Bacterial, yeast, parasitic, and viral enteropathogens in HIV-infected children from São Paulo State, Southeastern Brazil


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Abstract

We present here the frequency of enteropathogens in an HIV-infected children group and investigate their correlation with clinical and sociodemographic characteristics by collecting 100 stool samples from 55 HIV-seropositive Brazilian children. All specimens were processed according to standard methods for bacterial and yeast detection. A commercially available enzyme-linked immunosorbent assay was used to detect protozoan, and to perform virus detection, molecular tests were applied. Consumption of raw vegetables and fruits and severe immunosuppression were significantly associated with diarrhea. Cryptosporidium parvum was the commonest enteropathogen, followed by Candida albicans, enteropathogenic Escherichia coli, and astrovirus. The number of potential pathogenic agents identified in fecal specimens in asymptomatic HIV-seropositive infants is high, which raises the need for additional investigation in this area as well as in other Brazilian regions.

Keywords: AIDS; Pediatric; Enteric pathogens; Brazil

1. Introduction

The Southeastern Brazilian region concentrates 67.8% of the AIDS cases, and, associated with the Southern region, it shows the highest mortality rates (8.7/100.000 inhabitants) of the country because of this disease. The epidemic situation in Brazil follows worldwide tendencies presenting a growing number of cases because of heterosexual transmission and, as a consequence, an increase in the number of mother to child transmitted HIV infection. In Brazil, maternally acquired pediatric HIV infection indicates the Southeast as the region of greater AIDS incidence (63% of all cases) (Brazil, Ministry of Health, 2003).

One of the major health problems among HIV-seropositive patients is superimposed infections due to defect of immunity. Furthermore, intestinal pathogen infection, which is also one of the basic health problems in tropical regions, is common in these patients (Kumar et al., 2002). Among the possible AIDS clinical manifestations, diarrhea represents a major concern (Colebunders and Latif, 1991) mainly for the immunocompromised infantile population that is susceptible to classic pathogens as well as to different opportunistic agents (Monkemuller and Wilcox, 2000; Madhivanan et al.,...
2. Material and methods

The study was conducted from May 2003 to June 2004 by the staff of the Center for Microorganisms Investigation and Laboratory of Microbiology, São José do Rio Preto (SJRP), Southeastern Brazilian region, in the Infectious and Parasitical Diseases Pediatrics Service from Hospital de Base (HB). The HB is a tertiary care hospital catering to a large population from the Northwestern region of São Paulo (SJRP), Southeastern Brazilian region, in the Infectious and Laboratory of Microbiology, São José do Rio Preto (MI). The section of Evandro Chagas Institute, Belem, Para State, treatment. Viral analysis was conducted at the Virology Base (HB). The HB is a tertiary care hospital catering to a Parasitical Diseases Pediatrics Service from Hospital de Base (HB), Southeastern Brazilian region, in the Infectious and Laboratory of Microbiology, São José do Rio Preto (MI). In spite of the relevance of the association between AIDS and diarrhea, few studies were conducted to clarify the role of enteric pathogens in the HIV-seropositive infantile population. We present here the frequency of bacterial, yeast, parasitic, and viral enteropathogens in an HIV-infected children group from the Southeastern Brazilian region and also investigate the correlation of these agents with their clinical and sociodemographic characteristics.

2. Material and methods

The study was conducted from May 2003 to June 2004 by the staff of the Center for Microorganisms Investigation and Laboratory of Microbiology, São José do Rio Preto (SJRP), Southeastern Brazilian region, in the Infectious and Parasitical Diseases Pediatrics Service from Hospital de Base (HB). The HB is a tertiary care hospital catering to a large population from the Northwestern region of São Paulo State, Brazil, representing the regional center of AIDS treatment. Viral analysis was conducted at the Virology Section of Evandro Chagas Institute, Belém, Pará State, Brazil. Fifty-five infants were brought by their relatives or tutors to HB because of HIV follow-up. After an informed consent from all individuals was obtained, up to 3 fecal samples were collected in different medical interviews and with a minimum interval of 3 months. Only 1 sample was provided by the diarrheic children. Clinical data and additional information were obtained according to a protocol approved by the research board of the Faculdade de Medicina de São José do Rio Preto. The inclusion criteria were age between 1 and 13 years, belonging to any HIV risk category, and the presence of anti-HIV antibodies, measured by enzyme-linked immunosorbent assay and confirmed by Western blot.

