

## Drinking Water Quality, Feeding Practices, and Diarrhea among Children under 2 Years of HIV-Positive Mothers in Peri-Urban Zambia

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**Abstract.** In low-income settings, human immunodeficiency virus (HIV)-positive mothers must choose between breastfeeding their infants and risking transmission of HIV or replacement feeding their infants and risking diarrheal disease from contaminated water. We conducted a cross-sectional study of children < 2 years of age of 254 HIV-positive mothers in peri-urban Zambia to assess their exposure to waterborne fecal contamination. Fecal indicators were found in 70% of household drinking water samples. In a multivariable analysis, factors associated with diarrhea prevalence in children < 2 years were mother having diarrhea (adjusted odds ratio [aOR] = 5.18, 95% confidence interval [CI] = 1.65–16.28), child given water in the past 2 days (aOR = 4.08, 95% CI = 1.07–15.52), child never being breastfed (aOR = 2.67, 95% CI = 1.06–6.72), and rainy (versus dry) season (aOR = 4.60, 95% CI = 1.29–16.42). Children born to HIV-positive mothers were exposed to contaminated water through direct intake of drinking water, indicating the need for interventions to ensure microbiological water quality.

### INTRODUCTION

Unsafe drinking water is a major cause of diarrheal disease and death in young children in low-income countries. Diarrheal disease is a leading killer of children under 5 years old, accounting for an estimated 21% of deaths of children in developing countries; children under 2 years are especially vulnerable, accounting for the highest portion of morbidity and mortality.<sup>1</sup> It is estimated that almost 900 million people lack access to improved drinking water worldwide, and over 5 million of those people live in Zambia.<sup>2</sup>

Among people living with human immunodeficiency virus (HIV)—including almost 1 million estimated to be living in Zambia<sup>3</sup>—unsafe drinking water presents additional risks. First, people living with HIV (PLHIV) are especially vulnerable because of opportunistic infections from water-related pathogens,<sup>4</sup> such as cryptosporidiosis. Second, diarrheal disease may lead to intestinal malabsorption so that individuals on antiretroviral therapy (ART) are not acquiring essential nutrients and therapeutic dosages of medications.<sup>5</sup> Third, new mothers who are HIV-positive face a difficult choice to either breastfeed their infants and increase the risk of infecting them with the virus or provide formula or other replacement feeds with potentially unsafe water and increase the risk of diarrhea and other waterborne diseases.<sup>6</sup> Overall, up to 16% of infants born to HIV-positive women acquire HIV from breastfeeding alone, accounting for up to 44% of all mother to child HIV transmission.<sup>7</sup>

From 2006 to 2009, the World Health Organization (WHO) recommended that HIV-positive mothers exclusively breastfeed their infants for the first 6 months, except where replacement feeding was “acceptable, feasible, affordable, sustainable, and safe,” and then, replacement feeding was recommended; beyond 6 months, HIV-positive mothers were encouraged to cease breastfeeding and use complementary feeds.<sup>8</sup> In November 2009, these guidelines changed to recommend that HIV-positive mothers “exclusively breastfeed their infants for the first 6 months of life, introducing appropriate complementary foods thereafter, and continue breastfeeding for the first 12 months of life.”<sup>9</sup> Commercial infant formula is only

recommended when, among other conditions, “safe water and sanitation are assured at the household level and in the community.”<sup>9</sup> Zambia integrated these new recommendations into the 2010 National Protocol Guidelines for Integrated Prevention of Mother to Child Transmission of HIV that was published September 2010<sup>10</sup>; at the time of this research, Zambia had not yet adopted the revised guidelines.

Although infant feeding recommendations for HIV-positive mothers often involve impassioned discussions, both sides of the international debate fully endorse the urgent need for safe drinking water for young children born to HIV-positive mothers.<sup>11,12</sup> First, for the select HIV-positive mothers that do choose to replacement feed, “safe water and sanitation” is the first condition set in the new WHO guidelines.<sup>9</sup> Second, even for mothers that choose to breastfeed, infants may be exposed to waterborne pathogens; water treatment has been found to reduce diarrhea among breastfed children.<sup>13</sup> Third, children 6–24 months of age generally experience increased exposure to waterborne pathogens because of the introduction of complementary feeds, including drinking water, and heightened contact with their environment. Finally, young children that do contract the HIV virus will be more susceptible to water-related pathogens because of a weakened immune system and may particularly benefit from improved environmental conditions.

