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Feasibility of Using Flash-heated Breastmilk as an Infant Feeding Option for HIV-exposed, Uninfected Infants after 6 Months of Age in Urban Tanzania

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Abstract

Objective—Heat-treating expressed breastmilk is recommended as an interim feeding strategy for HIV-exposed infants in resource-poor countries, but data on its feasibility are minimal. Flash-heating (FH) is a simple in-home technique for heating breastmilk that inactivates HIV while preserving its nutritional and anti-infective properties. Our primary objective was to determine, among HIV-infected mothers, the feasibility and protocol adherence of FH expressed breastmilk after 6 months of exclusive breastfeeding.

Design—Prospective longitudinal

Participants—101 HIV-infected breastfeeding mothers

Setting—Dar es Salaam, Tanzania

Intervention—Peer counselors provided in-home counseling and support on infant feeding from 2 to 9 months postpartum. Mothers were encouraged to exclusively breastfeed for 6 months followed by FH expressed breastmilk if her infant was HIV-negative. Clinic-based staff measured infant growth and morbidity monthly and mothers kept daily logs of infant morbidity. FH behavior was tracked until 9 months postpartum using daily logs, in-home observations, and clinic-and home-based surveys. Bacterial cultures of unheated and heated milk samples were performed.

Results—Thirty-seven of 72 eligible mothers (51.4%) chose to Flash-heat. Median (range) frequency of milk expression was 3 (1–6) times daily and duration of method use on-study was 9.7 (0.1–15.6) weeks. Mean (SD) daily milk volume was 322 (201) mL (range 25–1120). No heated and 32 (30.5%) unheated samples contained bacterial pathogens.

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Conclusion—FH is a simple technology that many HIV-positive women can successfully use after exclusive breastfeeding to continue to provide the benefits of breastmilk while avoiding maternal-to-child transmission associated with non-exclusive breastfeeding. Based on these feasibility data, a clinical trial of the effects of FH breastmilk on infant health outcomes is warranted.

Keywords

HIV; infant feeding; breastmilk; pasteurization; feasibility; heat-treatment; Flash-heat; maternal-to-child transmission

Introduction

Of the 430,000 pediatric HIV infections acquired in 2008, 90% were attributable to maternal-to-child transmission (MTCT)¹; many of these were caused by breastfeeding^{2,3}. Yet replacement feedings have not improved HIV-free survival⁴ and have further resulted in poorer growth⁵ and greater diarrheal morbidity^{6,7} and mortality⁶. Because of breastmilk's critical importance, current World Health Organization (WHO) recommendations for infants born to HIV-infected (HIV+) mothers in developing countries focus on making breastfeeding safer, e.g. by encouraging exclusive breastfeeding (EBF), providing antiretroviral (ARV) prophylaxis, and heat-treating breastmilk⁸.

Heat-treating expressed breastmilk has been a WHO-recommended infant feeding option in the context of maternal HIV for the past decade^{8,9}. In the 2010 WHO guidelines, heat-treated breastmilk is recommended as an interim feeding strategy, e.g. during mastitis, when prophylactic ARVs are unavailable, or to assist mothers to stop breastfeeding. The guidelines note that programmatic data are scarce and call for more research on the feasibility of implementing and sustaining heat-treatment of breastmilk as a strategy to reduce postnatal MTCT⁸.

Flash-heating (FH) is a simple method for in-home breastmilk pasteurization. Briefly, a glass jar containing the milk is placed in a pan with water 2 finger-widths above the level of the milk. The water is heated over high heat and once the water reaches a rolling boil, the milk is removed from the water and cup-fed to the infant when cooled. Milk typically reaches a peak temperature of 72.9° C. Bacteriologic, virologic, immunologic and nutritional studies have indicated that it can be a safe feeding method^{10–13}.

The primary objective of this study was to evaluate the feasibility of HIV-infected mothers FH expressed breastmilk to feed their infants upon introduction of complementary foods. Specifically, we measured the uptake, frequency, and duration of use and adherence to the Flash-heat protocol.

