

Viral, Nutritional, and Bacterial Safety of Flash-heated and Pretoria Pasteurized Breast Milk to Prevent Mother-to-Child Transmission of HIV in Resource-Poor Countries: A Pilot Study

K A Israel-Ballard*¹, C J Chantry², R Donovan³, H Sheppard³, J R Carlson², B Lonnerdal⁴, A C Sage², B F Abrams¹

¹University of California, Berkeley, California, ²University of California, Davis Medical Center, Sacramento, California, ³California Department of Health Services, Richmond, California, ⁴University of California, Davis, California

*Corresponding author:

Kiersten Israel-Ballard, MPH
School of Public Health
University of California, Berkeley
Office: (510) 381-6335
Fax: (510) 849-4832
Email: ballardk@berkeley.edu

Revised Abstract

Background: Heat-treatment of expressed breast milk (BM) is an infant feeding option advocated by WHO, yet practical methods have not been systematically evaluated. The safety of two simple heating methods, Flash-heating (FH) and Pretoria Pasteurization (PP) is presented here.

Methods: Fresh BM from healthy, HIV negative women volunteers was aliquoted to unheated controls, FH (50mL BM heated in a 450mL water jacket until water boiled, then BM removed), or PP (450 mL of water brought to a boil, removed from heat, and 50mL BM placed in the water covered for 20 min), simulating field conditions with an aluminum pan as a water jacket, glass peanut butter jar containing the heated milk, and open flame (Figure 1).

HIV detection: 5 samples were spiked with 1x10⁶ copies/mL of HIV-1 subtype C. One mL samples were quantitatively assessed for reverse transcriptase (RT) activity.

Nutrition: Samples were analyzed for the impact of heat on vitamins (B₆, B₁₂, C, folate, riboflavin, thiamin) and on proteins (lactoferrin (LF), lysozyme).

Bacteriology: Storage safety for 12 h was studied by a) spiking BM with *E. coli* or *S. aureus* and aliquoting to heat then store or store then heat; b) and by measuring the effect of heat on antibacterial properties by heating BM then spiking.

Results: Comparison of RT activity for each sample is shown (Figure 3). FH nor PP significantly decreased vitamin concentrations. FH trended toward greater vitamin retention than PP. LF concentration decreased (p<0.05) with heat but the relative proportion of LF and lysozyme that survived digestion was similar for FH and PP. FH and PP, either before or after storage, eliminated the spiked bacteria. FH samples inhibited more bacterial growth than PP.

Conclusion: These pilot data suggest FH may be superior to PP in eliminating residual RT activity and in retaining nutritional and antimicrobial properties. These findings support FH as a safe and practical infant feeding option.

Introduction

Over one-third of perinatal HIV infections in sub-Saharan Africa occur postpartum through breastfeeding alone. The World Health Organization (WHO) recommends that HIV positive women should avoid breastfeeding when other feeding options are acceptable, feasible, affordable, safe, and sustainable¹, yet practical feeding alternatives that meet these criteria are desperately needed. Formula feeding has been associated with a 14-fold increase in diarrhea-associated mortality for all infants and a 25-fold increased risk in infants less than 2 months old². In contrast to the estimated 200,000-350,000 infants who contracted HIV through breastfeeding last year³, WHO estimates that 1.5 million infants died because they were not breastfed⁴. In addition, recent analysis documents that locally available alternatives to infant formula recommended by WHO/UNICEF are nutritionally inadequate⁵. Use of manually expressed, heat-treated breast milk is recommended by WHO, UNICEF, and UNAIDS⁶. Methods listed include 1) Holder Pasteurization (heating to 62.5°C for 30 minutes), which may not be feasible due to the need for temperature gauges and timing devices, and 2) Direct boiling (placing the milk in a pan with no water and boiling the milk directly), which most likely causes significant nutritional damage and has not been fully studied⁷. Here, we present data on the safety of **Flash-heat**⁸ and **Pretoria Pasteurization**⁹, simple and practical heating methods that could be used by mothers in developing countries. Previously we have reported data from focus groups suggesting that mothers in Zimbabwe might be willing to attempt manual expression and heat treatment of breast milk¹⁰. We have also shown that RT, rather than PCR, is the suitable method for detecting inactivated HIV post heat treatment¹¹.

Objective

To perform preliminary investigation and comparison of the virological, nutritional, and antimicrobial safety of the Flash-heat and Pretoria Pasteurization heating methods.