The need for safe drinking water is particularly critical considering that many mothers do not follow the WHO infant feeding recommendations. Despite clear recommendations to the contrary,<sup>14</sup> most breastfed infants receive replacement feeds or supplemental water in addition to breast milk in the first 6 months of life, regardless of their mother’s HIV status. Overall, only an estimated 38% of infants in low-income countries and 30% of infants in sub-Saharan Africa are exclusively breastfed for the first 6 months.<sup>15</sup> Exclusive breastfeeding may be particularly uncommon among HIV-positive mothers who have reason not to breastfeed to prevent virus transmission through breastmilk.<sup>16</sup> Providing infants with supplemental water is also a common practice in Africa; up to an estimated 70% of infants under 6 months may receive supplemental water,<sup>15</sup> apparently because of a belief that breast milk alone provides insufficient hydration in hot climates.<sup>17</sup> However, supplemental water is not recommended and has been found to double the risk of diarrheal disease in infants.<sup>18–20</sup>

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Although there is limited data in low-income settings, studies in Cote d'Ivoire and Thailand found both supplemental water and replacement foods to be contaminated.<sup>21,22</sup> In the cross-sectional study in Cote d'Ivoire among 120 households with children  $\leq 3$  years, *Escherichia coli* and coliform bacteria were detected in 41% and 74% of samples, respectively, despite source water being from a municipal supply of reasonable quality.<sup>21</sup> The study also reported that 90% of all infants had been given drinking water by 1 month of age and that only 3% of women who gave their child water treated it.<sup>21</sup>

We undertook this study to investigate the potential exposure to fecally contaminated drinking water either through replacement and complementary foods or supplemental water in children under 2 years born to HIV-positive mothers. We also sought to explore the association between drinking water quality, environmental conditions, and feeding practices with diarrheal disease. This study was intended to help determine whether this population could benefit from an intervention that encouraged HIV-positive mothers to treat their drinking water at the household level.

## METHODS

**Participant eligibility.** Women were eligible to participate in the study if (1) they were the primary caretaker for a child under 2 years of age at the time of recruitment, (2) the child was born to a mother who was HIV-positive, and (3) they resided in a household located in one of the designated peri-urban settlements in or around Lusaka, Zambia. Nurses and community volunteers from health clinics in each catchment area identified potentially eligible women through under 5 year clinics, nutrition programs, and ART programs and referred them to our field workers. The HIV status of the birthmother was confirmed through the child's health card, where children were listed as exposed based on clinical testing.

**Sample size.** The sample size calculations were based on an estimated 70% of the study population either procuring their drinking water from an improved source (60% in Zambia<sup>2</sup>) or effectively treating their drinking water at home before consumption (35% in Zambia<sup>23</sup>). A sample size of 50 households per site (250 total) would allow us to estimate the proportion of households with acceptable water quality at each site with a precision of at least  $\pm 14\%$  (95% confidence interval [CI] = 56–84%).

**Study sites.** The sites were selected in or around Lusaka based on limited access to piped water. We selected five initial sites in Lusaka District that were only partially serviced by the municipal system: Chipata, John Laing, Kanyama, Misisi, and N'gombe. Because of preliminary findings that the majority of participants in these sites used piped water, we extended the study to include two additional sites lacking piped water systems: Ngwerere in Chongwe District and Mahopo/Shantumbu in Lusaka District. All seven sites were at least partially unplanned settlements. Participants from the initial five sites were recruited from November 2009 to February 2010 (rainy season); recruitment in the additional two sites occurred in March and April of 2010 (dry season).

**Household questionnaires.** Mothers were interviewed to gather information regarding drinking water supply, treatment and storage, hygiene and sanitation practices; characteristics and feeding practices for children under 2 years, diarrhea prevalence in the previous 7 days for all household members,

and household demographics. For specific information on household drinking water, the respondent identified the main drinking water source, distance to water source, and household water treatment practices. Observations were made on whether water storage containers were covered and the method of obtaining water from container (pouring versus dipping with a cup). For specific information on sanitation, mothers were asked where their household goes to the toilet, if those facilities were private, shared, or public, and what method of disposal was used for children's stools. Households were asked if soap for hand washing was present at the time of interview; if so, households were asked to show the soap to the fieldworker. WHO/United Nations Children's Fund (UNICEF) Joint Monitoring Program (JMP) definitions were used to categorize drinking water sources and sanitation facilities as improved or unimproved<sup>24</sup> and child's stool disposal as safe or unsafe.<sup>25</sup>

Child characteristics included child's sex, child's HIV status (if known), child being cared for by birthmother (versus other caretaker), and child's age. For feeding practices, mothers were asked what the child was given to eat on the day of and the day before the interview. Exclusively breastfed at the time of interview was defined as the child only receiving breast milk and no other liquids or solids in the past 2 days, with the exception of oral rehydration salts or supplemental vitamins or medicines.<sup>26</sup> Mothers were also asked if they received counseling on infant feeding and if so, if they were told of mother to child transmission (MTCT) of HIV and given two feeding options. Diarrhea was defined using the WHO definition of three or more loose stools within 24 hours.<sup>27</sup> Data on housing characteristics and assets including electricity, radio, television, refrigerator, bicycle, car, telephone, fuel source, floor material, and agricultural land ownership were also gathered based on the asset questionnaires in the Zambia Demographic and Health Survey (ZDHS).<sup>28</sup>