Methods

Subjects and Design

In this prospective longitudinal study, women over 18 years who were currently breastfeeding infants aged between 6 weeks and 3 months and who were permanent local residents were recruited. Participants were from 4 community health center child immunization clinics in the Amana Municipal Hospital catchment area in the Ilala District of Dar es Salaam. The investigation, described as an infant feeding study, entailed weekly home visits by community health workers and monthly clinic visits. HIV status and CD4⁺ counts of mothers were confirmed by review of personal medical records. Women receiving or eligible for ARV therapy based on CD4⁺ < 200 were excluded in order to target infants

most likely to benefit from the intervention. Both HIV-infected mothers (n=101) and those with indeterminate or negative status (n=43) were enrolled to minimize stigma associated with study participation. HIV-infected participants were encouraged to disclose their status to a household member to gain support and facilitate open communication during home visits.

Written informed consent from all participating mothers was obtained. The study was approved by Institutional Review Boards at the National Institute of Medical Research and Muhimbili University of Health and Allied Sciences (MUHAS) in Tanzania and the University of California Davis and was registered at ClinicalTrials.gov (200513446).

Counseling Intervention

Home-based infant feeding counseling was provided by 'peer' counselors (11 females and 1 male) with previous experience as breastfeeding counselors in programs sponsored by the Tanzania Food and Nutrition Center but without biomedical training. For this study, they were provided with a one-week course on HIV and infant feeding training, based on WHO recommendations and Tanzanian National Policy (2007), which included additional training and demonstrations on FH. Periodic 'refresher' sessions were provided.

Peer counselors were asked to visit mothers weekly from 2 until 9 months postpartum. They encouraged EBF during the first 6 months of life and appropriate complementary feeding thereafter, instructed mothers on manual expression of breastmilk, and carried illustrated job aids supporting these messages. HIV-infected mothers were considered eligible for counseling on FH if they were breastfeeding an infant documented to be HIV-negative at 5 months of age. As an additional safety precaution, mothers were instructed to not feed heated milk to their infant until the first heated sample tested negative for bacterial pathogens. FH was demonstrated to eligible women in their homes, unless the mother requested demonstration at the clinic. Mothers eligible and interested in FH were given a plastic bucket containing an aluminum pan, glass jar and graduated plastic feeding cup. Those opting to use the method were encouraged to begin prior to introduction of complementary foods [in order to avoid mixed feeds (non-exclusive breastfeeding)], to heat their milk after each expression and to express and heat their milk as frequently and for as long as possible.

Data collection

Trained clinic-based nursing staff collected baseline demographic and infant feeding information at study entry and data on infant feeding, growth, and morbidity during subsequent monthly clinic visits. Infant morbidity and FH data were also captured daily by mothers using pictorial logs designed for low-literacy. For mothers who were FH breastmilk, peer counselors observed a FH episode biweekly if possible, measured the peak milk temperature and collected pre- and post-heat milk samples for bacterial cultures. Observation checklists of the procedure included noting hand washing, cleansing of jar and cup, appropriate water level in pan, removal of jar of milk from water upon water reaching a rolling boil and procedure duration. Using a pictorial log, mothers were asked to record milk volume expressed and heated during each episode (estimated by use of graduated cup), length of time required and whether time was estimated or measured. Biweekly, peer counselors surveyed the mothers in their homes about their FH experiences in the past 24 hours.

Infants with signs or symptoms of illness were referred to clinic staff. Infants testing HIV+ were referred to an HIV care and treatment center and their mothers encouraged to continue

breastfeeding. Telephone numbers of study nurses were given to mothers to call with queries or concerns.

Laboratory analyses

Qualitative HIV DNA PCR was performed on dried blood spots collected from infants 5 months old by the National HIV Reference Laboratory in the Department of Microbiology and Immunology at MUHAS utilizing the Amplicor HIV-1 DNA PCR assay version 1.5 (Roche Diagnostics, Branchburg, NJ) according to manufacturer's instructions.

