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Diarrhea in children less than two years of age with known HIV status in Kisumu, Kenya


1. Introduction

Diarrhea is a major cause of child morbidity and mortality in developing countries. It is estimated to account for approximately 1.9 million or 18% of deaths among children under 5 years of age annually, of which approximately 40% occur in the African region. Diarrhea is a common cause of morbidity and mortality among HIV-infected children. Approximately 2.1 million children under 14 years of age are estimated to be infected with HIV worldwide, and 90.5% of infected children are in sub-Saharan Africa. Most children acquire HIV-infection through vertical transmission from their mothers, either perinatally or through breastfeeding.

In 1997, we initiated a nested cohort study of diarrheal disease within a larger prospective cohort study examining the relationship between placental malaria and perinatal mother-to-child transmission of HIV in an urban/periurban area of western Kenya. The purpose of the study was to examine the burden of diarrheal disease in infants and young children born to HIV-infected and HIV-uninfected mothers. In the present analysis we examine the frequency and etiology of diarrhea among HIV-infected and HIV-uninfected children aged 0–23 months.
2. Materials and methods

2.1. Study site, enrollment, and study population

This study was conducted at an outpatient clinic of the Nyanza Provincial General Hospital (NPGH) in Kisumu, a city located on the shores of Lake Victoria in western Kenya with a population of approximately 300,000. NPGH is a 400-bed government referral hospital, providing healthcare mostly to the local low-income population. Study procedures have been described elsewhere. From November 1997 to August 2001, we questioned mothers about diarrhea in their children during routine and unscheduled visits. Between October 1999 and January 2000 the diarrhea sub-study was suspended for technical reasons. Enrollment stopped in August 2000 and follow-up visits ended one year later.

We obtained blood by finger prick for detection of HIV-DNA by PCR at the routine visits. Stool samples were collected from children who presented with diarrhea at routine or unscheduled visits. Caretakers of children for whom a stool culture was performed, were invited to return after 1 week to obtain the result and for follow-up of the child's diarrheal illness. Ill children were examined by a clinical officer or the study pediatrician and treated accordingly. At the time of the study, trimethoprim–sulfamethoxazole prophylaxis was not yet routinely provided to infants born to HIV-infected women. Children who did not return for routine follow-up appointments were visited at home. For children who were reported as having died, we obtained additional information using verbal autopsy. Verbal autopsy information was reviewed independently by three medical workers (clinical officers or doctors) who each assigned a diagnosis. If one diagnosis was given by two or more reviewers, it was assigned as the cause of death. If all three causes were different, a fourth reviewer was used to adjudicate.

2.2. Laboratory procedures

Stool specimens were obtained either as a whole stool sample or by rectal swab. Swabs were immediately placed in Cary–Blair transport medium. Whole stool samples and swabs were kept at 4 °C until same day transport to the Enterics Laboratory run jointly by the Kenya Medical Research Institute (KEMRI) and the Centers for Disease Control and Prevention (CDC); all specimens were processed within 6 hours of collection. Stool specimens were cultured by standard techniques for Shigella, Salmonella, Campylobacter and Vibrio cholerae; media, reagents, bacterial isolation techniques, and antimicrobial susceptibility testing were routinely quality controlled. We determined antimicrobial susceptibilities of Shigella and Salmonella to ampicillin, amoxicillin–clavulanate, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim–sulfamethoxazole by the Kirby–Bauer disk diffusion method. The results of bacterial cultures and antimicrobial susceptibility testing were returned to the clinician who was caring for the child within 1 week. We also collected stool for culture from 96 children without diarrhea in the week before or after specimen collection to assess the prevalence of potential pathogens in the absence of diarrheal disease ('controls'). A subset of all whole stool samples was tested for parasites and rotavirus. Detection of intestinal parasites was performed on concentrated stool samples following the formal–ether concentration method. Wet mounts of stool concentrates were examined by means of both bright-field and ultraviolet fluorescence microscopy. Stool concentrates were also stained by use of the trichrome technique and Kinyoun's modified acid-fast technique and examined using bright-field microscopy. In addition, stool concentrates were also tested for Giardia and Cryptosporidium using commercially available immunofluorescence assay (IFA) test kits. Stool specimens were tested for rotavirus with a commercial antigen detection kit (Premier Rotaculture, Merifluor, Meridian Bioscience, Inc., Cincinnati, OH, USA). Each sample was diluted in 1 ml of kit sample buffer and then tested in an enzyme immunoassay according to the manufacturer's instructions. Samples with an optical density reading of ≥0.150 at 450 nm were considered positive for rotavirus.