Stool samples were transported in Cary–Blair transport media for bacterial and yeast analysis. Another clean container was used for fecal collection for parasites and virus investigation. All specimens were examined on the day of collection according to standard bacteriologic and mycological procedures. Briefly, suggestive bacterial colonies were isolated from McConkey, Salmonella–Shigella, brilliant green (after enrichment in tetraethionate broth), and Columbia agar for isolation and identification of the following enteropathogenic bacteria: enteropathogenic Escherichia coli (EPEC), enterohemorrhagic E. coli (EHEC), enteroinvasive E. coli (EIEC), enterotoxigenic E. coli (ETEC), Shigella spp., Salmonella spp., Yersinia spp., and Campylobacter jejuni. Isolates identified by biochemical tests were serotyped by standard techniques (EPM-Mili and Oxidase stripes plus commercially available antisera; PROBAC, Brazil). Yeast strains were isolated on mycobiotic agar (DIFCO, Detroit, MI) followed by germ tube testing, morphologic aspects on corn-meal Tween 80 agar (CTA, Oxoid, Basingstoke, UK), and identification on CHROMAGAR Candida (Chromagar, Paris, France). A commercially available immunoenzymatic assay (Alexon BIOBRÁS, Brazil) to detect Cryptosporidium parvum, Giardia lamblia, and Entamoeba histolytica-specific coproantigen was used. To perform virus detection molecular tests, including rotavirus and astrovirus, we froze and stored 1 aliquot of each fecal sample, previously diluted in water for parasite investigation. Briefly, viral single-stranded RNA was extracted as described previously, including modifications (Boom et al., 1990; Cardoso et al., 2002). Reverse transcriptase (RT) polymerase chain reaction (PCR) for human astrovirus (HAstV) was carried out using primers (Mon 269 and Mon 270) directed toward the ORF2 region, and the amplification conditions described by Noel et al. (1995), except for the RT reaction where a random initiator (hexamer pd (N)6–50 A260 units; Amersham Biosciences, Freiburg, Germany) was used to obtain the complementary (c) DNA product. PCR products were resolved on a 1% agarose gel, followed by ethidium bromide staining, and photo documentation was done in a Gel Doc 1000 (BioRad, Hercules, CA). Samples showing a specific amplicon of 449 bp were considered as positive. Polycrylamide gel electrophoresis was carried out in Tris–glycine buffer, and rotavirus genome profile was defined after electrophoresis of extracted double-stranded RNA through vertical 5% acrylamide bisacrylamide gels, as described previously (Pereira et al., 1983).

Statistical analysis was performed using Epi-Info (version 6.0). To obtain the independence among proportions, we applied the χ² test or the Fisher exact test. The relationship between the clinical characteristics of participant children and the presence of enteropathogens as for the fecal aspect was assessed using the Wilcoxon rank sum test. The adopted significance level for statistical inference was 5%.

3. Results

Fifty-five children ranging from 1 to 13 years (mean age, 7.07 years) were enrolled in this study and provided 1 to 3 diarrheic or no diarrheic fecal samples that were searched for bacterial, yeast, parasitic, and viral enteropathogens. Among these, 58.2% were male, of which 28.1% presented diarrhea symptoms. As summarized in Table 1, sociodemographic characteristics of children and their families such as breast-feeding, pacifier use, living in rural area, and water source could not be associated with diarrhea. On the other hand, raw fruit and vegetable consumption was significantly associated to this condition (P = .009).
Table 2 shows clinical characteristics of the children and presence of enteropathogens as for the fecal aspect. Variables such as antiretroviral therapy or antibacterial chemoprophylaxis, previous hospitalization, or even the presence of enteric pathogens on the sample under study were not associated with diarrhea. However, severe immunosuppression, established according to TCD4+ cell count, showed to be an important factor related to this gastrointestinal symptom ($P = .0042$). The mean TCD4+ cell count in this group was $696.19 \pm 523.73$ cells/mm$^3$, and the cell counts varied from 1 to 2001 cells/mm$^3$.