Breastmilk aliquots were collected by the counselor in the mother's home using disposable, sterile pipettes. The first sample was taken after the community health worker's FH demonstration; subsequent samples were taken after mothers heated their milk. Aliquots of milk (2.0 mL) were placed in sterile vials prior to heating and again after FH and transported to the bacteriology lab at MUHAS on ice within 3 h of collection. Samples were stored at 2–8°C overnight and then placed at 37 °C the following morning. At 0, 3 and 6 h, aliquots of both unheated and Flash-heated breastmilk were each plated on MacConkey's, mannitol salt (MSA) and blood agars to detect coliforms, *S. aureus* and total colony counts, respectively.

Statistical Analysis

We estimated *a priori* that a sample size of 100 HIV-infected women would be necessary to obtain approximately 30 women who were willing to trial FH, out of an anticipated 75 women who would be eligible for Flash-heat counseling. We hypothesized that at least one-third of women would be willing to try FH. A woman was defined as a 'Flash-Heater' if she chose to use the method after the initial demonstration.

Student's *t*-test was used to compare means and Fisher's exact test was used to compare proportions. Growth was compared between groups with analysis of covariance, using change in z-scores at 9 months as the outcome and z-scores at 6 months as the covariate. Mean and median values were calculated for both an individual and by woman-day (unit of 1 woman heating milk for 1 day).

Results

Study population

One hundred-one HIV-infected, Tanzanian women were enrolled between March 1, 2008 and March 31, 2009. Their mean (SD) age was 27.5 (4.8) years (range 18–41), parity was 2.8 (1.1) and they were 2.4 (0.8) months postpartum (Table 1). Most were married or living with their partner (75.5%), had completed primary school (73.2%), and had disclosed their HIV status to a household member prior to study entry (75.3%). Most used charcoal for cooking fuel (89.7%), had pit latrines (86.7%), and used either a public tap (49.5%) or shallow well (32.0%) for water.

Eighty-six infants of mothers known to be HIV-infected were alive and in follow-up at 5 months of age when infants were tested for HIV (Figure 1). Of these, 72 tested HIV-negative, 13 tested HIV-positive and 1 infant had no record of testing. Fifty-four of the 72 (75%) mothers eligible for counseling on FH agreed to express milk and observe a Flash-heat demonstration.

The mean (SD) number of counselor visits prior to the infant reaching 6 months was 4.5 (6.2). Mothers eligible for FH received 9.7 (5.4) visits. Of those, mothers who chose to Flash-heat received 10.5 (5.7) visits vs. 8.9 (4.9) for those who opted not to. After 6 months,

Flash-heaters received an additional 5.3 (4.4) visits for a total of 15.8 (8.1) visits between 2 and 9 months.

FH behaviors

Uptake of Flash-heat—Thirty-seven of the 72 eligible mothers (51.4%) chose FH after their infant reached 6 months (Fig. 1). One infant died at 6.0 months, prior to the mother commencing FH. Mothers who elected to use FH did not differ in age, religion, parity or education from those who chose not to use FH but may have had lower socioeconomic status as reflected by less use of kerosene stoves, $p=0.042$ (Table 1) and anecdotal reports of the counselors. An increasing proportion of eligible mothers chose FH as the study progressed ($p=0.003$), i.e. 38.8% enrolled in the first 6 months of the study became Flash-heaters compared to 78.2% of women enrolled in the subsequent 7 months.

Data on FH—Data on FH behaviors are reported from daily logs and 24-hour recalls, and observations. Of the 36 women with FH experience, data are available from logs from 35 women covering 1,566 days of FH, from recalled experiences of 27 mothers covering 83 24-hour periods (Table 2) and peer counselors' observations of 80 episodes among 30 mothers expressing and FH milk (Table 3).