Women were initially screened for HIV with Serostrip HIV-1/2 (Saliva Diagnostic Systems Pte. Ltd, Singapore); a woman with a negative test result on screening with Serostrip HIV-1/2 was considered HIV-seronegative. A positive HIV test with Serostrip HIV-1/2 was confirmed with Capillus HIV-1/HIV-2 (Cambridge Diagnostics, Wicklow, Ireland); when both of these rapid tests for HIV antibodies were positive a woman was considered HIV-seropositive. In the case of inconclusive rapid tests results (e.g., Capillus test indeterminate or a discrepant result compared with the Serostrip HIV-1/2), a Western blot test was performed to determine HIV status. HIV-testing of children born to HIV-seropositive women was conducted using PCR amplification of proviral DNA extracted from peripheral blood mononuclear cells. The PCR testing of PCR-negative children was repeated at 12-week intervals and at the last routine visit of the child. For infants who tested positive we probed the blood specimens that we had collected monthly and determined the month of the first positive PCR test result. We retrospectively tagged stool specimens as coming from HIV-positive or HIV-negative children.

2.3. Definitions

HIV-positive children (HIV +/+ ) were children of HIV-seropositive mothers who had two or more consecutive positive PCR tests, with the first positive test detected within the first 4 months of life. Children of HIV-seropositive mothers were defined HIV-negative (HIV −/+ ) if they had two or more negative consecutive PCR tests, and the PCR test of the last visit was negative as well. We classified infants for whom we had insufficient PCR data to determine their status as indeterminate; these infants were excluded from the analysis. Children of HIV-negative women were defined as HIV-negative (HIV −−). We defined diarrhea as three or more loose or watery stools per day. We defined an episode of diarrhea as a new illness if it had been preceded by at least 7 days without diarrhea; the length of illness was the number of days during which a child experienced diarrhea during that episode. Bloody diarrhea was diarrhea with blood visible in the stool as reported by the mother. A diarrheal episode that lasted 14 days or more was defined as persistent diarrhea.

2.4. Analysis and statistical methods

This was a descriptive study; thus, no formal hypotheses were tested. Differences in proportions were analyzed using the chi-square test or Fisher's exact test when appropriate. Differences in means were compared by Student's t-test; differences in medians were compared using the Kruskal–Wallis test. We calculated incidence densities as number of diarrheal episodes per 100 person-years of observation, and estimated rate ratios (RR) and 95% confidence intervals (95% CI). We used the statistical programs SAS (the SAS system for Windows, v. 8.1: SAS, Inc.) and Epicalc (Epicalc 2000 v. 1.02, http://www.brixtonhealth.com/epicalc.html)
for all analyses. For all statistical tests, a two-sided $p$-value of $<0.05$ was considered significant.

2.5. Ethical review

The study protocol was approved by the institutional review boards at KEMRI (Nairobi, Kenya) and CDC (Atlanta, USA). Issues concerning case management during the course of the study have been discussed previously. Before enrollment in the study, informed consent was obtained from the mother for her own participation (during pregnancy) and for that of her child.