Methods

Fresh breast milk samples collected from healthy, volunteer mothers (not infected with HIV) in Berkeley and Sacramento/Davis, California in the United States

Heat treatments

Simple methods which could be implemented by mothers in their kitchens or over a fire
Field conditions were simulated (Figure 1)



Figure 1. Flash-heat experiment using simple equipment (same equipment used for Pretoria Pasteurization)

- 1:1 Hart 1 qt. aluminum pan from South Africa for 450mL water bath jacket
- 16 oz. glass peanut butter jar for 50mL breast milk
- a single burner butane stove to imitate intense heat of a fire

Flash-heat protocol

- Breast milk and water are heated together in a water bath
- When water boils, remove milk immediately from both the water and heat source

Pretoria Pasteurization protocol

- Boil the pan of water
- Remove it from the heat source
- Immediately place covered glass peanut butter jar of breast milk in the water for 20 minutes
- Water and milk temperatures tracked using DualLogR Thermocouple thermometer probes (Table 1 and Figure 2)

	Peak Temperature (C)	Time Above 56.0C
Flash-heat	72.9	6 min 15 sec
Pretoria Pasteurization	63.8	13 min 52 sec

Table 1. Typical temperatures reached by Flash-heat and Pretoria Pasteurization.

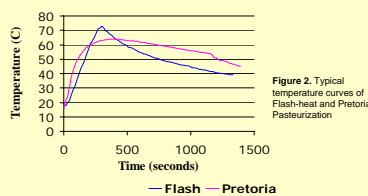


Figure 2. Typical temperature curves of Flash-heat and Pretoria Pasteurization

HIV Assays

- Five 120 mL samples were collected from mothers as described.
- Samples to be spiked with HIV were inoculated with 1 x 10⁶ copies/ml of cell-free clade C HIV
- The following study arms were assayed for HIV:
 - Unheated fresh, whole breast milk spiked with HIV
 - Flash-heated fresh, whole breast milk spiked with HIV
 - Pretoria Pasteurized fresh, whole breast milk spiked with HIV
 - Unheated PBS spiked with HIV
 - Flash-heated PBS spiked with HIV
 - Pretoria Pasteurized PBS spiked with HIV
 - Unheated fresh, whole breast milk NOT spiked with HIV
- Samples were assayed for HIV by Reverse Transcriptase (RT) enzymatic activity assay (ExaVir Quantitative HIV-RNase Reverse Transcriptase Load Kit, Cavid, Uppsala, Sweden)

Nutrition Assays

- Milk samples collected as described; sample size varied
- Study arms included:
 - Unheated fresh, whole breast milk
 - Flash-heated fresh, whole breast milk
 - Pretoria Pasteurized fresh, whole breast milk
- Vitamin Assays
 - HPLC methodology; Mayo Central Laboratory for Clinical Trials (Rochester, MN):
 - Ascorbic Acid
 - Thiamin
 - Riboflavin
 - Pyridoxal-5-phosphate (Vitamin B₆)
 - Folate (Bayer Advia; Centaur instrument)
 - Vitamin B₁₂ (Bayer Advia; Centaur instrument)
 - Protein Assays for Lactoferrin and Lysozyme
 - Lönmædal laboratory, Dept. of Nutrition, University of CA, Davis
 - Quantified by ELISA
 - In vitro digestion, SDS-Page and Western Blot

Bacteriology Assays

- To determine if heating was safer before versus after storage
 - 1100 mL of fresh breast milk pooled from ten mothers
 - Unspiked control removed
 - Remaining samples spiked with 1x10⁶ *E. coli* or *S. aureus*
 - Following study arms were subcultured at stated time points:
 - Flash-heat then store at 25°C for 0,4,8,12 hrs
 - Pretoria Pasteurized then store at 25°C for 0,4,8,12 hrs
 - Stored at 25°C for 0,4,8,12 hrs then Flash-heated
 - Stored at 25°C for 0,4,8,12 hrs then Pretoria Pasteurized
 - Unheated, unspiked control at 0,4,8,12 hrs
- To determine the effect of heat treatments on the natural antimicrobial properties of breast milk
 - Study arms included:
 - Unheated fresh, whole breast milk
 - Flash-heated fresh, whole breast milk
 - Pretoria Pasteurized fresh, whole breast milk
 - Saline controls
 - Each aliquot was then inoculated with 1 x 10⁶ CFU/ml of either *E. coli* (ATCC 25922) or *S. aureus* (ATCC 25923)
 - Samples were incubated at 25°C for 12 hours and then quantitative subcultures were performed

Results

HIV Assay Results

- RT assay results in 5 breast milk samples treated by 1) Flash-heat, 2) Pretoria Pasteurization, 3) No Heat, or 4) No Virus are shown (Figure 3)

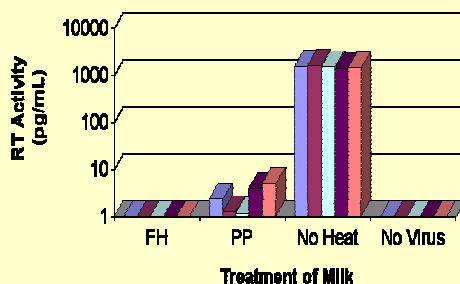


Figure 3. Comparison of RT assay results among Flash-heat, Pretoria Pasteurization, and unheated and unspiked controls in 5 different samples.