Among the 100 fecal samples tested, only 9% were classified as diarrheic stools, corresponding to 16.4% of all patients ($n = 55$). At least 1 intestinal potential pathogen was found in approximately 66% of the diarrheic stool samples, whereas around 54% of the nondiarrheic stool samples presented such agents. After performing subsequent fecal analysis for the same group of enteropathogenic agents, we were unable to detect a pattern of maintenance for bacteria, yeast, and virus or parasite species. The etiologic agents found in diarrheic and nondiarrheic stool samples are shown in Table 3. The high rates of positive stool samples were in samples without diarrhea. The EPEC was the most frequent bacterial pathogen (17%), followed by EIEC (5%), C. jejuni (1%), Shigella boydii (1%), and Shigella sonnei (1%). Yeast from the genus Candida was isolated from 37% of all fecal samples, and Candida albicans was the most frequent (32%), followed by Candida parapsilosis (2%), Candida tropicalis (2%), and Candida krusei (1%). One child carried, concomitantly, C. albicans and C. tropicalis. Molecular analysis revealed that 11% of the fecal samples were positive for astrovirus, exclusively in children without diarrhea. No rotavirus was detected. Cryptosporidium parvum was the commonest parasitic agent detected (62%), followed by G. lamblia (9%) and E. histolityca (1%). The enteropathogens detected among the 9 children presenting clinical signs of diarrhea showed the following frequencies: C. parvum 66%; C. albicans, 55%; EPEC, 11%; and S. sonnei, 11%.

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>D ($n = 9$)</th>
<th>ND ($n = 91$)</th>
<th>Significance ($P$)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age of enrollment (years)</td>
<td>8.18</td>
<td>6.97</td>
<td></td>
</tr>
<tr>
<td>Age range (years)</td>
<td>3–12</td>
<td>2–13</td>
<td></td>
</tr>
<tr>
<td>Breast-feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Pacifier use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Raw fruit and vegetable consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>77</td>
<td>.009</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Water source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Public reservoir$^b$</td>
<td>5</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Others$^c$</td>
<td>4</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Rural area</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>

D = diarrheic stool sample; ND = nondiarrheic stool sample.
$^a$ Comparisons by analysis of variance or Fischer exact test are shown only for differences approaching statistical significance.

$^b$ Provided and treated by the city public administration.

$^c$ Mineral, bottled or not (boiled or not).

Table 3

<table>
<thead>
<tr>
<th>Enteropathogens</th>
<th>D ($n = 9$)</th>
<th>ND ($n = 91$)</th>
<th>Significance ($P$)$^a$</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. jejuni</td>
<td>0</td>
<td>1</td>
<td>.910</td>
<td>1</td>
</tr>
<tr>
<td>EIEC</td>
<td>0</td>
<td>5</td>
<td>.617</td>
<td>5</td>
</tr>
<tr>
<td>EPEC</td>
<td>1</td>
<td>16</td>
<td>.525</td>
<td>17</td>
</tr>
<tr>
<td>ETEC</td>
<td>0</td>
<td>0</td>
<td>.648</td>
<td>0</td>
</tr>
<tr>
<td>S. boydii</td>
<td>0</td>
<td>1</td>
<td>.910</td>
<td>1</td>
</tr>
<tr>
<td>S. sonnei</td>
<td>1</td>
<td>0</td>
<td>.909</td>
<td>1</td>
</tr>
<tr>
<td>Yeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>5</td>
<td>27</td>
<td>.114</td>
<td>32</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>0</td>
<td>2</td>
<td>.827</td>
<td>2</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>1</td>
<td>1</td>
<td>.172</td>
<td>2</td>
</tr>
<tr>
<td>C. krusei</td>
<td>0</td>
<td>1</td>
<td>.910</td>
<td>1</td>
</tr>
<tr>
<td>Virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrovirus</td>
<td>0</td>
<td>11</td>
<td>.648</td>
<td>11</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>0</td>
<td>0</td>
<td>.009</td>
<td>0</td>
</tr>
<tr>
<td>Parasites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. parvum</td>
<td>6</td>
<td>56</td>
<td>.893</td>
<td>62</td>
</tr>
<tr>
<td>G. lamblia</td>
<td>0</td>
<td>9</td>
<td>.758</td>
<td>9</td>
</tr>
<tr>
<td>E. histolityca</td>
<td>0</td>
<td>1</td>
<td>.147</td>
<td>1</td>
</tr>
</tbody>
</table>

D = diarrheic sample; ND = nondiarrheic sample.
$^a$ Fischer exact test.
4. Discussion

Despite major efforts in scientific understanding of effective interventions that reduce children’s HIV infection, over the last year, global estimates for newly acquired HIV infections in this population remain high (Luo, 2000). The gastrointestinal tract plays a key role in AIDS pathogenesis, because of suppressed immunologic responses at the mucosal level that delay the intestinal nonspecific defense mechanisms (Yolken et al., 1991; Smith, 1993). In the infantile population, the resultant gastroenteric complications from HIV infections can contribute to nutritional deficiency with consequent aggravation of the immunologic conditions (Gassama et al., 2001). In developing countries, the most common causes of diarrhea are microorganism’s infections, but there is huge variation on the causal agent of this disease among different regions (Kelly, 1998).

