EFFICACY OF FLASH HEAT TREATMENT VS HOLDER PASTEURIZATION ON ANTIMICROBIAL ACTIVITY AND IMMUNOGLOBULIN A PRESERVATION IN DONOR BREAST MILK

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ABSTRACT

BACKGROUND: Human milk is considered the optimal nutritional source for infants.Due to the possibility of microbial contamination during collection and handling, milk is pasteurized to prevent transmission of pathogens. In low income areas where pasteurization is inaccessible, the pursuit for the best alternative in rendering donor milk safe remains.

OBJECTIVE: We aimed to determine and compare the efficacy of flash heat treatment and holder pasteurization in preserving human milk IgA while reducing bacterial contamination ofdonor breast milk.

METHODOLOGY: This is an experimental study which utilized pooled donor breastmilk from healthy mothers later subjected to bacterial analysis and immunoglobulin A level determination prior to and post flash heat treatment and pasteurization. Standardized scores were used to normalize population with unknown parameters. T-test comparison of means and Levene's test for equality of variances were used.

RESULTS: Twenty samples of aliquoted breastmilk were subjected to pasteurization and flash heat treatment, both yielding a statistically significant reduction in colony forming units using Blood agar and MacConkey plates. These sample groups also underwent IgA level determination using Bindarid Kit IgATM and there was no significant decline in IgA levels.

CONCLUSIONS: Flash heat treatment may be an alternative for holder pasteurization in providing safe and effective breastmilk.

KEYWORDS: Breastmilk, flash heat, immunoglobulin A, antimicrobial, pasteurization

INTRODUCTION

Human milk is considered the optimal nutritional source for infants. The World Health Organization recommends exclusive breastfeeding for the first six months of life. Breast milk is safe and contains antibodies that protect infants from common childhood illnesses. Immunoglobulin A (IgA), present in the colostrum and milk offers passive protection for the gastrointestinal system. When breastfeeding is not possible, pasteurized human donor milk is the best alternative.

Due to the possibility of microbial contamination during collection and handling, milk is pasteurized to prevent transmission of pathogens to the infant. Holder pasteurizationuseslow temperature long time pasteurization (LTLT), rendering the milk at 62.5 C for 30 minutes, by which most pathogenic organisms are inactivated.[(1)]Flash-heat mimics high-

temperature, short-time (HTST) pasteurization, wherein it imitates the intense heat of a fire, until the water reaches 100°C and is at a rolling boil. Milk is immediately removed from water bath and immersed in crushed ice. [2]Since contents of immunoglobulin in milk are known to be thermolabile compounds, heat denaturation has been observed in both pasteurization methods.

We aimed to determine andcompare the efficacy of flash heat treatment and holder pasteurization in preserving human milk IgA with the ability to reduce bacterial contamination of the donor breast milk.The data gathered from this paper will be developed to support the use of flash heat treatment in low income areas where pasteurization is inaccessible.

If breastfeeding is not possible, pasteurized donor human milk is considered the best alternative. However, human milk is a perfect culture media for microorganisms due to its relatively easy contamination. Microbial contamination can occur during collection and handling of the human milk, which paved the way for pasteurization for the prevention of transmission of pathogens to the infant.

Milk processing in North America follows guidelines set out by the Human Milk Banking Association of North America (HMBANA) [3]. HMBANA guidelines for operation of a donor milk bank uses Holder method of pasteurization for 30 minutes at 62.5°C, then immediately cooled down by immersing in crushed ice. [1,3] Similar guidelines from the Human Milk Banking Association of South Africa suggests the same manner of pasteurization since it inactivates Cytomegalovirus, Human Immunodeficiency Virus, Human Lymphotropic virus and kills most pathogenic bacteria found in breastmilk. [5]

In certain areas which lack facilities for pasteurization, flash heat method may be utilized. The former was described as ‘‘low tech’’ method of pasteurization appropriate for home use. [8] Flash-heat mimics high-temperature, short-time (HTST) pasteurization, wherein it imitates the intense heat of a fire, until the water reached 100°C and was at a rolling boil. Milk is immediately removed from the water bath and rapidly cooled by immersing in crushed ice. [2]

Prior to pasteurization of breast milk, it is first stored properly. Fresh raw milk can be kept safely at room temperature (25 □ C) for up to 6 hours. Fresh raw milk can be stored in a refrigerator at 4 □ C for 48 hours. Donor milk should be refrigerated as soon as possible after it has been expressed, this prevents bacteria multiplication and lipolysis. Milk thawed can be frozen immediately and then more added to the same container over 24 hours, provided that the fresh milk is well chilled. Donor milk should be chilled then frozen as soon as possible. Raw donor milk should be stored in a freezer (-18 □C) for a maximum of 3 months while waiting for pasteurization. At the Milk Bank, human milk must be stored in a separate freezer where the temperature is rigorously controlled. [5]