**Water quality.** For each participant, two water samples were collected: (1) stored drinking water in the household and (2) source drinking water directly from the source where the participant obtained their water. To assess fecal contamination, 125-mL samples were collected in Whirl-Pak bags (Nasco International, Fort Atkinson, WI) that included sodium thiosulfate to neutralize any chlorine. Samples were assayed for thermotolerant coliforms (TTC), a WHO-approved indicator of fecal contamination<sup>29</sup> using the membrane filtration method with membrane lauryl sulphate medium using a DeLagua field kit (Robens Institute, University of Surrey, Guilford, Surrey, United Kingdom). Source and household samples were tested for free and total chlorine residuals using a Hach color-wheel test kit (Hach Company, Loveland, CO). Bacterial assays were performed at the University Teaching Hospital, Lusaka. Source water was also tested for *Cryptosporidium* spp., a waterborne pathogen particularly prevalent in HIV-positive individuals<sup>30,31</sup> and resistant to chlorination.<sup>32</sup> Twenty-five source samples, representative of 10% of the study population, were sampled in March 2010 for *Cryptosporidium* spp. testing; 50-L samples were collected and processed in accordance with Environmental Protection Agency (EPA) Protocol 1622<sup>33</sup> using Envirochek sampling capsules (Pall, Ann Arbor, MI), and samples were assayed by Centre for Research into Environment and Health (CREH) Analytical laboratory in Horsforth, United Kingdom (<http://www.creh.org.uk/>). Stored drinking water in the household was not sampled for *Cryptosporidium* spp. because of the 50-L volume of water needed per sample.

**Data handling and analysis.** All data were double-entered into EpiData 3.1 and analyzed using Stata 10. Socioeconomic status (SES) was measured using an asset index created by combining data on household possessions and housing characteristics using principal component analysis.<sup>34</sup> Because bacterial loads followed a skewed distribution, TTC counts were grouped according to their  $\log_{10}$  values and analyzed categorically. Data were summarized using cross-tabulations and compared using  $\chi^2$  tests. To investigate water intake as a main exposure of interest, we examined feeding practices by what the child was given in the past 2 days: water, other non-nutritive liquids such as juice and tea, nutritive liquids such as milk or formula, and solid foods such as porridge.

We used logistic regression to investigate factors associated with the prevalence of diarrhea in children under 2 years. Potential determinants of diarrhea prevalence were examined using a conceptual framework with four levels: sociodemographic, child characteristics, environmental, and feeding practices.<sup>35</sup> Child's age was grouped into four categories to capture variations in feeding practices and diarrhea (< 3 months, 3 to < 6 months, 6 to < 9 months, and 9–24 months) and included in all models *a priori*, because it was expected that diarrhea prevalence would vary with age. Environmental factors included information on water, sanitation, and hygiene, water quality, season of interview, and whether the mother had diarrhea in the past 7 days.

Sociodemographic factors significantly associated with diarrhea ( $P < 0.10$ ) were included in a multivariable model; the remaining factors independently associated at  $P < 0.10$  were retained in a core model. Child characteristics were added to this core model one by one. Those factors with adjusted association with diarrhea that reached significance at  $P < 0.10$  were included in a multivariable model; those factors remaining significant at  $P < 0.10$  were retained. Associations with environmental factors and feeding practices were determined in a similar way. The final model excluded factors one at a time until all remaining factors were significant at the  $P < 0.05$  level. Because similar analysis was done to investigate factors associated with diarrhea prevalence in children < 5 years, logistic regression with generalized estimating equations (GEE) and an exchangeable correlation matrix to account for clustering within children at the household level was used.

**Ethics.** This study was approved by the ethics committees of London School of Hygiene and Tropical Medicine and the University of Zambia. Additional approval was obtained from the Ministry of Health, Republic of Zambia for recruitment through the health clinics in the study catchment area. Informed written consent to partake in the study was obtained from each participating woman. Additional steps were taken to ensure there would be no adverse impact from participating in the study: interviews were conducted in private, a trained nurse counselor was used full time by the study, and the HIV-positive eligibility criteria were not disclosed to community members aside from the health clinic staff involved in recruitment.

## RESULTS

**Enrollment.** Women (305) were screened for participation; 30 (10%) were ineligible, and 6 (2%) refused to participate. The remaining 269 participants were enrolled. However, 15 (6%) participants could not be located; therefore, data were collected on 254 households (Table 1).

TABLE 1

Household characteristics for all participants ( $N = 254$ ): demographics, water, sanitation, and hygiene practices, and infant feeding counseling and HIV progression

	<i>N</i> = 254	Percent
<b>Demographics</b>		
<b>Sites</b>		
Chipata	53	21
John Laing	25	10
Kanyama	35	14
Mahopo/Shantumbu	9	4
Misisi	53	21
N'gombe	54	21
Ngwerere	25	10
Median number of household members (range)	5	2–12
Median mother's age (range)	29	12–55
<b>Mother's marital status*</b>		
Married/living together	208	82
Single/divorced/widowed/separated	45	18
<b>Mother's education</b>		
None	27	11
Primary	152	60
Secondary or more	75	30
<b>Season</b>		
Dry	34	13
Rainy	220	87
<b>Water, sanitation, and hygiene</b>		
<b>Water source</b>		
Piped into home or yard	8	3
Public standpipe	193	76
Borehole	29	11
Protected dug well	15	6
Unprotected dug well	7	3
Other†	2	1
Time to water source 30 minutes or less*	214	87
<b>Water treatment</b>		
Chlorinate	119	47
Boil	107	42
Solar disinfection	18	7
Currently have treated water in home‡	2	1
Water storage container covered§	37	21
199	92	
<b>Method of obtaining water¶</b>		
Cup used to draw water	183	85
Pour water	32	15
<b>Sanitation*</b>		
Improved and private pit latrine	18	7
Improved pit latrine but shared	191	76
Unimproved pit latrine	30	12
No toilet	14	6
Safe disposal of child's stool*	236	94
Soap present in household§	104	48
<b>Infant feeding counseling and HIV</b>		
Mother received counseling on infant feeding*	227	92
<b>When mother received counseling (<i>N</i> = 227)</b>		
Was told of MTCT	224	98
Given two feeding options	217	95
Asked about water source*	23	10
Asked about fuel	15	9
Asked about fridge	15	9
<b>Mother's CD4 count</b>		
≤ 350	24	9
> 350	35	14
Do not know	195	77
Mother currently on ART**	145	63