Duration of FH—Median (range) duration of FH documented by daily log use during the study was 9.6 weeks (range 1 day to 15.6 weeks); mean (SD) duration of FH during the study was 8.7 (5.3) weeks. Notably, at least 4 women reported FH beyond the period of study follow-up (3 months), 1 of whom continued to do so for an additional 9 months, until her infant was 18 months old. Thus, the reported mean duration of FH during the study is shorter than mean duration of actual use.

Hygiene—In 24-hour recalls, all mothers reported cleansing the utensils. During FH sessions observed by counselors, mothers washed their hands with soap and water 100% of the time. Utensils were washed 95% of the time, with soap and/or boiling water in 78.8% of episodes and with only running water 16.2% of the time.

Breastmilk expression—During FH observations, milk was expressed into the jar used for heating (as recommended) 21.5% of the time, and into a cup or glass during all other episodes. Mean time for expression was 16.7 (7.5) min.

The median frequency of milk expression was three times daily: daily mean (SD) was 2.6 (0.8) based on log results and 3.3 (1.5) times based on 24-hour recall.

Median milk volume per episode was 120 mL based on daily logs and 24-hour recall, and 100 mL during observation. According to recall data, median volumes were higher for fourth and fifth daily expressions (180 mL) than for the first through third expressions (100 mL), i.e. women who expressed more frequently also expressed greater volumes. The linear association between total daily milk volume and number of expressions daily was statistically significant, $p=0.006$ (Figure 2). Median (range) total daily volumes were 300 (25–1120) mL from logs and 360 (10–1200) mL from recalls. When controlling for differences between individuals, there was no significant within-individual relationship between age of infant and daily milk volume expressed.

Heating and feeding expressed milk—Mothers heated and fed milk as frequently as they expressed, suggesting milk was not combined from more than one expression for heating. Reported time lapse between expressing and heating milk ranged from 10 minutes to 7.5 hours (79% of intervals were measured; 21% estimated).

During FH sessions observed by counselors, kerosene was the most frequently used fuel (42.3%), followed by charcoal. In all but two observations, the water in the pan was at the proper level; the milk was removed from the pan prior to the water boiling in only one episode. Observed mean time required for heating was 12.0 (4.6) minutes and mean peak milk temperature was 79.3 (4.4) °C (range 68.0 – 88.8).

While most often mothers fed the heated milk to the infant immediately (71.6% of reports), heated milk was stored in the jar used for heating (9.9%), a cup (12.3%) or bottle (1.2%) anywhere from 5 minutes to 7.7 hours. Stored milk was reported to be covered 78.6% of the time. Mother reported re-using the water 95% of the time, most often for cooking (50.0%) or bathing (40.8%).

During observed FH sessions, infants were fed the milk with a cup in all cases except once each using a spoon and bottle; water was added to the milk in 3.8% of observations, always after heating the milk.

Milk bacteriology—Bacterial cultures were performed on 105 pre- and post-heated samples from 61 mothers. Of the 105 samples collected prior to heating, 44 (41.9%) samples had bacterial growth with a median colony forming units (CFU)/mL of 2.4×10^3 [range 80- too many too count (TMTC)]. All but one of the contaminated samples were positive for coliforms; median coliform count was 2.0×10^3 CFU/mL (range 50- TMTC). Thirty-two samples (30.5%) contained pathogens. Twenty-six samples (24.8%) were positive for *S. aureus* [median concentration 3.0×10^3 (range 90 – 7.8×10^3)]; additional pathogens isolated included *E. coli* and *Klebsiella* and *Bacillus* species. One sample was negative prior to heating and positive for *S. epidermidis* and coliforms after heating, (total colony count 3.4×10^3 CFU/mL). All other 104 heated samples yielded negative bacterial cultures. Of the 44 mothers who provided more than one sample, 3 mothers' samples were contaminated on multiple occasions.

In order to evaluate the risk for staphylococcal food poisoning if unheated samples were stored prior to heating, the growth rate of *S. aureus* was calculated over 6 h at 37° C. Mean (SD) log CFU/mL of those samples testing positive for *S. aureus* was 3.22 (0.04) at 0 hours and at 6 hours it was 3.44 (0.26), $p < 0.003$. No samples reached a concentration of 10^4 CFU/mL.

