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Destruction of *Mycobacterium paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in Raw Milk by a Commercial On-Farm High-Temperature, Short-Time Pasteurizer

J. R. Stabel,[1] S. Hurd,[2] L. Calvente,[3] and R. F. Rosenbusch[3]
[1]USDA-ARS, National Animal Disease Center, Bacterial Diseases of Livestock Research Unit, Ames, IA 50014
[2]Preharvest Food Safety and Enteric Diseases Research Unit, Ames, IA 50010
[3]Iowa State University, College of Veterinary Medicine, Ames 50014

ABSTRACT

The 2002 NAHM's Dairy Survey indicated that 87.2% of dairy farms in the United States feed waste milk to their neonatal calves. Although cost-effective, this practice can lead to increased calf morbidity and mortality due to ingestion of pathogenic agents. In an effort to reduce the risk of infection, dairy producers are implementing on-farm pasteurization of the waste milk as a control procedure before feeding the milk to calves. In the present study, the efficacy of a commercial high-temperature, short-time (HTST) on-farm pasteurizer unit to destroy *Mycobacterium paratuberculosis, Salmonella enterica* spp., and *Mycoplasma* spp. in raw milk was evaluated. Replicate experiments were run for 3 isolates of *M. paratuberculosis*, 3 serovars of *Salmonella* (*derby, dublin, typhimurium*); and 4 species of *Mycoplasma* (*bovis, californicum, canadense, serogroup* 7) at 2 different levels of experimental inoculation. In addition, HTST pasteurization experiments were performed on colostrum experimentally inoculated with *M. paratuberculosis*. After culture of the pasteurized milk samples, no viable *M. paratuberculosis, Salmonella*, or *Mycoplasma* were recovered, regardless of species, strain, or isolate. Pasteurization of colostrum was also effective in the destruction of *M. paratuberculosis* but resulted in an average 25% reduction in colostral immunoglobulin. These results suggest that HTST pasteurization is effective in generating a safer product to feed to young calves.
(**Key words:** waste milk, pasteurization, neonatal calf)

Abbreviation key: HEYM = Herrold's egg yolk medium.

INTRODUCTION

Waste milk is a major source of nutrition for neonatal calves on dairy farms in the United States. This was recently documented in a survey conducted by the National Health Monitoring System (NAHMS) that reported 87.2% of United States dairy farms feed waste milk to their neonatal calves (USDA, 2002). Although cost effective, this practice can lead to increased calf morbidity and mortality due to ingestion of pathogenic agents. It has been demonstrated that infected cows shed viable *Mycobacterium paratuberculosis* and *Salmonella* spp. in their colostrum and milk (Smith et al., 1989; Spier et al., 1991; Sweeney et al., 1992; Streeter et al., 1995). Contamination of milk from fecal matter containing either *M. paratuberculosis* or *Salmonella* shed by infected dams is another potential method of disease transmission from dam to calf. In addition, waste milk from mastitic cows may harbor mycoplasma agents that are primary causes of calf respiratory problems (Butler et al., 2000; Gonzalez and Wilson, 2003). The presence of these pathogens on dairy farms results in a major management problem for producers feeding waste milk to their calves. A recommendation for controlling the spread of infectious disease within a herd is to feed colostrum from noninfected dams or milk replacer. However, this recommendation creates an additional expense to a producer who must dump the waste milk and purchase commercial sources of colostrum or replacer products. Today, more producers are willing to implement control measures such as on-farm pasteurization of waste milk to destroy potential pathogens. This practice improves their long-range economic outlook by reducing losses due to calf morbidity and cost-outlay for commercial products. The effectiveness of an on-farm batch pasteurizer unit (65.5°C for 30 min) was demonstrated to destroy *M. paratuberculosis* in waste milk (Stabel, 2001). On-farm pasteurization of waste milk held at 65°C for 10 min also destroyed common mastitic mycoplasma such as *Mycoplasma bovis, M. californicum*, and *M. canadense* (Butler et al., 2000). In the present study, the efficacy of a commercial HTST pasteurizer unit in the destruction of *M. paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk and *M. paratuberculosis* in colostrum was evaluated.