\* Data missing for less than or equal to nine households (marital status: one missing; time to water source: nine missing; sanitation: one missing; safe disposal of child's stool: three missing; mother received counseling: six missing; mother asked about water source: one missing).

† Other water sources included bottled water (one household) and surface water (one household). The household drinking bottled water was classified as having improved drinking water because of an improved secondary water source as defined by the WHO.

‡ Data missing for 79 households on currently having treated water.

§ Data missing for 37 households on water storage container covering and having soap present.

¶ Data missing for 39 households on method for obtaining water.

|| Data missing for 67 households on whether mother was asked about fuel and fridge during counseling.

\*\* Data missing for 24 households on whether mother is on ART.

**Demographics.** The median maternal age was 29 years (range = 12–55 years), and 208 (82%) were married or living with a partner (Table 1). Most women (89%) had received at least some education, and the median household size was five members (range = 2–12).

From the 254 participating households, there were 267 children under 2 years, including four sets of twins, five dependents (cared for in addition to birth children), and four sets of siblings all under 2 years. There were 108 children < 6 months of age (median = 1 month), 157 children 6–24 months (median = 12 months), and 2 children with missing age data. There were 374 children under 5 years of age (median = 1 year).

**Water, sanitation, and hygiene.** One hundred ninety-three households (76%) used a public standpipe as their primary drinking water source (Table 1). Of the eight (3%) households with unimproved sources, all were from the two sites lacking a piped water supply (Ngwerere or Mahopo/Shantumbu). All 254 households stored water in the home for drinking. For household water treatment, 119 (47%) of women interviewed reported treating their drinking water to make it safer to drink: 107 (42%) chlorinated, 18 (7%) boiled, and 2 (1%) used solar disinfection (Table 1). Of households that treated their water, 57 (48%) women responded that water was unsafe without treatment, and 41 (34%) reported treating water to improve health. Only 37 (21%) households had treated drinking water in the house during the time of interview.

Although 209 (83%) households had improved pit latrines facilities, 191 of these were shared with other households, rendering them unimproved sanitation according to the JMP definition<sup>24</sup>; only 18 (7%) households were classified as having improved sanitation access. Sanitation facilities were more likely to be unimproved in Ngwerere and Mahopo/Shantumbu compared with the other five areas (74% versus 9%, respectively;  $P < 0.001$ ). Soap was present during the household visit in 104 (48%) households.

**Infant feeding counseling and HIV.** Overall, 227 (92%) mothers reported having received antenatal counseling on infant feeding. Of these women, 224 (98%) were told of MTCT, and 217 (95%) were told about replacement feeding options. However, only 23 (10%) of mothers recalled being asked about their water source, 15 (9%) of mothers recalled being asked about fuel, and 15 (9%) of mothers recalled being asked about refrigeration (all resources considered necessary for safe replacement feeding under applicable WHO guidelines).<sup>36</sup>

**HIV status of children under 2 years.** The 267 children under 2 years were all born to HIV-positive mothers as determined by the eligibility criteria; 5 (2%) of 254 women enrolled were not the birthmothers of the child, because the mother was either deceased or too ill to care for the child. In these circumstances, the HIV status of the caretaker was unknown, although the birthmother was confirmed to be HIV-positive. HIV testing results were known for 142 (53%) children, of whom 33 (23%) were positive (Table 2). For the 125 of unknown HIV status, either the mother did not know or did not wish to share the results.

**Feeding of children under 2 years.** Feeding practices were examined separately for children < 6 months and children 6–24 months because of complementary feeds being recommended for children after 6 months of age (Table 2). Overall, mothers reported breastfeeding 233 (89%) children; furthermore, 102 (94%) children < 6 months and 65 (42%) children 6–24 months were still being breastfed. Among those mothers that had ever breastfed, 168 (80%) mothers chose to breastfeed,