Stigma—Mothers reported hiding milk expression in 78.3% of the time and FH in 76.0% of the time; 92.6% of mothers hid these practices at some point – usually from neighbors (78.5% of reports of hiding expression and 65.0% of those hiding FH), with fewer reports of hiding from family (21.5%, 26.6%) or friends (7.7%, 16.7%). Most (71.2%) mothers reported that someone in their family or household knew they were FH; most often this was the infant's father (64.9%) with the most common reactions being “supportive” (41.1%), “neutral” (35.7%) and “curious” (21.4%). Forty-six percent of mothers surveyed explained to others why they FH milk; half of these mothers gave the reason of killing HIV and one-third said it was because of a breast infection or problem. Ten percent of mothers reported newly disclosing their HIV status during the study.

Other Outcomes

Infant health—There was no statistically significant difference in number of morbidities (number of days ill with each symptom: diarrhea, cough, runny nose, fever, vomiting, rash or ear infection) between 6 and 9 months in infants who received vs. those who did not receive Flash-heated breastmilk, but there was a trend towards fewer ear infections in those receiving heated breastmilk, $p = 0.08$. Growth was also not significantly different between the 2 groups of infants.

Breast and nipple pain—After 6 months postpartum, there were 4 reports of breast or nipple pain within the past 2 weeks from 3 mothers; 2 who did not Flash-heat compared to 1 Flash-heater $p=0.58$.

Infant diet—Dietary data from 24-hour recalls ($n=217$) after 6 months of age for the 34 infants fed heated breastmilk vs. the 28 who were not revealed no difference in the mean daily number of servings of meat [0.46 (0.39) vs. 0.45 (0.49), $p=0.56$], meat stew [0.05 (0.18) vs. 0.11 (0.27), $p=0.46$] or eggs [0.01 (0.09) vs. 0.06 (0.20), $p=0.24$]. However, infants not receiving expressed, heated breastmilk received a slightly greater mean (SD) number of daily servings of other animal milks than did those receiving human milk, 1.5 (0.9) vs. 1.0 (0.7), $p=0.03$.

In 3 of 83 (3.6%) surveys of Flash-heaters, the infant was fed directly at the breast in the last week, from 2 to 10 times.

Discussion

This study has several important findings. Foremost, a substantial percent (51.4%) of HIV-infected mothers whose infants tested HIV-negative at 5 months were willing to express and Flash-heat their breastmilk upon introduction of complementary foods. This was a greater proportion than the 33% we anticipated. It is notable that the percentage of mothers that chose to Flash-heat dramatically increased over the course of the study, possibly as a result of the counselors' greater experience. Second, we observed that mothers were able to successfully follow the FH protocol and the heated milk was bacteriologically safe. Third, the amount of breastmilk provided to these infants substantially contributed to their diet. The mean daily volume of 322 mL constitutes approximately 34% of caloric needs for an average 6 month infant (weight 7.6 kg)¹⁴, and increased by 2- to 3-fold the mean amount of animal milk they otherwise received (one serving daily). Fourth, most mothers sustained this practice for at least 2 months, with duration varying widely from 1 day to 1 year. Lastly, these mothers succeeded in FH with only modest support from trained peer counselors rather than healthcare providers, suggesting this counseling could be sustainable in terms of costs and workforce capacity at a larger scale in resource limited settings.

In addition to the wide variation in duration of method use, mothers in our study reported a huge range of milk volume expressed daily, from a mean of only 46 mL to nearly a liter. Anti-infective effects of breastmilk are dose-responsive and even partial breastfeeding has been documented to decrease infections in infants^{15,16}. There may be a threshold effect, however, and the lower amounts of daily breastmilk expressed by some mothers in this study may not provide significant immunoprotection while the greater amounts almost certainly would¹⁵. Of note, this feasibility study was not powered to detect differences in growth and morbidity, and should not be used to infer that clinically significant differences would not occur in a larger trial.