METHODOLOGY

This was an experimental study using pooled donor breast milk from healthy reproductive aged women.Breast milk samples were obtained by donation of healthy reproductive aged group women. Donors should be mothers who have established lactation and are meeting their own infant’s needs and have volunteered to donate surplus

breast milk. Donor milk was chilled then frozen in a chest freezer as soon as possible at a temperature of -23 to -18 □C for a maximum of 48 hoursbefore it is pasteurized. At the Milk Bank, donor milk wasstored in a separate freezer where the temperature is rigorously controlled.These milk samples were thawed and pooled together. The pooled expressed milk wasequallydivided to make 10 bottles per group, with 50ml of milk sample per bottle. Each sample bottle was randomly designatedinto 2 groups: 10 bottles for the flash heat treatment group, and another 10 bottles for the holder pasteurization. An initial bacteriologic culture (using blood agar and McConkey agar) and Immunoglobulin A (IgA) level determination were done prior to intervention.

Aliquot milk samples, 0.5ml each from the two groups were subjected to microbiologic analysis before and after interventions (flash heat treatment and holder pasteurization). Milk samples will were inoculated on Blood agar and McConkey agar, to facilitate growth of gram positive and gram negative organisms respectively. Samples were stored for at least 48 hours in the incubator and thereafter subjected to colony count.Aliquot of milk samples, 0.005ml (5µl) per well from the different groups were subjected to IgA level determination by radio-immunodiffusion assay (RIA) using Bindarid Kit IgA™ before and after the interventions (flash heat treatment and holder pasteurization). In the study done by Vergara, identical IgA kits and reagents were used. A liquid control serum and high calibrators were run in each plate to ensure all kits are performing correctly. A 'double fill' of the sample well were done to ensure adequate sampling as the kits are not designed for measuring low concentrations of IgA in breastmilk was but designed to measure serum IgA levels. After an incubation period of 48 hours at room temperature, samples were analyzed. Ring diameters were measured to the nearest 0.1mm using an RID plate reader. Images of ring diameters in each kit were sent verified by the manufacturer as positive diffusion. Measurements of ring diameter corresponds to a specific IgA concentration reported in milligram per liter (mg/L) as seen in Appendix A.Mean difference between the experimental groups were determined.

The remaining aliquot of pooled donor milk (49ml) were placed in a 2oz plastic bottle, sealed, and immersed in a water bath. The water bath, including the 2oz plastic bottle were heated over a butane stove burner to replicate the intense heat of a fire until the temperature reaches 100C.Upon reaching 100C the plastic bottles were immediately removed from the water bath and immersed in crushed ice until the temperature reaches37C. The sample, upon reaching a temperature of 37C, were brought to the laboratory for bacteriologic analysis and IgA level determination.

The remaining aliquot of pooled donor milk (49ml) werepasteurized using the existing method of pasteurization at the Philippine Children's Medical Center milk bank, using the ASTI table top microprocessor pasteurizer. Temperature was set at 62.5C for 30 minutes. After which, the milk samples were immersed in crushed ice. The milk samples were brought to the laboratory for microbiologic analysis and IgA level determination once cooled.

T-test comparison of means for two independent samples were used in comparing the sample groups.Levene's test for equality of variances was used to

test if the null hypothesis that population variances are equal or homogenous. Standardized scores were used to normalize population with unknown parameters. This study expresses a confidence interval of 95%.

Results:

	Pre Holder Pasteurization (Raw Milk)	**Post Holder Pasteurization**
1	3,700 CFU/ml	No growth
2	6,200 CFU/ml	No growth
3	9,800 CFU/ml	No growth
4	8,400 CFU/ml	No growth
5	7,900 CFU/ml	No growth
6	10,400 CFU/ml	No growth
7	6,500 CFU/ml	No growth
8	6,500 CFU/ml	No growth
9	4,200 CFU/ml	No growth
10	600 CFU/ml	No growth

Table 1. Bacteriologic analysis on holder pasteurization group reported in colony forming units per milliliter (CFU/ml).