3. Results

3.1. Characteristics of the study population

During the study period, 682 children contributed 610 child-years of observation (CYO) periods with a median per-child observation time of 6 weeks (95% CI 5–8 weeks), with no difference by HIV status of the child/mother pair (data not shown). The median weaning age was 94 weeks (95% CI 89–97 weeks). Use of antibiotics in the 2 weeks preceding a visit was reported in 62 routine visits (0.8%) and was more common among HIV-positive children (2.7%) compared to the other groups (HIV+/+ children median 28 weeks (range 4–88) vs. 48 weeks (range 4–96) among HIV−/− children and 52 weeks (range 4–96) among HIV−/+ children; $p < 0.01$ both comparisons, Kruskal–Wallis test). Factors associated with a shorter follow-up time ($<48$ weeks) were child-related and included being male, born prematurely, HIV-infected, and dying during the study period (Table 1).

In this study population, breastfeeding was common, but only exclusively for a short period; water (plain or in combination with sugar and/or salt), or other foods were introduced at a median age of 6 weeks (95% CI 5–8 weeks), with no difference by HIV status of the child/mother pair (data not shown). The median weaning age was 94 weeks (95% CI 89–97 weeks). Use of antibiotics in the 2 weeks preceding a visit was reported in 62 routine visits (0.8%) and was more common among HIV-positive children (2.7%) compared to the other groups (0.6% and 0.4% among HIV−/− and HIV−/+ children, respectively; compared to HIV+/+ children, both $p < 0.01$).

3.2. Diarrhea disease characteristics, pathogens, and sensitivity

A total of 1209 episodes of diarrhea were reported (Table 2), corresponding to 198 episodes per 100 CYO. HIV+/+ children

### Table 1
Characteristics of participating mother/child pairs ($N = 682$) by follow-up time, Kisumu, 1997–2001

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Total number of mother/child pairs (%)</th>
<th>Number of pairs with a follow-up time of the child of &lt;48 weeks ($n = 327$)</th>
<th>Number of pairs with a follow-up time of the child of ≥48 weeks ($n = 355$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young age at delivery (&lt;20 years)</td>
<td>206 (30.2)</td>
<td>105 (32.1)</td>
<td>101 (28.5)</td>
</tr>
<tr>
<td>Luo ethnicity</td>
<td>582 (85.3)</td>
<td>275 (84.1)</td>
<td>307 (86.5)</td>
</tr>
<tr>
<td>Urban residence</td>
<td>528 (77.4)</td>
<td>254 (77.7)</td>
<td>274 (77.2)</td>
</tr>
<tr>
<td>Completed primary school</td>
<td>474/680 (69.7) [69.5]</td>
<td>220 (67.3)</td>
<td>254/353 (72.0) [71.5]</td>
</tr>
<tr>
<td>Not married at the time of delivery</td>
<td>158 (23.2)</td>
<td>76 (23.2)</td>
<td>82/353 (23.2) [23.1]</td>
</tr>
<tr>
<td>Low/medium quality house</td>
<td>523 (76.7)</td>
<td>255 (76.0)</td>
<td>268 (75.5)</td>
</tr>
<tr>
<td>Electricity in residence</td>
<td>136/680 (20.0) [19.9]</td>
<td>66 (20.2)</td>
<td>70/353 (19.8) [19.7]</td>
</tr>
</tbody>
</table>

**Child characteristics**

| Firstborn | 266 (39.0) | 136 (41.6) | 130 (36.6) |
| Low birth weight | 38 (5.6) | 21 (6.4) | 17 (4.8) |
| Premature | 52/680 (7.7) [7.6] | 33 (10.1) | 19 (5.4)$^a$ |
| Male | 348 (51.0) | 180 (55.1) | 168 (47.3)$^a$ |

**Child/maternal HIV status**

| HIV-positive child/HIV-positive mother | 93 (13.6) | 58 (17.7) | 35 (9.9)$^a$ |
| HIV-negative child/HIV-positive mother | 407 (59.7) | 191 (58.4) | 216 (60.9) |
| Child of HIV-negative mother | 182 (26.7) | 78 (23.9) | 104 (29.3) |
| Death during surveillance | 78 (11.4) | 62 (19.0) | 16 (4.5)$^a$ |