Milk volumes at the lower end of the range expressed by mothers in our study likely relate, in part, to relatively infrequent expression (mean 2.3 episodes recorded daily) as milk production declines if the breasts are not emptied regularly and thoroughly^{17,18}. Accordingly, we expect that mothers with more frequent expression would be better able to maintain a robust milk supply, producing greater mean milk volumes and sustaining milk production for longer durations. This expectation is supported by our data whereby mean reported volumes of milk expression for the fourth and fifth episodes in the previous day were greater than mean reported volumes for the first through third episodes, i.e. women expressing more frequently had both larger volumes per expression and larger daily volumes.

One possible strategy that may enable mothers to express milk more frequently would be for them to ‘batch heat’ milk from two or more expressions. This method, involving heating more than one jar of milk in a pan simultaneously, is currently under investigation (K. Israel-Ballard, personal communication). However, it will be necessary to document if peak milk temperatures with ‘batch heating’ are similar to those attained when heating a single jar (the method used during virologic safety studies) prior to recommending this method.

In all contaminated specimens that we tested, bacterial growth that occurred during 6 hours of storage was minimal, albeit statistically significant, and did not result in bacterial counts considered potentially unsafe (10^5 CFU/mL)¹⁷; heating eliminated all pathogens. We surmise that the one sample that was positive for bacteria after heating was contaminated after heating, as the pre-heated sample was bacteriologically negative. Nevertheless, if milk is to be stored, we recommend that milk be heated prior to storage, when possible, to further minimize the *theoretical* risk of staphylococcal food poisoning. It is also important to note that current recommendations are that expressed human milk may be stored for 6 to 8 hours at ambient temperatures up to 25°C¹⁹, with conflicting evidence regarding storage safety at higher temperatures^{20,21,22}. We have previously demonstrated that FH breastmilk does not significantly impact the antimicrobial properties of the milk²³ but note the need for more work to determine the ‘shelf-life’ of expressed breastmilk, heated or unheated, at ambient temperatures above 25°C.

The proportion of mothers who chose to express and heat-treat their milk was somewhat less than the 66% of 30 eligible mothers who did so in a similar study in rural Zimbabwe²⁴. The high rate of uptake in the Zimbabwean study may have been due, in part, to a social marketing campaign that promoted expression and heat-treatment of breastmilk for any mother (even those who are HIV-negative). Our study was performed in the absence of such generalized messages. Although that approach likely decreases stigma, such messages may have unintended consequences, e.g. creating a barrier for HIV-negative women who might otherwise leave fresh expressed breastmilk for their infant when separated. Another salient difference between our study and that of Mbuya *et al* (2010) is that in our study, education and support about FH was provided by lay counselors with a much less intensive home visit schedule (mean of 15.8 visits over 7 m) than did the nurse counselors in Zimbabwe (21 visits over 8 w). Use of lay counselors could be a more cost-effective and sustainable practice given the dearth of health care workers in countries hardest hit by HIV/AIDS²⁵ and where limited resources make breastfeeding imperative²⁶.

There are several limitations to this study. This population was self-selected, as we enrolled willing participants in a research study on infant feeding, and therefore does not necessarily represent the population of all HIV-infected mothers in the area. Women with the most supportive home environments may have enrolled, potentially resulting in a greater proportion of mothers opting to try FH. Alternatively, the burden of study participation may have deterred mothers who would otherwise have chosen to provide Flash-heated breastmilk to their uninfected infants. Further, some of the questionnaires were administered by the counselors who were supporting EBF and FH, possibly resulting in reporting bias.