	Pre Flash Heat (Raw Milk)	**Post Flash Heat**
1	4,900 CFU/ml	300 CFU/ml
2	6,600 CFU/ml	No growth
3	8,300 CFU/ml	100 CFU/ml
4	11,000 CFU/ml	100 CFU/ml
5	6,800 CFU/ml	300 CFU/ml
6	5,000 CFU/ml	300 CFU/ml
7	1,700 CFU/ml	100 CFU/ml
8	1,700 CFU/ml	300 CFU/ml
9	7,300 CFU/ml	300 CFU/ml
10	900 CFU/ml	300 CFU/ml

Table 2. Bacteriologic analysis on flash heat treatment reported in colony forming units per milliliter (CFU/ml).

Using MacConkey and Blood agar plates, bacteriologic analysis was done. Blood agar plates were used to isolate gram positive organisms which may also be derived from normal bacterial flora, while MacConkey agar plates were used to isolate gram negative microorganisms. Some colonies that grew out of the inoculum were considered as contaminants and were therefore not counted. Tables 1 and 2 showthat in the pretreatmentdetermination (raw milk), there is a significant amount of bacterial growth as compared to post treatment (holder pasteurization and flash heat treatment).

	Raw Milk	Post Holder Pasteurization	Post Flash Heat Treatment
Control	3,930 mg/L	3,930 mg/L	3,930 mg/L
High concentration	9,080 mg/L	9,080 mg/L	9,080 mg/L
1	2,620 mg/L	605 mg/L	605 mg/L
2	605 mg/L	1,510 mg/L	2,620 mg/L
3	1,510 mg/L	1,510 mg/L	605 mg/L
4	1,510 mg/L	2,620 mg/L	605 mg/L
5	1,510 mg/L	1,510 mg/L	2,620 mg/L
6	1,510 mg/L	605 mg/L	1,510 mg/L
7	2,620 mg/L	605 mg/L	1,510 mg/L
8	605 mg/L	5,450 mg/L	1,510 mg/L
9	2,620 mg/L	605 mg/L	605 mg/L
10	605 mg/L	1,510 mg/L	2,620 mg/L

Table 3. Immunoglobulin A level determination in raw milk (pre treatment) and post treatment (holder pasteurization and flash heat treatment) reported in milligram per liter (mg/L).

Immunoglobulin A level measurement was based on ring diffusion in the agarose gel which corresponds to a specific IgA concentration reported in milligram per liter (mg/L). IgA levels showed minimal decrease in mean concentrations. Table 3 shows that IgA levels of raw milk were lower as compared to its respective IgA level concentration post treatment. In this experiment, aliquoted milk samples were used and treated (holder pasteurization and flash heat). Before and after undergoing treatment process, milk samples are re-pooled into a bigger container to ensure homogeneity and thereafter re-distributed to smaller containers for experimentation. Hence *mean* Immunoglobulin A concentrations were determined.

Both holder pasteurization and flash heat treatment, with p-values of 0.00 and 0.001 respectively, have significantly decreased bacterial load therefore suggests that both were effective in decreasing bacterial contamination of breastmilk after each treatment. The immunoglobulin A concentration post treatment, with p-values of 0.881 and 0.815, for holder pasteurization and flash heat treatment respectively, despite a notable drop in IgA concentrations, are not statistically significant.

Levene's test for equality of varianceswas used to test if the null hypothesis that population variances are equal or homogenous. If the resulting p-value of Levene's test is less than some significance level, the obtained differences in sample variances are unlikely to have occurred based on random sampling from a population with equal variances.[10]Holder pasteurization and flash heat treatment have no significant difference and therefore suggests that both are effectivein decreasing bacterial load on donor breast milk. Both treatment groups are effective in maintaining immunoglobulin A levels.

DISCUSSION

This study aimed to determine whether flash heat treatment is as effective as holder pasteurization in decreasing bacterial load while preserving immunoglobulin A levels in donor breastmilk. It is most beneficial in low income and resource limited areas

wherein pasteurization is not available. Young et al advocated the use of flash heat treated milk as an infant feeding option as recommended by the World Health Organization as a strategy to reduce vertical transmission.[12]Flash heat treated breast milk is recommended as temporary feeding strategy during mastitis, or when prophylactic antiretroviral drugs are unavailable.[12]Promoters included successful breastmilk expression, infant health after initiation of flash heat, and inability to pay for milk, while barriers included doubt about the safety or importance of pasteurized breastmilk, and difficulties with expressing milk.