### Table 2
Episodes of diarrhea among children aged <2 years by infant/maternal HIV-status and age group, Kisumu, 1997–2001

<table>
<thead>
<tr>
<th>Follow-up time (years)</th>
<th>Episodes of diarrhea</th>
<th>Incidence density$^a$</th>
<th>Rate ratio (95% CI)</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>610.2</td>
<td>1209</td>
<td>198</td>
<td>1.71 (1.44–2.04)</td>
</tr>
<tr>
<td>HIV +/+</td>
<td>65.5</td>
<td>210</td>
<td>321 [318]</td>
<td>0.98 (0.85–1.11)</td>
</tr>
<tr>
<td>HIV −/+</td>
<td>369.4</td>
<td>672</td>
<td>182</td>
<td>Reference</td>
</tr>
<tr>
<td>HIV −/−</td>
<td>175.2</td>
<td>327</td>
<td>187</td>
<td>Reference</td>
</tr>
</tbody>
</table>

**Infants (age 0–364 days)**

| All | 352.9 | 749 | 212 | 1.64 (1.32–2.03) | <0.01 |
| HIV +/+ | 42.6 | 139 | 326 [323] | 0.98 (0.83–1.17) | 0.44 |
| HIV −/+ | 209.1 | 409 | 196 | Reference |
| HIV −/− | 101.2 | 201 | 199 | Reference |

**Children in their 2nd year of life**

| All | 257.3 | 460 | 179 | 1.82 (1.36–2.44) | <0.01 |
| HIV +/+ | 22.9 | 71 | 310 [309] | 0.96 (0.78–1.19) | 0.39 |
| HIV −/+ | 160.3 | 263 | 164 | Reference |
| HIV −/− | 74.0 | 126 | 170 | Reference |

HIV +/+, HIV-positive children; HIV −/+; HIV-negative children of HIV-positive mothers; HIV −/−; children of HIV-negative mothers; CI, confidence interval.

$^a$ Diarrhea per 100 child-years of observation.
percent of the children with infection with of diarrhea by bacterial species detected, but children with mono-infection with Campylobacter (16/109 or 14.7% among HIV +/+ children) and Campylobacter and Salmonella three times (one HIV +/+ and two HIV –/– children, no bloody diarrhea reported).

The prevalence of any bacterial infection, and of Campylobacter, Salmonella, and Shigella, detected in 91 stool samples of 67 children (43% male) without diarrhea at the time of visit or in the week before or after the visit, was lower than, but not statistically significantly different from, the prevalence among children with active diarrhea (p = 0.40, 0.34 and 1.00 for Campylobacter, Salmonella, and Shigella, respectively, Table 3).

Shigella and Salmonella species were largely resistant to available antibiotics (trimethoprim–sulfamethoxazole, tetracycline, ampicillin, chloramphenicol, and streptomycin), but demonstrated little resistance to ciprofloxacin, nalidixic acid, gentamicin, kanamycin, and ceftriaxone, antibiotics that were generally not available in this area (Table 4). Sample sizes were inadequate to meaningfully compare susceptibility patterns of pathogens by child HIV status.

A stool culture was obtained for 630 (66.5%) active diarrheal episodes (Table 3). Stool cultures were less likely to yield a bacterial pathogen in HIV +/+ children compared to HIV –/– children (p = 0.01, Table 2); however, there was no statistically significant difference observed between HIV –/– and HIV +/+ children (RR 0.98, 95% CI 0.85–1.11, p = 0.36). The combined ratio for HIV –/– and HIV +/+ children was 183 episodes per 100 CYO. The median duration of diarrheal illnesses was 5 days (range 1–36 days) and was not statistically different by the child’s HIV status (p < 0.01). Fever (77.0%) was the most common symptom accompanying the diarrheal illness; HIV +/+ children with diarrhea experienced 321 episodes per 100 CYO and were significantly more likely to have episodes of diarrhea compared to HIV –/– children, who experienced 187 episodes per 100 CYO (RR 171, 95% CI 1.44–2.04, p < 0.01, Table 2); however, there was no statistically significant difference observed between HIV –/– and HIV +/+ children (RR 0.98, 95% CI 0.85–1.11, p = 0.36). The combined ratio for HIV –/– and HIV +/+ children was 183 episodes per 100 CYO. The median duration of diarrheal illnesses was 5 days (range 1–36 days) and was not statistically different by the child’s HIV status (p = 0.36 and 0.33, respectively). Rotavirus infection was less common among children with mono-infection with Shigella (4/25, p = 0.05) or Salmonella (3/19, p = 0.08).