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Corresponding author: J. R. Stabel; e-mail: jstabel@nadc.ars.usda.gov.

Figure 1. Bettermilk commercial high-temperature, short-time (HTST) pasteurizer unit (Bettermilk, Inc., Winona, MN).

MATERIALS AND METHODS

Experimental protocol. A commercial HTST pasteurizer unit (Bettermilk, Inc., Winona, MN) (Figure 1) was utilized to evaluate the efficacy of pasteurization in the destruction of *M. paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk. Fresh raw milk was obtained from Jersey cows housed at the National Animal Disease Center, Ames, IA, on the day of each experimental run. Twelve liters of raw milk were used for each experiment. An aliquot of fresh milk was reserved from the 12 L prior to each experiment to serve as a negative control. The remainder of the milk was placed in the holding vessel of the pasteurizer unit and inoculated with the appropriate level of pathogen. After careful mixing, an aliquot was reserved to serve as a nonheated positive control. The milk was then subjected to the standard protocol of heat treatment at 71.7°C for 15 s. Upon completion of the cool-down at ambient temperature, samples were collected at 2, 3, and 4 min.

Colostrum was treated at temperatures ranging between 64 and 71.7°C, then it was drained into stainless steel buckets and allowed to sit at ambient temperature for 30 min. Samples of colostrum were obtained at times 0, 10, 20, and 30 min during this period after pasteurization for culture. A negative control colostrum sample was obtained before inoculation of the colostrum with *M. paratuberculosis*, and a positive control sample was taken before heat treatment of the inoculated colostrum.

Mycobacterium paratuberculosis inoculation of milk and colostrum. Three isolates of *M. paratuberculosis* were selected for evaluation in this study; strain 19698, a well-characterized laboratory strain originally isolated at the National Animal Disease Center from a cow with clinical Johne's disease; and strains 167 and 6112, both propagated from primary isolations from cows with clinical disease that were necropsied at the National Animal Disease Center. Strain 19698 had been passed multiple times and was laboratory adapted, whereas strains 167 and 6112 had been passed 2 and 3 times, respectively. Two inoculum levels were evaluated for each strain of *M. paratuberculosis*: 10^2 and 10^6 cfu/mL of milk. Strains of *M. paratuberculosis* were propagated in Middlebrook 7H9 medium (Becton Dickinson, Cockeysville, MD) supplemented with 2 mg/L of mycobactin J (Allied Monitor, Fayette, MO) and 10% oleic acid-albumin-dextrose complex enrichment (Difco, Detroit, MI) plus 0.05% Tween 80 (Sigma Chemical Co., St. Louis, MO) until log phase of growth (A_{540nm} = 0.2 to 0.4) was attained. Cells were harvested by centrifugation at 10,000 × *g* for 20 min, washed with 1× PBS, 0.01 *M*, pH 7.4), and then resuspended to the appropriate concentration to achieve the high (10^6) and low level (10^2) of inoculum when added to the raw milk. Three replicates of each experiment (strain and inoculum level) were performed. Frozen colostrum was obtained from noninfected dairy cows on-site and from a farm in Minnesota with no history of Johne's disease. Only one isolate of *M. paratuberculosis* (strain 19698) at a concentration of 10^5 was utilized in the colostrum experiments.