To our knowledge, this is the 1st survey yet done of enteropathogens detected in stools from Brazilian HIV-seropositive infants. Although the cases reported here can be representative of the HIV-infected children community from Northwestern São Paulo State region, the obtained data can be viewed as a start point for further comparisons within the diverse Brazilian regions and also within other developing countries. Furthermore, they provide important clues to the future understanding, prevention, and control of such relevant coinfections around the world.

There were high rates of positive stool samples (91%) in children without diarrhea, underlining the difficulties of determining the cause of an episode of diarrhea. EPEC and S. sonnei were associated with the rise of diarrhea in 2 children, C. albicans in 5, and C. parvum in 6. Other well-known enteropathogens such as G. lamblia, E. histolytica, astrovirus, or EIEC strains were isolated without statistical significance. The high rate of enteropathogen detection in asymptomatic infants can be explained by the well-established medical care assistance for these children and their mothers’ antiretroviral efficient treatment (Weber et al., 1999), both performed at the Infectious and Parasitical Diseases Pediatrics Service from HB. Moreover, SJRP, from where the most of the evaluated children proceed, is the biggest city of the Northwestern São Paulo State region and presents an efficient system of basic sanitation in almost the totality of its districts. Nevertheless, the major concern of our finding regarding asymptomatic carriers is that enteropathogens are being maintained in a high percentage in this population, as described for other enteropathogens in adults from Peru (Black et al., 1989) and also in children from Tanzania (Gascón et al., 2000). Other reasons incriminated can be the lack of virulence factors from several lines of some enteropathogens, as described for Campylobacter spp. (Klipstein et al., 1985) or G. lamblia (Flanagan, 1992), and/or acquired immunity from previous infections at an earlier age, because most of the children (60%) were above 5 years old.

One of the possible collateral effects of the highly active antiretroviral therapy (HAART), especially of the protease inhibitors (PI), is diarrheic manifestation (Weber et al., 1999; Lemberg et al., 2002). However, therapy with PI could not be related to this gastrointestinal disturb in the studied population. On the other hand, severe immunosuppression was associated with this manifestation in the HIV-seropositive children, as well as primarily verified for the adult HIV-positive population (Gassama et al., 2001; Galli et al., 1995; Kakai et al., 1995; Brink et al., 2002). Indeed, severe immunosuppression is referred as a predisposing condition for infections, mainly bacterial (Gassama et al., 2001; Onyemelukwe and Musa, 2002), and it is also considered a death predictor for the HIV-carrier child (Torres et al., 1991). There was no significant predominance of specific enteropathogen in any of the 3 immunity levels. This study shows the importance of the association of intestinal infection with diarrhea symptoms in the low immunity group of HIV-infected infants. However, in most of the HIV-seropositive individuals, we could show a nonsignificant higher detection of C. parvum and C. albicans, as firstly demonstrated for a non-HIV-infected children population (Koffi-Akoua et al., 1989).

Although it has been reported, differences between diarrhea occurrences in urban and rural areas (Coker and Poznansky, 1997) were not verified in the studied sample, probably because of the small number of diarrheic samples and/or children proceeding from farming areas. Breast-feeding is considered a preventive practice toward diarrhea (Newburg et al., 2003; Vieira et al., 2003). However, in our study, it was not possible to verify such association. In spite of the fact that according to the International AIDS policies, breast-feeding is not recommended in HIV-infected newborns (Committee on Pediatric AIDS, 1995), a significant percentage of our studied children were breast-fed. In fact, such protection seems to be effective up to 12 months (WHO, 2000), a period of life not included in our study population. In support to our findings for the HIV-seropositive infants from SJRP, the use of pacifiers did not influence diarrhea onset in Brazilian children, even when fecal coliforms were present in 49% of the cultured devices (Tomasi et al., 1994). On the other hand, the morbidity pattern in a pacifier-user group of Indian children revealed that 70.5% had diarrhea, and pacifier use was significantly associated to this disease (Bhat et al., 1991). The level of contamination of fruits and vegetables in developing countries is high, and enteropathogenic microorganisms can be introduced by irrigation with contaminated water of the sewer or by inadequate manipulation in domestic environment. Another frequently associated variable as introductory of enteric pathogens is the water source for household consumption (Plate et al., 2004). In this study, raw food consumption showed significant association with diarrhea occurrence, in contrast to the observed regarding source of water consumption. These findings corroborate the review performed for
Lanata (2003), where higher levels of food contamination is associated with diarrhea and not the source of water domestic consumption, in different reports. Whether additional unconsidered factors can mislead conclusions regarding the role of different infantile diarrhea risk contributors remains to be established.