Nutrition Assays Results

- No significant difference in vitamin concentration in Flash-heat or Pretoria Pasteurization samples compared to unheated samples (Vitamin A was also assessed but data not shown due to an interfering substance) (Table 2)
- Reduction in concentrations of vitamins C and E was observed
- B vitamins and folate concentrations tended to increase with heat treatment
- Significant reduction in lactoferrin concentration observed for both heat treatments
- Non-significant reductions in lactoferrin and lysozyme % digestion observed for both heat treatments

	Flash	Pretoria	No Heat
Vitamin B12 (pg/mL) (n=6)	862.8 (667.0)	862.3 (651.1)	724.5 (488.0)
Vitamin C (mg/dL) (n=5)	4.2 (2.5)	3.6 (2.2)	5.3 (1.6)
Vitamin E (mg/L) (n=9)	2.7 (2.0)	2.4 (1.3)	3.0 (1.2)
Pyridoxal-5-phosphate (ug/L) (n=5)	29.0 (17.2)	28.0 (17.4)	28.8 (17.3)
Folate (ng/mL) (n=5)	18.7** (3.8)	19.0* (1.5)	13.2 (1.2)
Riboflavin (ug/L) (n=5)	728.0 (1142.9)	704.4 (1094.5)	667.2 (955.0)
Thiamin (nmol/L) (n=4)	18.0 (9.1)	17.8 (8.2)	12.3 (4.6)
Lactoferrin concentration (ELISA, mg/ml) (n=10)	0.214* (272)	0.686* (248)	1.341 (0.154)
Lactoferrin digestion (% digested) (n=9)	84.0 (10.1)	80.3 (9.8)	87.7 (12.5)
Lysozyme digestion (% digested) (n=9)	70.6 (21.0)	75.8 (14.9)	89.0 (14.1)

Table 2. Nutrient assay results (*p<0.05, compared to non-heated samples.)

Bacteriology Assays Results

- Heat then store versus store then heat
 - Both Flash-heat and Pretoria Pasteurization eliminated spiked *S. aureus* or *E. coli* and maintained bacterial inhibition whether heating before vs. after storage
- Effect of heat on antimicrobial properties
 - Greater inhibition by Flash-heat compared to Pretoria Pasteurization of *E. coli* and *S. aureus* growth (by factors of 2.9 and 2.2, respectively)
 - Unheated breast milk had greater inhibition of *E. coli* and *S. aureus* growth over 12 hrs post inoculation, compared to Flash-heat (by factors of 23.81 and 3.78, respectively) and to Pretoria Pasteurization (by factors of 68.25 and 8.22, respectively)

Discussion

- Flash-heat method appeared superior to Pretoria Pasteurization method in eliminating residual RT activity
- Nutrition
 - Post heat treatment increase in thiamin, riboflavin, vitamin B₆, folate, and vitamin B₁₂ may be due to release of vitamins from binding proteins in the milk
 - Power to detect 25% decrease in riboflavin, thiamin, and B₆ only
 - Significant reduction in lactoferrin may not actually impair its biological activity¹²
 - Lactoferrin and lysozyme should remain biologically active in the infant GI tract
- Bacteriology
 - Lack of refrigeration in developing countries mandates that safe storage must be possible for heat-treated breast milk
 - Safest option may be to store first then heat-treat prior to feeding, as the heat treatment would also destroy any potential contamination¹³
- Limitations
 - Small sample size; need confirmation in a larger sample size of naturally infected breast milk from HIV positive mothers in developing countries
 - Impact of heat treatments on cell-associated HIV-1 and on bioactivity of lactoferrin, lysozyme, and other anti-infective proteins (e.g. sIgA) not assessed
 - Acceptability and feasibility of heat-treating breast milk not addressed

Conclusions

- Virology
 - Flash-heat and Pretoria Pasteurization inactivated ≥ 3 logs cell-free HIV-1 RT activity
- Nutrition
 - No significant decreases were observed with either heat treatment for most nutrients assayed, but the small sample size precludes detecting differences which may be clinically significant
 - There was a trend towards higher concentrations of vitamins B₁₂, C, E, and lactoferrin digestion retained by the Flash-heat method compared to Pretoria Pasteurization
- Bacteriology
 - Flash-heat method was more inhibitory to bacterial growth than Pretoria Pasteurization
 - Heating destroys *E. coli* or *S. aureus* contamination
- Overall Safety of Heat-treated Breast Milk
 - These findings support Flash-heat as a potentially safe and practical infant feeding option that merits further research

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