Culture of M. paratuberculosis from milk and colostrum. After heat treatment, control and test samples were centrifuged in 50-mL polypropylene tubes at 1200 × *g* for 30 min at 4°C. The whey was discarded, and remaining pellet and cream fractions were resuspended in 1 mL of 1× PBS. This suspension was further diluted 10-fold in PBS. The original suspension and the diluted suspension (100 μL each) of each sample were inoculated in duplicate onto Herrold's egg yolk medium (**HEYM**) as previously described (Stabel, 2001). Samples were incubated at 37°C, and cfu were recorded at 4, 8, and 26 wk. Colonies were confirmed as *M. paratuberculosis* by nested PCR and acid-fast stain. A suspect colony was picked using a sterile disposable loop and placed into 1-mL TE buffer (10 m*M* Tris-HCl, pH 8.0; 1 m*M* EDTA, pH 8.0), and PCR analysis was performed as previously described (Stabel et al., 2002). Smears of suspect colonies were dried onto glass slides and examined after Ziehl-Neelsen staining for the presence of acid-fast bacteria.

Milk sample suspensions and their dilutions (500 μL) were also inoculated into BACTEC medium (Becton-Dickinson,Franklin Lakes, NJ) containing 0.1 mL of mycobactin J, 1 mL of 50% egg yolk suspension (Difco)

and 0.1 mL of PANTA antibiotic supplement (Becton-Dickinson). Samples were incubated at 37°C, and growth was monitored each week for 8 wk. A growth index greater than 30 was indicative of bacterial respiration and growth (Damato and Collins, 1990).

Colostral immunoglobulin. Total IgG in colostrum samples was determined in pre- and postpasteurization samples (T = 30 min after heat treatment) using the Bovine IgG Vet-RID kit (Bethyl Laboratories, Montgomery, TX). Colostrum samples were diluted 1:10 with sterile water and 5 μL of diluted sample was placed in the test wells on the plate. Bovine IgG standards (5 μL; 625, 2500, and 5000 mg/mL) were run on each plate concurrently. Samples and standards were allowed to incubate at room temperature for 18 h according to the manufacturer's recommendation. The preciptin ring diameters of the reference standards, and test samples were measured to the nearest millimeter. A reference curve was constructed and utilized to determine the concentration of IgG in the test samples.

Salmonella samples and culture. Three serovars of *S. enterica* were obtained from cattle: *S. dublin, S. derby*, and *S. typhimurium*. Briefly, 1 mL of an overnight culture of *Salmonella* spp. was inoculated into 100 mL of lactose broth (EM Science, Darmstadt, Germany). Cultures were expanded at 37°C while shaking at 220 rpm for 3.5 h. Cultures were centrifuged at 18,000 × *g* for 20 min, and the pellet resuspended in 1× PBS to the desired concentration. Two inoculum levels were evaluated for each serovar of *Salmonella*: 10^2 and 10^6 cfu/mL of milk. Three replicates of each experiment (serovar and inoculum level) were performed. Negative (uninoculated milk) and positive (inoculated milk) control samples were taken in each experiment and cultured for the specific serovar of *Salmonella*.

After heat treatment, samples were direct plated (100 μL of milk) onto XLT4 (Becton-Dickinson, Sparks, MD; *S. derby; S. typhimurium*) or Chromogar (CHROMagar, Paris, France; *S. dublin*) and incubated for 24 h at 37°C. On d 2, suspect colonies were picked, streaked onto Rambach agar (CHROMagar), and incubated for 24 h at 37°C. After incubation, a BBL crystal microbial identification sytem (Becton-Dickinson) was run on any suspect colonies. In addition, samples were enriched by adding 1 mL of milk to each of the following: 9 mL of tetrathionate broth (Becton-Dickinson; TET) and 9 mL of buffered peptone water (Remel, Lenexa, KS; BPW). Samples were incubated at 37°C for 24 h, then 0.1 mL of the enriched samples were transferred, respectively, to 10 mL of Rappaport-Vassiliadis medium (Becton Dickinson). Samples were further incubated at 42°C for 24 h. The same protocol as described above was followed for direct plating of samples by plating on XLT4 agar or Chromagar (*S. dublin*) and brilliant green sulfa agar (all from Becton Dickinson), followed by Rambach agar, and BBL crystal analysis.