Anotherstudy done by Israel-Ballard et al entitled Bacterial Safety of Flash Heated and Unheated Expressed Breastmilk During Storage, compared bacterial growth between flash heat treated samples against unheated samples during storage at room temperature for 0, 2, 6, and 8 hours. Total colony counts were performed and identified Escherichia coli, Staphylocuccus aureus and Group A and Group B streptococci. Unheated samples had a significantly higherbacterial growth at each time point. It was also evident in the same study that unheated samples had a significantly higher rate of bacterial propagation over time than flash-heated samples. No pathogenic growth was observed in the flash-heated samples, while the unheated samples showed growth of Escherichia coli and Staphylococcus aureus, therefore suggesting that storage of flash-heated breastmilk is safe at room temperature for up to 8hours. [13] In the study of RT Vergara, both flash-heat treated and pasteurized samples showed growth of Pseudomonas aeruginosa, Escherichia coli, Enterobacter and Klebsiella pneumonia prior to treatment. In the same study, there were no growth noted from 0 to 6 hours of incubation. After 8 hours post flash heat treatment, there were notable growth of Enterobacter spp at 100 CFU/ml. After 24 hours of incubation, growth of Diptheroids and coagulase negative Staphylococcus amounted to 100 CFU/ml. After 48 hours of incubation, growth of Enterobacter spp amounted to 100 CFU/ml. For pasteurization group, Diptheroids grew after 24 hours and at 48 hours Enterobacterspp and Diptheroids were noted amounting to 100 CFU/ml.[11]This paper proposes a parallel outcome. As seen on Table 1 and 2, we can observe that prior to treatment, there are approximately 600 – 11,000 colony forming units per ml (CFU/ml) bacterial growth on MacConkey or Blood Agar. Also on the same table, we noted that for the gold standard, holder pasteurization, there were no noted significant growth of bacterial pathogens after treatment. Prior to colony count, the investigators noted very minimal growth of colonies post holder pasteurization on the Blood agar which were away from inoculating site. To verify truepresence of pathogenic organisms, inspection of MacConkey agars were done. The absence of colonies on MacConkey agar signifies absence of gram negative organisms and therefore are considered contaminants only. Bacteriologic analysis of donor milk post flash heat treatment group showed a significant decrease in bacterial count.

A similar study by RT Vergara mentioned earlier in this study, concluded that flash heat treatment is capable of rendering breast milk bacteriologically safe, therefore suggesting that it can be used as an alternative to holder pasteurization. [11] Immunoglobulin A levels werealso measured but were undetected due to adaptation in measurement. The IgA kits were not specifically designed for breastmilk. In that said study, storage of

milk ranged from 3 – 6 months prior to experimentation. The investigators in this studyparticularly used freshly collected expressed breast milk samples stored up to 48 hours only. Undetectable levels of immunoglobulin A may be attributed to prolonged storage. It may also be indicative of poor sample application technique or moving the plates too quickly before the samples have diffused in properly. Sample application was done differently in this study. Secretory IgA are noted to be very high in colostrum for the first few days, and decline rapidly. Double filling of sample wells was done as the levels of IgA in breast milk can be estimated as low as approximately 364mg/L on the first five days and 142 mg/L from 30 days and beyond.[14] The measuring range of the IgA kit used is 545 – 5450 mg/L,therefore asingle fill of the sample rings, could be inadequate in detecting IgA levels.Chantry et al also suggested that our "low tech" version of pasteurization, namely flash heat treatment did not essentially denature immunoglobulins as compared with the gold standard, holder pasteurization.[2]One of theirobjectives was to evaluate the effects of each treatment on the concentrations of breast milk IgA. In the 20 samples analyzed, as shown in Table 3, both holder pasteurization and flash heat treatment group induced no statistically significant decrease in IgA concentration. These results suggest that both heat treated milk samples would still contain considerable passive protection.

CONCLUSION

Flash heat treatment is a simple, "low tech" method which may be comparable to the gold standard, holder pasteurization in decreasing microbial contamination of donor breast milk while preserving essential immunoglobulins such as immunoglobulin A. This study was successful despite using IgA kits not specifically designed for breast milk. Better immunoglobulin A level determination will result from breastmilk specific IgA kits. Prolonged storage of milk may result in significant reduction of immunoglobulin A levels.

Thus, flash heat treatment may be utilized in far flung areas and low income communities as alternative for holder pasteurization in providing safe and effective breast milk. Werecommend future researchers to use immunoglobulin A specific kit in measuring IgA levels. Future researches can also use colostrum for in measuring immunoglobulin A levels. Studies can be done comparing bacterial growth and IgA concentrations in freshly collected milk, stored milk, and frozen milk in different periods of time.

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