The absence of a regular detection of particular microorganism’s species in subsequent samples from the same patient suggests that in HIV-seropositive children, there is a fluctuation in the gastrointestinal microbiota or pathogenic agent’s constitution, probably because of the multidrug chemoprophylaxis (sulfamethoxazole plus trimethoprim) and/or treatment to which this group is constantly exposed. Another possible explanation relies on laboratorial methodology limitations and also sample availability.

Bacterial enteropathogens, in this study, were identified in 23% of all diarrhea cases and almost equally often (25%) in the subjects without diarrhea. In HIV-uninfected diarrheic children from the same area, bacterial enteropathogen detection occurred in 48% (Almeida et al., 1998), and for the same group of patients from São Paulo city (capital—450 km apart from SJRP), in 55% (Gomes et al., 1991), whereas in Porto Velho, Rondônia State, this detection occurred in 40.7% of the cases (Orlandi et al., 2001). The results of our study showed that EPEC is the 3rd most frequently isolated enteropathogen after C. parvum and C. albicans. There is evidence that EPECs are now unusual in most industrialized countries, but they continue to be an important pathogen in many developing countries in urban and rural areas (Trabulsi et al., 1985a). EPEC strains were most frequent in children under 5 months of age (El-Sheikh and El-Assouli, 2001). We could not confirm this association because our children aged above 1 year old, but our results show that E. coli isolation rate averaged 17% among HIV-infected infants aged up to 5 years old without diarrhea and 11% in HIV-children aged among 3 and 5 years old. Detection of ETEC was not achieved. This result is in accordance with the low prevalences previously reported for ETEC in no HIV-serum positive children from the same area (Almeida et al., 1998), from São Paulo, São Paulo State (Trabulsi et al., 1985b), and in diarrheic infants from Northwestern Brazil (Orlandi et al., 2001) and Northeastern metropolitan (Lima et al., 2000). Its role as a causative agent of diarrhea in Brazilian children remains to be determined. S. sonnei was associated with an increased risk of diarrhea in only 1 child (7 years of age). Its role as causative agent of diarrhea in Northwestern São Paulo State children is still to be evaluated. It is worthy of note that EPEC and S. sonnei were also the main bacterial agents isolated in a large survey of 1836 non–HIV-infected infants from Ribeirão Preto, São Paulo State (250 km apart from SJRP), showing clinical signs of acute diarrhea (Medeiros et al., 2001). Together, these data suggest that both bacteria represent endemic pathogens in this region. Other bacterial gastrointestinal detected besides EPEC and S. sonnei were E. coli (60.4%), Pseudomonas spp. (22.7%), Klebsiella spp. (18.8%), Citrobacter spp. (15.8%), Alcaligenes spp. (14.8%), Proteus spp. (9.9%), Enterobacter spp. (6.9%), Edwardsiella spp. (1.9%), and Providencia spp. (0.9%).

The importance of Candida spp. as a colonizer of the gastrointestinal mucosa is unquestionable in the HIV-seropositive children, especially of those named “nonalbicans”, which had been isolated in 9% of our study patients. Candida spp., in particular, C. albicans, emerge in cases of HIV-infected children with diarrhea (Rozikiewicz et al., 2005). The isolation of C. albicans in stools of patients from different countries such as Senegal (Gassama et al., 2001), Cameroon (Same-Ekobo et al., 1997), India (Anand et al., 1998), Ivory Cost (Therizol-Ferly et al., 1989), and Poland (Same-Ekobo et al., 1997) yielded prevalence rates of 7.6% to 39.1%. Our results are comparable to the highest levels reported because we could detect 35% of this yeast. Indeed, Candida sp. is referred as a gastrointestinal opportunistic pathogen, and some authors incriminate such yeast as the unique agent responsible for diarrhea onset (Koffi-Akoua et al., 1989; Chaudhury et al., 1996), whereas others consider it important in the etiology of infantile diarrhea only in cases of association to other microorganisms (Koffi-Akoua et al., 1989; Forbes et al., 2001). Anyhow, its importance relies on the increasing incidence of acquired or intrinsic resistance isolates from oral and esophageal candidiasis and also candidemia.