Mycoplasma samples and culture. Four major species of *Mycoplasma* that have been implicated in bovine mastitis were evaluated in this study, including: *M. bovis*, strains Jasper, UCD9, and 1135-6; *M. californicum*, strains ST6, and Cs 687; *M. canadense*, strain 275C; and *M.* serogroup 7, strains PG50 and Cs 826C. *Mycoplasma* strains were propagated by growing in Friis broth (Knudtson et al., 1986) containing 200 μg/mL of bacitracin, 100 μg/mL of thalium acetate, and 67 μg/mL cefoperazone, to inhibit bacterial growth. After 48 h of incubation at 37°C, each broth culture was sampled with a sterile toothpick and streaked onto a Friis agar plate containing the same concentrations of bacterial inhibitors to check growth of the *Mycoplasma*. Cells were harvested by centrifugation at 25,000 × *g* for 20 min at 4°C, washed twice with PBS, then resuspended to 10× of the original concentration. Suspensions were passed 3 times through a 25-g needle to disperse clumps. After addition of 10% fetal bovine serum, suspensions were snap-frozen and stored at −70°C. Aliquots of each strain were thawed prior to each experiment and added to the raw milk to achieve the appropriate concentration. Two inoculum levels were evaluated for each strain of *Mycoplasma*: generally, 10^2 and 10^6 cfu/mL of milk. Two replicates of each experiment (strain and inoculum level) were performed. Negative (uninoculated milk) and positive (inoculated milk) control samples were taken in each experiment and cultured for the specific strain of *Mycoplasma. Mycoplasma canadense*, strain 275C, was demonstrated to be the most heat resistant strain of mycoplasma in a previous pasteurization study.

After heat treatment, samples were cultured by inoculating 100 μL into 900 μL of Friis broth in multiwell plates, and plates were incubated at 37°C in 2% CO_2 atmosphere. After 48 h of incubation, each broth culture was sampled with a sterile toothpick and streaked onto a Friis agar plate containing bacterial inhibitors as previously described. Plates were incubated for 2 to 3 d at 37°C in 2% CO_2, and then examined under low magnification for the presence of mycoplasma colonies on the streaked areas.

RESULTS

The temperature changes over time during standard operation of the HTST pasteurizer unit are depicted in Table 1. Temperature of the raw milk was 19°C at time 0 and ascended rapidly to a temperature of 61.7°C by 1 min of circulation through the unit. The set temperature of 71.7°C was reached after 9 min of circulation, at which time it was diverted into the heating tube for

Table 1. Time and temperature control (°C) displays during HTST processing of raw milk using the Bettermilk (Bettermilk, Inc., Winona, MN) pasteurizer unit.

	Time (min)	Milk holding tank	Entry into heating tube	Product temperature
Prepasteurization	0	26		20
	1	26		62
	2	32		57
	3			57
	4	39	62	62
	5			65
	6		67	68
	7			69
	8		71	71
	9		72	72
Postpasteurization	1			72
	2			72
	3			72
Rinse			65	

a 15-s period. Temperature of the unit held at the set temperature with only minor fluctuation of 1 to 2°C after the milk product was treated.

Serial titration of positive control and test samples was performed for milk inoculated with *M. paratuberculosis* to allow optimal detection of potential survivors. The detection limits of the positive controls for the HEYM solid medium and the BACTEC liquid medium system are depicted in Table 2. On HEYM agar medium we were able to detect between 1 to 10 cfu/mL of milk. The BACTEC liquid system detected 1 cfu/mL after 6 wk of incubation. Therefore, the sensitivity of detection of *M. paratuberculosis* was very high, and it was unlikely that any survivors were missed.

Results of the heat treatment of the raw milk containing *M. paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. indicate that the commercial HTST unit is effective in the destruction of these pathogens, regardless of inoculum level (Tables 3, 4, and 5). The negative control milk sample (nonheated, uninoculated raw milk) was culture positive on several experimental runs with the *Salmonella* strains, but upon subculture this proved to be a contaminant. No evidence of contaminants was indicated in the heated milk samples.