The prevalence of any bacterial infection, and of Campylobacter, Salmonella, and Shigella, detected in 91 stool samples of 67 children (43% male) without diarrhea at the time of visit or in the week before or after the visit, was lower than, but not statistically significantly different from, the prevalence among children with active diarrhea (p = 0.40, 0.34 and 1.00 for Campylobacter, Salmonella, and Shigella, respectively, Table 3).
respectively, \( p = 0.91 \), but children with rotavirus were more likely to have persistent diarrhea (14.3% vs. 3.5%, \( p = 0.03 \)).

A subset of 394 consecutive diarrhea samples provided by 240 children were examined for parasites. No Isospora, Cyclospora, Blastocystis hominis, Strongyloides, or Schistosoma infections were detected. Giardia intestinalis was identified once (HIV –/– child), Cryptosporidium spp and hookworm twice (0.5%; Cryptosporidium: one HIV +/+ and one HIV –/– child; hookworm: two HIV –/– children), Entamoeba histolytica and Trichuris trichiura three times (0.8%; E. histolytica: one in each child group; T. trichiura: all three infections in HIV –/– children), and Ascaris lumbricoides was detected four times (three HIV –/– and one HIV –/– child).

3.3. Persistent diarrhea

Sixty-six episodes of diarrhea among 62 children lasted \( \geq 14 \) days (5.5% of all diarrhea episodes; 10.8 per 100 CYO among all children); 51 of these episodes (77.3%) occurred in the first year of life (follow-up time as in Table 2). HIV +/+ infants were significantly more likely to experience persistent diarrhea (30.5 per 100 CYO) than children in the other groups (7.8 per 100 CYO for HIV –/– children and 9.7 per 100 CYO for HIV –/– children, \( p < 0.01 \)). Stool cultures were available for 46 episodes; Campylobacter, Shigella, and Salmonella were detected in 6 (13%), 5 (11%), and 3 (7%) episodes, respectively.

3.4. Diarrhea and child death

Seventy-eight children died during follow-up; 36 were HIV +/+ (38.7% of HIV +/+ participants), 32 were HIV –/– (7.9% of HIV –/– participants), and 10 were HIV –/– (5.5% of HIV –/– participants). The majority of deaths occurred in the first year of life (58, 74.4%). Information on the cause of death was available for 74 children (94.9%). Diarrhea was diagnosed as the terminal illness for 28 children (37.8%), and was not a significantly more common cause among HIV +/+ children who died (14, 41.2%), compared to HIV –/– children (11, 36.7%) or HIV –/– children who died (3, 30%, overall Fisher’s exact test \( p = 0.85 \)). There was no statistically significant difference in the duration of the last diarrheal illness before death by HIV status (HIV +/+ children: median 14 days, range 2–30 days; HIV –/– children: median 11 days, range 1–33 days; and HIV –/– children: median 10 days, range 3–31 days, Kruskal–Wallis test \( p = 0.88 \)).

3.5. Discussion

In this study population, diarrhea was more common among HIV-positive than HIV-negative children in whom it was more likely to be accompanied by fever and poor feeding. Stool cultures from HIV-positive children with diarrhea were less likely to yield routine bacterial pathogens than stool cultures from children of HIV-negative women. Susceptibility testing of the routine bacterial pathogens we isolated showed high levels of resistance to the antibiotics commonly used in the community. Diarrhea was identified as the direct cause of death for 37.8% of all children regardless of HIV status.