TABLE 2  
Demographics, health, and feeding practices for children under 2 years

Demographics	Children < 6 months (N = 108)	Children 6–24 months (N = 157)	Total (N = 267)
Median age (IQR*) in months†	1 (0.5–3)	12 (9–16)	7 (2–15)
Male‡	54 (50%)	79 (51%)	134 (50%)
Mother's birth child‡	107 (99%)	148 (95%)	257 (97%)
Health			
Diarrhea in past 7 days‡	11 (10%)	58 (37%)	70 (26%)
HIV status			
Negative	22 (20%)	87 (55%)	109 (41%)
Positive	4 (4%)	28 (18%)	33 (12%)
Unknown	82 (76%)	42 (27%)	125 (47%)
Feeding practices‡			
Ever breastfed	103 (95%)	128 (84%)	233 (89%)
Current feeding (today/yesterday)			
Exclusively breastfed§	86 (81%)	7 (5%)	93 (36%)
Water	10 (9%)	142 (93%)	153 (59%)
Milk (formula or fresh)	10 (9%)	31 (21%)	41 (16%)
Juice, tea, or other liquids	1 (1%)	57 (38%)	58 (22%)
Porridge or other solids	12 (11%)	142 (93%)	155 (59%)

\*IQR = interquartile range.

†Age data missing for two children.

‡Data missing for less than or equal to eight children (male: one missing; mother's own child: one missing; diarrhea: two missing; currently breastfed: two missing; exclusive breast-feeding: eight missing; ever breastfed: five missing; water: six missing; juice: seven missing; porridge: six missing).

§Defined as currently breastfed and not given water, milk, other liquids, or other solids the day of and day before the interview.

because they simply wanted to or because breast milk was best for babies. Other reasons for breastfeeding were that formula was not affordable (27 women, 13%) and they were advised to breastfeed during counseling (11 women, 5%). Exclusive breastfeeding was currently practiced for 86 (81%) children < 6 months and 7 (5%) children 6–24 months.

Supplemental water had been given to 10 (9%) children < 6 months and 142 (93%) children 6–24 months within the past 2 days. Only two households reported giving their children different water than was used by the rest of the household for drinking; both reported giving their children bottled water. For the preparation of formula, powdered milk required the addition of water and was often but not always boiled. Tea, porridge, and other solids effectively always involved boiling the water during preparation. Juice was generally made by adding drinking water to a juice concentrate (often made from artificial flavors and colorants), resulting in direct exposure of drinking water.

**Water quality testing.** Water samples were obtained from 234 (92%) households and 85 (33%) source samples (Table 3). The source water was not always available because of piped water only running intermittently or sources being locked during certain hours of the day. For 34 (11%) plates where TTC levels were too numerous to count, a value of 500 coliform forming units (CFUs)/100 mL (the upper detection limit) was assigned. Overall, 163 (70%) household samples had some level of fecal contamination (geometric mean = 195 TTC/100 mL; 95% CI = 141–270 TTC/100 mL). Among source samples, TTC were present in 48 (56%) samples (geometric mean = 2.0 TTC/100 mL; 95% CI = 1.7–2.4 TTC/100 mL). Although contamination was generally higher in household samples compared with source samples (70% versus 56% with some level of fecal contamination), there was only weak evidence for this difference ( $P = 0.12$ ). The areas without piped water,

TABLE 3

Water quality testing results for household and source drinking water samples

	Household samples (N = 234)	Source samples (N = 85)
Fecal contamination (TTC/100 mL)		
0	71 (30%)	37 (44%)
1–10	21 (9%)	16 (19%)
11–100	29 (12%)	11 (13%)
101–1,000	50 (21%)	9 (11%)
> 1,000	63 (27%)	12 (14%)
Free chlorine residual (mg/L)*		
0	151 (65%)	47 (57%)
> 0 to < 0.2	60 (26%)	9 (11%)
0.2–2.0	23 (10%)	27 (33%)
<i>Cryptosporidium</i> spp.† present	N/A	3 (12%)

\* Data missing for two source samples.

† *Cryptosporidium* spp. testing for 25 source samples. Positive samples included 124 oocysts in 50 L (2.5 oocysts/L), 6 oocysts in 50 L (0.12 oocysts/L), and 1 oocyst in 50 L (0.02 oocysts/L).

Ngwerere and Mahopo/Shantumbu, had more contamination compared with the other sites for both household samples (93% versus 66% with some level of fecal contamination;  $P = 0.017$ ) and source samples (81% versus 45% with some level fecal contamination;  $P < 0.001$ )

Recommended levels of free chlorine, defined as  $\geq 0.2$  mg/L 24 hours after treatment,<sup>37</sup> were detected in 23 (10%) household samples and 27 (33%) source samples (Table 3). There was no detectable free chlorine in 151 (65%) household samples and 47 (57%) source samples. In Ngwerere and Mahopo/Shantumbu, there was no detectable chlorine in 25 of 30 (83%) household samples and 26 of 27 (96%) source samples. Overall, source water was more likely to have any detectable free chlorine residual compared with household water (43% versus 35% of samples, respectively;  $P < 0.001$ ); 60% of the households that reported chlorinating their water had no detectable free chlorine in their household water.

Source samples from 9 dug wells, 11 standpipes, and 5 boreholes were tested for *Cryptosporidium* spp. (Table 3). Oocysts were detected in three (12%) samples, two from wells (with levels of 2.5 and 0.12 oocysts/L) and one from a borehole (0.02 oocysts/L).