Heat treatment of colostrum in the lower temperature range (63.9 to 66.7°C) did not immediately destroy the *M. paratuberculosis* present (Table 6; time 0). However, recovery of *M. paratuberculosis* from colostrum was reduced by 2 $\log_{10}$ after 10 min of incubation in the buckets and achieved a nadir of < 3 cfu/mL of milk after 30 min. Increasing the temperature range (68.3 to 70.8°C) completely abrogated recovery of viable *M. paratuberculosis* from the colostrum, even at time 0 (Table 6). Total IgG was reduced by HTST pasteurization of colostrum regardless of temperature regime (Table 7). Initial colostral IgG averaged 68.4 mg/mL (range: 62 to 78 mg/mL) across all experiments. At the lower temperature range, a 22% reduction in colostral IgG was observed after 30 min in the bucket. Colostral IgG decreased even more (27%) after treatment of colostrum at the higher temperatures. Postpasteurization colostral immunoglobulin concentration averaged 51.3 and

Table 2. Titration of viable *Mycobacteria paratuberculosis* from high positive control sample(1×10^5 cfu/mL) on BACTEC[1] and HEYM[2] media.

	Weeks of incubation					
Culture medium	1	3	4	6	7	8
BACTEC						
No dilution	999	999	999	999	999	999
10^{-1}	89	876	999	999	999	999
10^{-2}	16	999	999	999	999	999
10^{-3}	9	39	172	999	999	999
10^{-4}	6	3	7	144	999	999
10^{-5}	8	5	3	303	999	999
10^{-6}	7	8	3	39	999	999
HEYM	4	8	24			
No dilution	C	C	C			
10^{-1}	C	C	C			
10^{-2}	T	T	T			
10^{-3}	T	T	T			
10^{-4}	50	64	65			
10^{-5}	1	2	2			
10^{-6}	0	0	0			

[1]The cut-off for a positive growth index for the detection of *M. paratuberculosis* in BACTEC medium is designated as >30; GI = 999 indicates overgrowth in the media.

[2]Designations are C = contamination and T = too numerous to count (100 cfu/slant). HEYM = Herrold's egg yolk medium.

Table 3. Destruction of *Mycobacterium paratuberculosis* after HTST heat treatment at 71.7°C for 15 s.

	High level of inoculum		Low level of inoculum	
Strain	Prepasteurization	Postpasteurization	Prepasteurization	Postpasteurization
19698 (lab)	8.2×10^4	ND[1]	6.0×10^1	ND
	7.8×10^4	ND	1.3×10^2	ND
	2.3×10^3	ND	2.3×10^2	ND
167 (wild)	1.9×10^5	ND	6.3×10^2	ND
	2.1×10^5	ND	4.2×10^2	ND
	2.2×10^4	ND	8.2×10^2	ND
6112 (wild)	1.9×10^6	ND	5.4×10^1	ND
	5.9×10^5	ND	4.3×10^2	ND
	6.8×10^5	ND	2.1×10^2	ND

[1]ND = Not detected.

45.3 mg/mL, respectively, for the low and high temperature range.