To explain the increase in diarrhea among HIV-infected children reported by us and others,\(^{2,3,5}\) some authors have hypothesized that HIV may induce changes in the intestinal immune system that increase vulnerability to enteric infections or that HIV itself may cause diarrhea.\(^{4,6,11}\) The HIV disease stage-related release of cytokines may affect enterocyte secretion of chloride, or may more directly affect the epithelial barrier function,\(^{11}\) and in this way reduce the threshold that diarrheal pathogens must overcome to cause illness. HIV-infected children may also live in environments with greater risk of exposure to pathogens, or may use more antibiotics which can induce diarrhea.

Several strategies have been examined to reduce diarrhea among HIV-infected and uninfected children. Use of water chlorination and safe water storage can reduce episodes of diarrhea among HIV-infected children <5 years of age by 30%\(^ {12} \). Among HIV-infected persons of all ages, combining safe water practices with trimethoprim–sulfamethoxazole prophylaxis reduced episodes of diarrhea by 67%\(^ {12} \). However, among HIV-uninfected children <3 years of age, safe water storage and use of water chlorination was not associated with protection from diarrhea, suggesting that other routes of exposure (e.g., hand–mouth contact) may be important in this younger age group. The effect of trimethoprim–sulfamethoxazole prophylaxis on diarrhea in HIV-infected children is not clear; a South African study associated its use with an increased risk of diarrhea in both HIV-infected and uninfected infants,\(^ {13} \) whereas in a Ugandan study its use was associated with a non-significant decrease in the incidence of diarrhea among HIV-infected children <5 years of age (0.84, 95% CI 0.41–1.70).\(^ {9} \) Vitamin A and zinc supplementation have both been associated with reduced risks of diarrhea morbidity and reduced overall child mortality in developing countries;\(^ {14,15,16,17} \) this effect seems to extend to HIV-infected children as well,\(^ {16,17} \) and may be a promising additional strategy to reduce diarrhea morbidity and overall mortality.

There were several limitations to our study. Due to limited local laboratory capacity at the time the study was conducted, we did not test routinely for the full complement of viral or parasitic pathogens, or for diarrheagenic Escherichia coli, which have been established as important causes of pediatric diarrhea.\(^ {18,19} \) Intestinal parasitism with geohelminths in the age group studied is typically low, and generally increases around 2 years of age.\(^ {20} \) Infection with protozoa can be common in this age group, but is more variable depending on the type and source of drinking water, especially for *Giardia intestinalis* and *Cryptosporidium spp*.\(^ {21} \) Because diarrhea surveillance was not instituted at the start of the larger cohort study, and was interrupted during the study, we were not able to assess the importance of recurrent diarrhea. The prevalence of *Campylobacter*, *Salmonella*, and *Shigella* in stool samples of children without diarrhea was similar compared to children with diarrhea, raising cautions regarding the interpretation of a positive stool culture in children with diarrhea; the mere presence of a bacterial pathogen may not be a sufficient factor to explain diarrhea.\(^ {2} \) We were unable to obtain complete information on every child’s diarrhea history; however, we do not feel that any particular bias was introduced by the missing data. One should also interpret cautiously our reported \( p \)-values, given the relatively large number of tests performed and the low number of positive tests in some comparison groups.

In conclusion, diarrhea was more common among HIV-infected children, even though bacterial pathogens such as *Campylobacter* and *Shigella* were recovered less frequently from their stool samples, suggesting that other pathogens (e.g., viruses, parasites, diarrheagenic *E. coli*) or other causes (e.g., malabsorption, metabolic enteritis) may be important in this population. Improving access to safe water, basic sanitation and hygiene, and exploring vitamin A and zinc supplementation could help all children, but may in particular benefit HIV-infected children in whom diarrheal illness was more frequent, more severe, and more frequently persistent.

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**Conflict of interest:** No conflict of interest to declare.

**References**