**Diarrhea prevalence.** Women reported that, during the 7 days before the interview, 70 (26%) children under 2 years experienced diarrhea (Table 2), analogous to at least 13 episodes of diarrhea per child per year. Diarrhea prevalence was 22% (83/372) for all children under 5 years, 13% (163/1250) for all household members, and 7% (17/242) for the women interviewed.

**Factors associated with diarrhea prevalence in children under 2 years.** In the crude analysis, children under 2 years were more likely to have diarrhea if their mother had diarrhea in the past 7 days (odds ratio [OR] = 6.55, 95% CI = 2.35–18.25,  $P < 0.001$ ) and during the rainy (versus dry) season (OR = 3.24, 95% CI = 1.10–9.52,  $P = 0.02$ ). Feeding practices associated with diarrhea were giving water (OR = 10.05, 95% CI = 4.37–23.09,  $P < 0.001$ ), solids such as porridge (OR = 8.12, 95% CI = 3.69–17.90,  $P < 0.001$ ), or other non-nutritive liquids such as juice (OR = 2.62, 95% CI = 1.40–4.89,  $P = 0.003$ ) in the past 2 days. Children that had never been breastfed were more likely to have diarrhea than those children that had ever been breastfed (OR = 4.16, 95% CI = 1.88–9.19,  $P < 0.001$ ). Children were less likely to have diarrhea if there was soap present in their household (OR = 0.53, 95% CI = 0.29–0.98,  $P = 0.04$ ) (Table 4). Children in households with poor water

TABLE 4

Unadjusted regression analysis for diarrhea in children under 2 years

Covariates	N with diarrhea	Percent with diarrhea	Crude OR	95% CI	P
<b>Demographics</b>					
<b>Site*</b>					
Chipata	13/58	22	1		0.02
John Laing	8/25	32	1.63	0.57–4.62	0.02
Kanyama	5/37	14	0.54	0.18–1.67	0.02
Mahopo/Shantumbu	1/10	10	0.38	0.04–3.32	0.02
Misisi	20/54	37	2.04	0.89–4.66	0.02
N’gombe	20/55	36	1.98	0.87–4.52	0.02
Ngwerere	3/26	12	0.45	0.12–1.75	0.02
<b>Season</b>					
Dry	4/36	11	1		0.02
Rainy	66/229	29	3.24	1.10–9.52	0.02
<b>Socioeconomic quintiles</b>					
Lowest	19/69	28	1		0.34
Low	15/38	39	1.72	0.74–3.97	0.34
Middle	11/54	20	0.67	0.29–1.57	0.34
High	13/52	25	0.88	0.39–1.99	0.34
Highest	12/52	23	0.79	0.34–1.82	0.34
<b>Water, sanitation, and hygiene</b>					
<b>Water source</b>					
Improved	68/256	27	1		0.77
Unimproved	2/9	22	0.79	0.16–3.90	0.77
<b>Reported treating water</b>					
No	40/143	28	1		0.53
Yes	30/122	25	0.84	0.48–1.46	
<b>Currently have treated water</b>					
No	42/146	29	1		0.53
Yes	7/37	19	0.58	0.24–1.42	0.21
<b>Water storage</b>					
Closed	55/211	26	1		0.82
Open	4/17	24	0.87	0.27–2.79	0.82
<b>Method of obtaining water</b>					
<b>Dipping with cup</b>					
Pouring	51/193	26	1		0.79
Pouring	8/33	24	0.89	0.38–2.10	0.79
<b>Toilet</b>					
Unimproved	68/248	27	1		0.13
Improved	2/17	12	0.35	0.08–1.58	0.13
<b>Child’s stool disposal</b>					
Safe	67/246	27	1		0.53
Unsafe	3/15	20	0.67	0.18–2.44	0.53
<b>Soap present</b>					
No	37/117	32	1		0.04
Yes	22/111	20	0.53	0.29–0.98	0.04
<b>Mother’s characteristics</b>					
<b>Mother on ART</b>					
Yes	42/154	27	1		0.59
No	21/87	24	0.85	0.46–1.55	0.59
<b>Mother had diarrhea in past 7 days</b>					
No	55/235	23	1		< 0.001
Yes	12/18	67	6.55	2.35–18.25	< 0.001
<b>Child demographics</b>					
<b>Age (months)</b>					
< 3	4/76	5	1		< 0.001
3 to < 6	7/32	22	5.04	1.36–18.68	< 0.001
6 to < 9	8/38	21	4.80	1.34–17.15	< 0.001
9 to < 24	50/117	42	13.43	4.60–39.21	< 0.001
<b>Sex</b>					
Male	40/134	30	1		0.20
Female	30/131	23	0.70	0.40–1.21	0.20
<b>HIV status (if known)</b>					
Negative	36/109	33	1		0.27
Positive	14/32	44	1.58	0.71–3.53	0.27
<b>Infant feeding</b>					
<b>Breastfeeding</b>					
Ever breastfed	53/232	23	1		< 0.001
Never breastfed	16/29	55	4.16	1.88–9.19	< 0.001

(continued)