DISCUSSION

We were able to demonstrate in the present study that HTST pasteurization is effective in the destruction of *M. paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in waste milk. Previously, we reported complete destruction of *M. paratuberculosis* occurred in waste milk treated at 65.5°C for 30 min using an on-farm batch pasteurizer (Stabel, 2001). Indeed, there was no recovery of any viable *M. paratuberculosis* organisms before the unit reached 65.5°C, indicating that progressive heating of the substrate during the period of time required to reach the target temperature can effectively reduce the presence of pathogenic contamination as well. Similarly, heat treatment of discard mycoplasma-containing mastitic milk at 65°C for 2 min in a batch pasteurizer unit destroyed *M. bovis* and *M. californicum* present (Butler et al., 2000). Milk was held at 65°C for 10 min before destruction of *M. canadense* was confirmed. Raising the temperature to 70°C shortened the time required for inactivation to 1 min for *M. bovis* and *M. californicum* and 3 min for *M. canadense.* The results from that study demonstrate that batch pasteurization was effective for all 3 species of *Mycoplasma*, although *M. canadense* was more heat-resistant than the other 2 strains. Other studies have indicated that *Mycoplasma* was successfully destroyed if treated at 74° to 76°C for 15 to 20 s (HTST) or 65.5°C for 30 min (batch) (Bray et al., 2001).

Although there are no published studies on record evaluating pasteurization of waste milk for the destruction of *Salmonella* spp., it is recognized that IMI with *S. dublin* and *S. typhimurium* are problematic in some dairy herds and that some cows may excrete up to 10^5 organisms per milliliter of milk (Ogilvie, 1986; Smith et al., 1989; Spier et al., 1991). Salmonellosis is a well-known contributor to the morbidity and mortality of neonatal calves, and feeding raw waste milk may play a significant role in exacerbating transmission of this infection between cows and calves.

Batch pasteurization has proven effective for the destruction of *M. paratuberculosis* and *Mycoplasma* and, therefore, can provide a clean product to feed to calves. This method of pasteurization also has the advantage that waste milk can be directly piped into the unit and

Table 4. Destruction of *Salmonella* spp. during HTST heat treatment at 71.7°C for 15 s.

		High level of inoculum		Low level of inoculum	
Species	Strain	Prepasteurization	Postpasteurization	Prepasteurization	Postpasteurization
S. derby	NVSL	2.0×10^6	ND[1]	2.5×10^3	ND
	2681b	2.0×10^6	ND	1.5×10^3	ND
		2.0×10^6	ND	9.0×10^2	ND
S. dublin	NVSL	6.0×10^6	ND	NP[2]	ND
	3129	9.0×10^6	ND	NP	ND
		3.3×10^7	ND	NP	ND
S. typhimurium	NVSL	2.1×10^7	ND	NP	ND
	5372	2.1×10^7	ND	NP	ND
		2.0×10^7	ND	NP	ND

[1]ND = Not detected.

[2]NP = Not performed.

Table 5. Destruction of *Mycoplasma* spp. during HTST heat treatment at 71.7°C for 15 s.

		High level of inoculum		Low level of inoculum	
Species	Strain	Prepasteurization	Postpasteurization	Prepasteurization	Postpasteurization
M. bovis	1135-6	1.0×10^6	ND[1]	1.0×10^2	ND
	UCD9	1.0×10^6	ND	1.0×10^2	ND
	Jasper	1.0×10^6	ND	1.0×10^2	ND
M. californicum	Cs657	1.0×10^6	ND	1.0×10^2	ND
	ST6	1.0×10^6	ND	1.0×10^2	ND
M. canadense	275C	1.0×10^6	ND	1.0×10^2	ND
M. serogroup 7	CS826	1×10^6	ND	1.0×10^2	ND
	PG50	2×10^4	ND	1.0×10^1	ND

[1]ND = Not detected.

held until the producer is ready to perform the heat treatment of the milk. However, it typically required about 1.5 h for the complete heating process, requiring nearly 1 h for the unit to reach the target temperature, followed by 30 min of heat treatment at the hold temperature. Commercial HTST pasteurization units for use on-farm may be more efficient processing tools for daily pasteurization of waste milk. The HTST unit utilized in the present study took minimal time (9 min) to reach the target temperature, and the product required only 15 s for complete inactivation of the pathogens tested due to the flow-through processing method. For optimal performance and maintenance of the machine, it was recommended that the unit be cleaned after each run. However, the cleaning cycle was completely automatic and required only 20 min. Therefore, the time and labor required for operation of this unit was significantly reduced compared to the batch pasteurization unit. However, both methods are equally effective in reducing potential pathogens in waste milk and can be recommended for the practice of controlling neonatal infections within a herd.

