic *Escherichia coli* strains in Brazilian children by PCR. *J Clin Microbiol 33*: 1371–1374.
11. Frankel G, Giron JA, Valmassoi J, Schoolnick GK, 1989. Multigene amplification: simultaneous detection of three virulence gene in diarrhoeal stool. Mol Microbiol 3: 1729–1734.
12. Riley LW, 1988. Infection diseases associated with *Escherichia coli.* Balows A, Hausler WJ, Ohashi M, Turano A, eds. *Laboratory Diagnosis of Infections Diseases. Principles and Practice.* First edition. Volume 1. New York: Springer-Verlag, 237–261.
13. Yamamoto T, Tamura T, Yokota T, 1984. Primary structure of heat-labile enterotoxin product by *Escherichia coli* pathogenic for humans. *J Biol Chem 259:* 5037–5044.
14. Venkatesan MM, Buysse JM, Kopecko DJ, 1989. Use of *Shigella flexneri Ipa.c* and *Ipa H* sequence for the general identification of *Shigella* spp. and enteroinvasive *Escherichia coli. J Clin Microbiol 27:* 2687–2691.
15. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KRL, 1990. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. *J Clin Microbiol 28:* 540–545.
16. Donnenberg MS, Giron JA, Nataro JP, Kaper JB, 1992. A plasmid-encoded type IV fimbrial gene of enteropathogenic *Escherichia coli* associated with localized adherence. *Mol Microbiol 6:* 3427–3437.
17. Germani Y, Bégaud E, Le Bouguenec C, 1997. Detection of the *Escherichia coli* attaching and effacing gene (*eae A*) in enteropathogenic strains by polymerase chain reaction. *Res Microbiol 148:* 177–181.
18. Schmidt H, Knop L, Franke S, Aleksic S, Heesemann J, Karch H, 1995. Development of PCR for screening of enteroaggregative *Escherichia coli. J Clin Microbiol 33:* 701–705.
19. Le Bouguenec C, Archamband M, Labigne A, 1992. Rapid and specific detection of the pap, afa, and sfa adhesin-encoding operons in uropathogenic *Escherichia coli* strains by PCR. *J Clin Microbiol 30:* 1189–1193.
20. Sack DA, Huda PKB, Daniel RR, Spira WM, 1980. Microtiter ganglioside enzyme linked immunosorbent assay for *Vibrio* an *Escherichia coli* heat-labile enterotoxins and antitoxin. *J Clin Microbiol 11:* 35–40.
21. Ashkenazi S, Cleary TG, 1989. Rapid method to detect Shiga like toxin 1 based in binding to globotriosyl ceramide (Gb3), their natural receptor. *J Clin Microbiol 27:* 1145–1150.
22. Germani Y, De Rocquigny H, Bégaud E, 1994. *Escherichia coli* heat-stable enterotoxin (STa) biotin enzyme linked immunosorbent assay (STa-biotin ELISA). *J Immunol Meth 173:* 1–5.
23. Vial PA, Robins Brown R, Lior H, Prado V, Kaper JB, Nataro JP, Maneval D, Elsayed A, Levine MM, 1988. Characterization of enteroadherent-aggregative *Escherichia coli* a putative agent of diarrheal disease. *J Infect Dis 158:* 70–79.
24. Bauer AW, Kibby WMM, Sherris JC, Turk M, 1996. Antibiotic susceptibility testing by a standardized single disk method. *Ann J Clin Pathol 45:* 493–496.
25. Gruenewald R, Blum S, Chan J, 1994. Relationship between human immunodeficiency virus infection and salmonellosis in 20-to 59-year-old residents of New York City. *Clin Infect Dis 18:* 338–363.
26. Levine WC, Buehler JW, Bean NH, Tauxe RV, 1991. Epidemiology of nonthyphoidal *Salmonella* bacteremia during the human immunodeficiency virus epidemic. *J Infect Dis 164:* 81–87.
27. Paquet C, Perea W, Grimont F, Collin M, Guillod M, 1993. Etiology of haemorrhagic colitis epidemic in Africa. *Lancet 342:* 175.
28. Germani Y, Soro B, Vohito M, Morel O, Morvan J, 1997. Enterohaemorrhagic *Escherichia coli* in the Central African Republic. *Lancet 349:* 1670.
29. Mathewson JJ, Jiang ZD, Zumla A, Chintu C, Luo N, Calamari SR, Genta RM, Steephen A, Schwartz P, Dubut HL, 1995. HEp-2 all adherent *Escherichia coli* in patient with human immunodeficiency virus-associated diarrhea. *J Infect Dis 171:* 1636–1639.
30. Mayer HB, Wanke CA, 1995. Enteroaggregative *Escherichia coli* as possible cause of diarrhea in an HIV-infected patient. *N Engl J Med 332:* 273–274.
31. Thea DM, St Louis ME, Atido U, Kanjinga K, Kembo B, Matondo M, Tshiamala T, Kamenga C, Dovachi F, Brown C, Rand WM, Keusche G, 1993. A prospective study of diarrhea and HIV-1 Infection among 429 Zairian infants. *N Engl J Med 329:* 1696–1702.
32. Bhan MK, Raj P, Levine MM, 1989. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. *J Infect Dis 159:* 1061–1064.
33. Cravioto A, Tello A, Navarro A, Ruiz J, Villafan H, Uribe F, Eslava C 1991. Association of *Escherichia coli* HEp-2 adherence patterns with type and duration of diarrhea. *Lancet 337:* 262–264.
34. Ginzburg ST, Chang BJ, Elliot SJ, Benke V, Gracey M, 1993. Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* isolated from aboriginal children from the Kimberley region of Western Australia. *J Infect Dis 167:* 755–758.
35. Navaro JP, Yikang D, Cooksons, Cravioto A, Savarino S, Guers LD, Levine MM, Tacket CO, 1995. Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers. *J Infect Dis 171:* 465–468.
36. Adal KA, Sterling CR, Guerrant RL, 1995. *Cryptosporidium* and related species. Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, Guerrant RL, eds. *Infections of the Gastrointestinal Tract.* New York: Raven Press, 1107–1128.
37. Bogaerts J, Lepage P, Rouvray D, Vandepitte J, 1984. *Cryptosporidium* sp, a frequent cause of diarrhea in Central Africa. *J Clin Microbiol 20:* 874–876.
38. Huicho L, Sanchez D, Contreras M, 1993. Occult blood and fecal leukocytes as screening test in childhood infectious diarrhea: an old problem revisited. *Pediatr Infect Dis J 12:* 474–477.
39. Soave R, 1996. *Cyclospora*: an overview. *Clin Infect Dis 23:* 429–437.
40. Connor BA, Shlim DR, Sholes JV, Rayburn JL, Reidy J, Rajah R, 1993. Pathologic changes in the small bowel in nine patients with diarrhea associated with a coccidia-like body. *Ann Intern Med 119:* 377–382.
41. Rabeneck L, Gyorkey F, Genta R, 1993. The role of microsporidia in the pathogenesis of HIV-related chronic diarrhea. *Ann Intern Med 119:* 895–899.