Flash-heated and Pretoria Pasteurized destroys HIV in breast milk & Preserves Nutrients!

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Abstract

..... our findings suggest that these 2 simple heating methods, which do not require technical equipment, may be capable of HIV denaturation while retaining many of the protective elements of breast milk. FH may be more reliable than PP (Pretoria pasteurization) for complete viral destruction and may have less impact on nutritional and antimicrobial properties…

Introduction

Over one-third of prenatal 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 sustainable[1], 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 old[2]. In contrast to the estimated 200,000-350,000 infants who contracted HIV through breastfeeding last year[3], WHO estimates that 1.5 million infants died because they were not breastfeed[4]. In addition, recent analysis documents that locally available alternatives to infant formula recommended by WHO/UNICEF are nutritionally inadequate[5]. Use of manually expressed, heat-treated breast milk is recommended by WHO, UNICEF, and UNAIDS[6]. 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 studied[7]. 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 milk[10]. We have also shown that RT, rather than PCR, is the suitable method for detecting inactivated HIV post heat treatment[11].

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)

  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.

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

- Water and milk temperatures tracked using DuaLogR Thermocouple thermometer probes (Table 1 and Figure 2).

HIV Assays

- Five 120 ml samples were collected from mothers as described.
- Samples to be spiked with HIV were inoculated with 1 x 108 copies/ml of cell-free clade C HIV
- The following study arms were assayed for HIV:

1) Unheated fresh, whole breast milk spiked with HIV

2) Flash-heated fresh, whole breast milk spiked with HIV

3) Pretoria Pasteurized fresh, whole breast milk spiked with HIV

4) Unheated PBS spiked with HIV

5) Flash-heated PBS spiked with HIV

6) Pretoria Pasteurized PBS spiked with HIV

7) Unheated fresh, whole breast milk NOT spiked with HIV

- Samples were assayed for HIV by Reverse Transcriptase (RT) enzymatic activity a

ssay (ExaVir Quantitative HIV-Reverse Transcriptase Load Kit,avidi, Uppsala, Sweden)

Nutrition Assays

- Milk samples collected as described; sample size varied
- Study arms included:

1) Unheated fresh, whole breast milk

2) Flash-heated fresh, whole breast milk

3) Pretoria Pasteurized fresh, whole breast milk

- Vitamin Assays

HPLC methodology; Mayo Central Laboratory for Clinical Trials (Rochester, MN):
Ascorbic Acid
Thiamin
Riboflavin
Pryidoxal-5-phosphate (Vitamin B6)

	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.

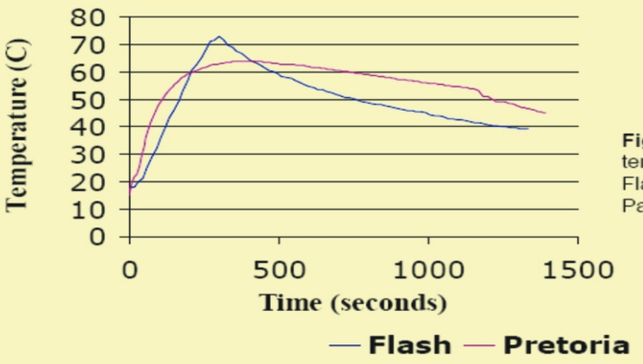

Figure 2. Typical temperature curves of Flash-heat and Pretor Pasteurization

Folate (Bayer Advia: Centaur instrument)
Vitamin B12 (Bayer Advia: Centaur instrument)

- Protein Assays for Lactoferrin and Lysozyme

Lonnerdal 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:

1) Flash-heat then store at 25ºC for 0, 4,8,12 hrs

2) Pretoria Pasteurized then store at 25ºC for 0,4,8,12 hrs

3) Stored at 25ºC for 0,4,8,12 hrs then Flash-heated

4) Stored at 25ºC for 0,4,8,12 hrs then Pretoria Pasteurized

5) 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:

1) Unheated fresh, whole breast milk

2) Flash-heated fresh, whole breast milk

3) Pretoria Pasteurized fresh, whole breast milk

4) 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 25ºC 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)

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.

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 12hrs 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

Virology

- 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.

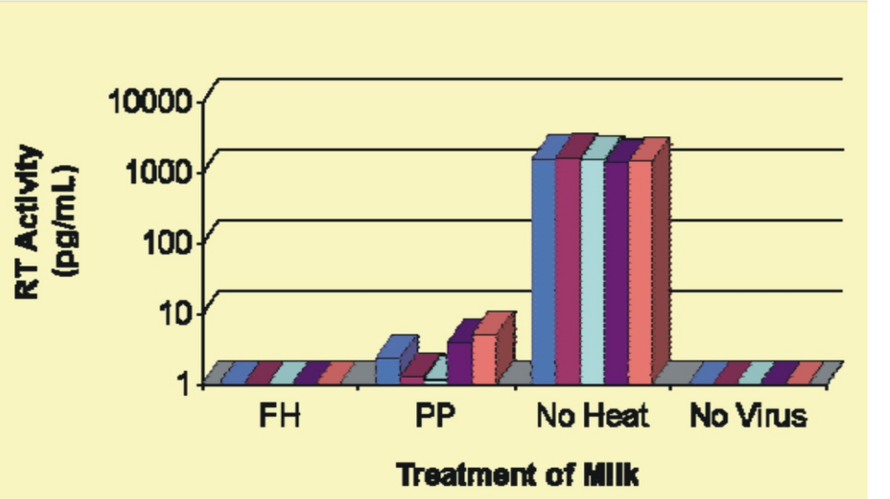

Figure 3. Comparison of RT assay results among Flash-heat, Pretoria Pasteurization, and unheated and unspiked controls in 5 different samples.

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

	Mean (sd)		
	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)	13.3 (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 (6.8)	12.3 (4.6)
Lactoferrin concentration (ELISA, mg/ml) (n=10)	0.214* (.272)	0.826* (.246)	1.341 (0.154)
Lactoferrin digestion (% digested) (n=9)	84.0 (10.1)	80.3 (8.8)	87.7 (12.5)
Lysozyme digestion (% digested) (n=9)	70.6 (21.0)	75.8 (14.9)	89.0 (14.1)

- 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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