A major concern of producers is whether there are economic benefits associated with pasteurization of waste milk or colostrum. In addition, there is concern about the reduction in immunoglobulin content in colostrum that has been heat-treated. We demonstrated an average reduction in colostral IgG of 25% regardless of heat treatment regime. Similar results were reported after heat treatment of colostrum in 57-L batches for 30 min at 63°C (Godden et al., 2003). Pasteurization has reduced the immunologlobulin content of colostrum to varying degrees, although it is not known if this affects the health of the calf (Meylan et al., 1995; Godden et al., 2003). Godden et al. (2003) demonstrated that feeding 2 L of pasteurized colostrum to calves at the first feeding resulted in significantly lower serum IgG concentrations (9.7 mg/mL) compared to calves fed unpasteurized colostrum (19.1 mg/mL). Alternatively, when calves were fed 4 L at the first feeding, there was no difference in serum IgG between the 2 groups (16.1 vs. 13.5 mg/mL for unpasteurized and pasteurized colostrum, respectively). A study comparing the performance of calves fed pasteurized or nonpasteurized colostrum and waste milk found that feeding pasteurized products to calves significantly increased their mean weight gain (Jamaluddin et al., 1996). Treatment costs for illness and mortality rates were reduced for calves fed the pasteurized milk and colostrum, whereas costs of pasteurization were negligible, suggesting a significant economic advantage for producers.

Producers may be wary of pasteurizing colostrum with an HTST unit due to potential gelling of the product within the tubing during the heating process. In

Table 6. Recovery of *Mycobacterium paratuberculosis* in colostrum after HTST heat treatment at a low and high temperature range for 15 s.[1]

Time after heat treatment (min)	Low temperature (63.9–66.7°C)		High temperature (68.3–70.8°C)	
	Milk temperature in the bucket	cfu/mL	Milk temperature in the bucket	cfu/mL
T = 0	62.8°C	1.4×10^4	65°C	< 1
T = 10	56.1°C	2.3×10^2	61.7°C	ND[2]
T = 20	53.3°C	4.1	56.7°C	ND
T = 30	51.7°C	2.2	54.7°C	ND

[1]Results are averaged from 3 replicate runs.

[2]ND = Not detected.

Table 7. Colostral IgG concentrations (mg/mL) before and after HTST pasteurization.

Time after heat treatment (min)	Low temperature (63.9–66.7°C)		High temperature (68.3–70.8°C)	
	Milk temperature in the bucket	IgG (mg/mL)	Milk temperature in the bucket	IgG (mg/mL)
T = 0	62.8°C	67.3	65.0°C	62.0
T = 30	51.7°C	51.3	54.7°C	45.3

preliminary experiments at a set temperature of 71.7°C (range 68 to 72°C), there was no gelling of the product within the holding tube, but gelling was observed when the product was allowed to sit in the bucket. To circumvent this problem, subsequent experiments were designed to run at lower temperatures than the typical set point of 71.7°C. After heating, the product was diverted into stainless steel buckets rather than running through the cooling tubes. Allowing the heated product to sit an additional 30 min in the bucket provided a heat sink that effectively increased the destruction of *M. paratuberculosis*. When the experiments were conducted with the temperature ranges reported within this paper (63.9 to 66.7°C and 68.3 to 70.8°C), the product remained fluid and did not coagulate even after sitting in the buckets for 30 min.

In conclusion, HTST pasteurization of raw milk is effective for the destruction of *M. paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk and effectively destroys *M. paratuberculosis* in colostrum. This information provides dairy producers with an alternative to purchasing commercial replacement products, resulting in reduced costs. More importantly, pasteurization of waste milk significantly reduces calf morbidity and helps break the transmission cycle of infectious pathogens on-farm.

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