In summary, our findings add significantly to understanding the feasibility of heat-treatment of breastmilk and reinforce those from Zimbabwe that suggest FH is feasible for many HIV-infected women. Although extended ARV prophylaxis for either the breastfeeding mother or her breastfed child is currently considered to be the optimal strategy for PMTCT⁸, the low coverage of ARV prophylaxis in sub-Saharan Africa²⁷ suggests the need for complementary PMTCT strategies. As with all medical interventions in resource-limited settings, antiretroviral agents are sometimes temporarily unavailable due to ‘stock-outs’; it will be important for mothers to be aware of the WHO recommended option to express and heat-

treat their breastmilk during this time of increased transmission risk. In addition to the four scenarios for which use of heat-treated breastmilk is currently recommended by WHO⁸, Flash-heated breastmilk could also be provided after ARV cessation at 12 months to further prolong provision of safe breastmilk to these vulnerable infants. Indeed, breastmilk continues to have dual nutritional and immunoprotective importance beyond the first year, the latter evidenced by the 1.6 fold increase in mortality from infectious causes amongst non-breastfed infants in resource poor settings compared to their breastfed counterparts in the second year of life²⁸.

Based on the feasibility data to date, a clinical trial of the effects of Flash-heated breastmilk on infant health outcomes is warranted. Only with such information will it be possible to provide both PMTCT programs and the families they serve with evidence-based recommendations. Finally, as countries seek to implement the 2010 WHO guidance, rigorous monitoring and evaluation around their inclusion of heat treatment within PMTCT programs is necessary to appropriately guide scale-up of such strategies.

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Figure 1.
Flow diagram of study population

Figure 2.
Mean Daily Milk Volume by Daily Number of Episodes of Milk Expression

Table 1

Socio-demographic Characteristics of HIV(+) Women in Flash-heat Study

Demographic Characteristic	All HIV(+)women enrolled in study (n=101)	HIV (+) women with documented HIV (-) Infants (n=72)		p-value FH vs. No FH
		Choose FH (n=37) **	Choose no FH (n=35)	
Mean (SD)				
Age (years)	27.5 (4.8)	27.2 (5.3)	28.0 (4.3)	0.49
Parity	2.8 (1.1)	2.9 (1.3)	2.8 (1.0)	0.74
Number in Household				
Adults	2.6 (1.2)	2.5 (0.8)	2.6 (1.1)	0.81
Children	2.8 (1.3)	3.0 (1.6)	2.5 (1.0)	
●	36	7	15	0.26
Number of subjects (Percent of subjects ^{##})				
Infant Gender				
Male	46 (46.0)	20 (54.0)	15 (42.9)	0.34
Female	54 (54.0)	17 (46.0)	20 (57.1)	
●	1			
Education [§]				
None	9 (9.3)	5 (13.9)	2 (5.7)	0.39
Primary	71 (73.2)	26 (72.2)	26 (74.3)	
Secondary	15 (15.5)	4 (11.1)	7 (20.0)	
Higher	0 (0)	0 (0)	0 (0)	
Other	2 (2.1)	1 (2.8)	0 (0)	
●	4	1	0	
Married or Living with Partner				
Yes	74 (75.5)	27 (75.0)	26 (74.3)	0.95
No	24 (24.5)	9 (25.0)	9 (25.7)	
●	3	1	0	
Previous HIV disclosure to household member ^{&}				
Yes	73 (75.3)	29 (80.6)	23 (65.7)	0.16
No	24 (24.7)	7 (19.4)	12 (32.3)	
●	4	1	0	
Source of water				
Public tap	48 (49.5)	18 (50.0)	20 (58.8)	0.82
Shallow well	31 (32.0)	11 (30.6)	10 (29.4)	