As for viral analysis, astrovirus was detected in non-diarrheic samples (11%), but the same pathogen was not identified in diarrheic samples. HAstVs have been increasingly identified as important etiologic agents of acute gastroenteritis in children up to 5 years old (Glass et al., 1996) and also in HIV-infected adults (Grohmann et al., 1993). Interestingly, our results found positivity in HIV-infected infants above 5 years old. In Brazilian children, astrovirus was detected in 5% and 3% of nondiarrheic infants’ samples from the Northeastern (Gabbay et al., 2005) and Southeastern (Timentensky et al., 1993) regions, respectively. In addition, approximately the same percentage (4%) was obtained for Brazilian children with acute gastroenteritis from the central region (Cardoso et al., 2002). All these frequencies were lower than that reported in our study. The rotavirus is considered the most important viral agent in diarrheic episodes in children less than 5 years old, and its prevalence ranges from 29% to 45% (Parashar et al., 2003, 2006). In studies conducted with diarrheic HIV-positive patients, the rotavirus was detected in 13.6% of the cases that occurred in a study carried out in Hamburg, Germany (Albrecht et al., 1993), and also in 2.5% of samples obtained in a health units from Belém, Brazil (Gabbay et al., 2001). Nonetheless, this virus was not detected in researches conducted in Baltimore, Venezuela, and Brazil (Kaljot et al., 1989; Gonzalez et al., 1998, Machado et al., 1996), as it happened in the present investigation. Another factor to be considered refers to the material used for the rotavirus detection; due to the insufficient amount of feces, a water-diluted fecal suspension was used initially for parasite...
Parasitic agents have also been reported as important etiologic agents of childhood diarrhea (Tangermann et al., 1991). Strong evidence suggests the C. parvum as an important community-disseminated protozoan in day care centers and hospitals (Guerrant, 1997). This coccidoid infects from 6% to 94% of HIV-infected individuals in developing countries (Same-Ekobo et al., 1997; Anand et al., 1998; Therizol-Ferly et al., 1989; Hashem et al., 1997), and the official global prevalence of cryptosporidiosis in HIV-infected patients reported by the Brazilian Ministry of Health is 0.7% (Brazil, 1997). Interestingly, we could reveal this opportunistic pathogen in 62% of the studied samples, which greatly differs from our previous report regarding this protozoan prevalence in an HIV-infected adult population from the same area (9%) (Cardoso et al., 2004). This difference suggests that there is an age-related maintenance that influences the distribution of this pathogen. Such situation can be due to the well-recognized differential effectiveness of the immune response. The necessity of routine investigation in this aspect is therefore noticed. Regarding G. lamblia prevalence, our investigation showed that giardiasis does not occur in an expressive frequency in HIV-seropositive children, because it was only accounted for 9% of all nondiarrheic samples. For G. lamblia, this is surprising, because in some regions of São Paulo State, up to 40% of the population carries this protozoan, although often without apparent symptoms (Cimerman et al., 1999). On the other hand, low frequencies of this infection can be related to the fact that the elimination of the parasitic forms of this protozoan is intermittent (Schnack et al., 2003). E. histolytica is estimated to be infecting around 10% of the world population as a whole (Walsh, 1986), where it is considered to be the 3rd leading parasitic cause of death (Khan et al., 1986). In this study, its prevalence was only 1% in the HIV-infected infants, which is lower than that reported in children from other parts of Brazil (Póvoa et al., 2000; Beneton et al., 2005). The low prevalence of the last 2 parasites, in accordance to previous work in adult HIV seropositive in Northwest São Paulo State (Cardoso et al., 2004), confirms that these protozoan are not to be endemic in this area.

In conclusion, the number of potential pathogenic agents identified in feces of the asymptomatic HIV-seropositive infants is high, and the frequency of enteropathogens in this Northwestern native population of São Paulo State is similar to that one observed for the non–HIV-infected children. The asymptomatic infection may be a resultant of mechanisms of immunologic tolerance, intraspecific variations in the virulence, or drawn-out liberation of microorganisms after the last episode of diarrhea (Brink et al., 2002). These data raise the pressing necessity of additional studies in different regions of the country, aiming at the clarification of the importance of each of these agents in the etiology of the diarrhea in the seropositive infantile population. The study revealed that although most of these individuals showed no gastrointestinal disorders such as diarrhea, they still harbor these enteropathogens and act as carriers. However, some observed particularity raise important questions for the future prevention of the diarrhea in this group, such as the identification of other pathogenic agents, beyond subgroups, variants, and pertinent factors of virulence to those identified here.

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