TABLE 4  
Continued

Covariates	N with diarrhea	Percent with diarrhea	Crude OR	95% CI	P
Water (today/yesterday)					
No	7/108	6	1		< 0.001
Yes	62/151	41	10.05	4.37–23.09	< 0.001
Porridge or solids (today/yesterday)					
No	8/106	8	1		< 0.001
Yes	61/153	40	8.12	3.69–17.90	< 0.001
Juice/tea/liquids (today/yesterday)					
No	45/202	22	1		0.003
Yes	24/56	43	2.62	1.40–4.89	0.003
Milk (today/yesterday)					
No	55/219	25	1		0.20
Yes	14/40	35	1.61	0.78–3.29	0.20
Water quality					
Household water TTC					
≤ 10	22/96	23	1		
11–499	17/63	27	1.24	0.60–2.58	0.56
500+	27/87	31	1.51	0.78–2.92	0.22
Household water free chlorine					
0	42/160	26	1		0.84
< 0.2	18/61	30	1.18	0.61–2.26	0.84
≥ 0.2	6/25	24	0.89	0.33–2.37	0.84
Source water TTC					
≤ 10	13/55	24	1		0.82
11+	9/35	26	1.12	0.42–2.98	0.82
Source water free chlorine					
0	13/51	25	1		0.92
< 0.2	2/10	20	0.73	0.14–3.89	0.92
≥ 0.2	7/27	26	1.02	0.35–2.97	0.92

\*Mahopo/Shantumbu and Ngwerere were studied in the dry season; the other five sites were studied in the rainy season.

quality (TTC ≥ 500) were more likely to have diarrhea than those children in households with better water quality (TTC ≤ 10), although the association was not significant (OR = 1.51, 95% CI = 0.78–2.92,  $P = 0.22$ ).

Diarrhea was also significantly associated with site ( $P = 0.02$ ) and child's age ( $P < 0.001$ ). However, two sites, Mahopo/Shantumbu and Ngwerere, were both studied in the dry season, whereas the others were studied in the rainy season. Therefore, variation between sites could not be completely separated from season. The season variable rather than site was considered in the multivariable model.

In the age-adjusted multivariable analysis, factors that were independently associated with diarrhea prevalence were the mother having diarrhea in the past 7 days (adjusted OR [aOR] = 5.18, 95% CI = 1.65–16.28,  $P = 0.003$ ), the child being given water in the past 2 days (aOR = 4.08, 95% CI = 1.07–15.52,  $P = 0.03$ ), the child never being breastfed (aOR = 2.67, 95% CI = 1.06–6.72,  $P = 0.04$ ), and rainy (versus dry) season (aOR = 4.60, 95% CI = 1.29–16.42,  $P = 0.008$ ) (Table 5).

**Factors associated with diarrhea prevalence in children under 5 years.** Child feeding practices were not collected for children over 2 years; therefore, only demographic variables, child's characteristics, and environmental factors were examined as covariates. In the multivariate analysis, only child's age ( $P < 0.001$ ) and the mother having diarrhea in the past 7 days (aOR = 10.15, 95% CI = 3.48–29.59,  $P < 0.001$ ) were independently associated with diarrhea prevalence in children under 5 years.

## DISCUSSION

To our knowledge, this is the first study to examine water quality exclusively in households with young children born to

TABLE 5  
Multivariable regression analysis for diarrhea in children under 2 years

Covariates	Adjusted OR	95% CI	P
Mother had diarrhea in past 7 days			
No	1		0.003
Yes	5.18	1.65–16.28	0.003
Water (given today/yesterday)			
No	1		0.03
Yes	4.08	1.07–15.52	0.03
Breastfeeding			
Ever breastfed	1		0.04
Never breastfed	2.67	1.06–6.72	0.04
Season			
Dry	1		0.008
Rainy	4.60	1.29–16.42	0.008
Age (months)			
< 3	1		0.13
3 to < 6	4.90	0.98–24.26	0.13
6 to < 9	2.20	0.36–13.62	0.13
9 to < 24	4.32	0.76–24.39	0.13

HIV-positive mothers. One of the key findings is the identification of factors associated with diarrhea in children < 2 years in this study: mother having diarrhea, child given water, child never being breastfed, and rainy (versus dry) season. There was some level of drinking water contamination in the majority of our households; 70% of household samples had detectable levels of fecal contamination. The finding that the majority of household water samples exhibited contamination is particularly concerning, because 97% of our households were drinking from water sources deemed by the JMP to be improved and 47% reported treating their drinking water in the household. These results provide evidence that even improved water sources are not necessarily safe to drink, and households that report treating their water may not be doing it consistently or effectively.

Replacement feeding was not widely practiced by our study participants; 94% of infants under 6 months were currently breastfed, and 81% were currently exclusively breastfed. These percentages may be particularly high as a result of our infants under 6 months being at the younger end of the range (median age = 1 month); exclusive breastfeeding is more common for young infants. Although the new WHO infant feeding guidelines, which recommend HIV-positive mothers to continue breastfeeding up to 2 years after birth, had not been officially implemented in Zambia at the time of this study, 42% of our children 6–24 months were currently being breastfed, implying that the new WHO infant feeding guidelines may be more acceptable to HIV-positive mothers who may prefer to continue breastfeeding beyond 6 months. Although replacement feeding with milk or formula was not widely practiced, 59% of children under 2 years were given water directly in the past 2 days, a potential exposure pathway for waterborne pathogens. Of the replacement and complementary feeds, porridge was the most common, which includes boiling during the preparation process. Other foods and liquids generally did not result in direct exposure to potentially contaminated drinking water, with the exception of juice made from concentrate that required the addition of water.