Demographic Characteristic	All HIV(+)women enrolled in study (n=101)	HIV (+) women with documented HIV (-) Infants (n=72)		p-value FH vs. No FH
		Choose FH (n=37) **	Choose no FH (n=35)	
Pond	0 (0)	0 (0)	0 (0)	
Piped to home	1 (1.0)	1 (2.8)	0 (0)	
Other	17 (17.5)	6 (16.7)	4 (11.8)	
●	4	1	1	
Toilet facility				
Flush latrine	10 (10.2)	2 (5.6)	6 (17.1)	0.15
Pit latrine	85 (86.7)	33 (91.7)	28 (80.0)	
VIP latrine	2 (2.0)	0 (0)	1 (2.9)	
None/bush	1 (1.0)	1 (2.8)	0 (0)	
●	3	1	0	
Cooking fuel##				
Firewood	16 (16.5)	8 (22.9)	4 (11.4)	0.21
Kerosene stove	21 (21.7)	4 (11.4)	11 (31.4)	0.042
Charcoal	87 (89.7)	30 (85.7)	31 (88.6)	1.00
Gas	0 (0)	0 (0)	0 (0)	--
Electrical	3 (3.1)	2 (5.7)	0 (0)	0.49

● Data missing;

* Percent may not add to 100 due to rounding;

Percent is of subjects with known status;

\$ Highest level of formal education;

& At entry;

£ Multiparous women only;

* At 6 m; FH=Flash-heating; VIP= Ventilated Improved Pit (Blair Toilet);

** 1 infant expired on 6 m birthday -mother's choice was FH, but never occurred – included here;

Percent exceed 100% as some mothers reported more than one fuel source;

Table 2

Flash-heat Behaviors Based on Maternal Daily Logs and 24-Hour Recalls

	Data from daily logs		Data from 24 h recall interviews	
	by day (n=1566)	by person (n=35) [‡]	by interview [#] (n=83)	by person (n=27) [‡]
Episodes/day [^]				
Express				
Mean (SD)	2.6 (0.8)	2.3 (0.7)	3.3 (1.5)	2.9 (1.3)
Median (Range)	3 (1–6)	2.1 (1 – 4)	3 (1–7)	3 (1 – 6.2)
Heat				
Mean (SD)			3.4 (1.4)	2.9 (1.3)
Median (Range)	N/A	N/A	3 (1–7)	3 (1 – 6.2)
Feed				
Mean (SD)			3.6 (1.8)	3.3 (1.6)
Median (Range)	N/A	N/A	3 (1–11)	3 (1 – 8.8)
Volume per episode (mL) [^]				
Mean (SD)	125 (55) mL	108 (46)	128 (56)	N/A
Median	120	104	120	100–180 ^{&}
Range	(10–300)	(30–190) [*]	(10–200)	N/A
Daily volume (mL) [^]				
Mean (SD)	322 (201) mL	266 (164)	430 (339)	341 (278)
Median	300	225	360	233
Range	(25–1120)	(30–689) [*]	(10–1200)	(34–986)
Method duration (w)				
Mean	N/A	8.7 (5.3)	N/A	N/A
Median		9.7		
Range		(0.1–15.6)		

[^] Based on 4,043 episodes of FH as reported by logs and 259 episodes of FH reported in 24-hour recall;[#] As reported for past 24 hours on periodic interviews;^{*} Represents range of maternal means;[‡] Not all mothers who Flash-heated filled out daily logs or were surveyed;[&] Median volume first episode/day 100 mL increased to 180 mL for 5th episode of day

Table 3

Flash-heat Behaviors Based on Observed Episodes

	Observed Experiences (n=80)*
	% of episodes
Washed hands with soap and water	100.0
Cleaned utensils #	
Soap	77.6
Boiling	50.0
Running water only	16.2
Not washed	5.0
Milk Expression Appeared...	
Comfortable	88.3
Uncomfortable	7.8
Painful	3.9
Easy or very easy	91.0
Slightly difficult or difficult	9.1
Heat source	
Kerosene	42.3
Charcoal	39.7
Firewood	16.7
Gas stove	1.2
Water level	
Correct	97.5
Too high	1.2
Too low	1.2
	Mean (SD)
Duration (minutes)	
Milk expression	16.7 (7.5)
Milk heating	12.0 (4.6)
Peak milk temperature (°C)	79.3 (4.4)
Milk volume/expression (mL)	121 (58)

* Observations of 30 mothers;

Some mothers used soap and boiled utensils.