Diarrhea prevalence in the past 1 week was an alarming 26% in children under 2 years. Breast milk is known to be protective against diarrhea,<sup>38</sup> and children in our study that had ever been breastfed were less likely to have diarrhea in the past 7 days after adjusting for other covariates. We found that diarrhea

prevalence in children under 2 years and under 5 years was strongly associated with the mother having diarrhea in the past 7 days. This finding is consistent with other research, which reported that children under 2 years were five times as likely to have diarrhea if their mother had diarrhea ( $P < 0.001$ ).<sup>39</sup> This association is of particular concern in HIV-affected areas; mothers with HIV may be more likely to have diarrhea and therefore, more likely to pass diarrhea onto their children. Previous research found that HIV-positive adults are seven times more likely to have diarrhea and HIV-positive children under 5 years are four times more likely to have diarrhea compared with HIV-negative individuals.<sup>4</sup> Therefore, the association of diarrhea in mothers and children may provide additional risks for children in households with HIV-positive mothers. However, it is also possible that the mothers were more likely to have diarrhea as a result of their children having diarrhea; we cannot establish a causal relationship with this cross-sectional study.

Children under 2 years were more likely to have diarrhea if they were given water in the past 2 days compared with children that were not given water (after adjusting for other covariates). Previous research has found supplemental water to double the risk of diarrheal disease in children  $< 6$  months, particularly when water quality was poor.<sup>18–20</sup> In our study, diarrhea in children under 2 years was not significantly associated with household water quality, although diarrhea was higher in children from households with poor water quality (TTC  $\geq 500$ ) compared with children from households with better water quality (TTC  $\leq 10$ ) in the crude analysis. A previous meta-analysis found no clear relationship between point of use water quality and general diarrhea, although interventions were found to reduce diarrhea.<sup>40</sup> In our study, it is possible that the TTC indicator did not fully capture water contamination; diarrhea may come from other pathogens such as the protozoa *Cryptosporidium* spp. not inactivated by chlorination<sup>32</sup> or viruses such as rotavirus that may not be fully reflected in the TTC count. Additionally, it is also possible that children are more likely to be given water as a result of having diarrhea, particularly if mothers believe that breast milk does not provide adequate hydration. However, this does not seem to be a normal cultural practice; from ZDHS data, roughly one-third of children are given more liquids when they have diarrhea, one-third of children are given about the same amount of liquids, and one-third of children are given fewer liquids.<sup>41</sup> Therefore, the association between children's diarrhea and water intake may be at least partially attributed to contaminated water, although this cannot be verified from our study.

This study has certain limitations. As with any cross-sectional study, a causal relationship cannot be determined between the outcome (diarrhea) and other covariates. It is possible that reported diarrhea and feeding practices may be altered by recall bias. Because recruitment was conducted from health clinics rather than randomized, our study may not have captured the most vulnerable population that does not regularly access health clinics; however, clinics are generally well used with 99% of mothers in urban Zambia receiving antenatal care during their last pregnancy.<sup>41</sup> Seasonal water quality variations were not captured, because sampling was only conducted one time for each household. Variations in sites cannot be fully separated from seasonal variations because of the sequential method of site sampling. There may be some residual confounding as a result of not being able to adjust for site-level effects. Finally, our study was conducted only in and around Lusaka

and may not be generalizable to other locations with different water quality and practices. However, despite these limitations, our study provides new insight on the exposure to fecal contamination in young children born to HIV-positive mothers.

Strategies to reduce diarrhea among young children in resource-limited settings should consider environmental health improvements, such as improved water supply, water quality, sanitation, and hygiene. The potentially higher diarrhea prevalence among HIV-positive mothers and the association between children's and mother's diarrhea provide evidence that environmental health improvements may be particularly relevant for households with HIV-positive individuals. Although the research is limited, there is promising evidence that both children born to HIV-positive mothers and PLHIV may benefit from safe drinking water and other environmental health improvements.<sup>39,42–44</sup> In areas where the source water is of relatively good quality, such as in some of our study sites, protecting drinking water during storage may be sufficient to ensure household water quality. Safe storage containers have the potential to substantially reduce recontamination of drinking water in the household at low cost and subsequently, reduce diarrheal disease.<sup>45</sup> Improved water quality interventions, such as treating water at the household level, may be particularly beneficial in areas that are dependent on unsafe drinking water sources. Water quality interventions should include treatment methods that inactivate the full array of microbiological pathogens, including *Cryptosporidium* spp., which is of particular concern for HIV-positive populations. High-quality filters that are effective against all waterborne microbes, including chlorine-resistant cysts, and do not present some of the challenges of proper dosing may be especially suitable in these settings, despite their higher up-front cost. Additional investigations in the form of randomized, controlled trials are needed to examine water, sanitation, and hygiene interventions for households with PLHIV and their children.

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