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# On-Farm Batch Pasteurization Destroys *Mycobacterium paratuberculosis* in Waste Milk

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## ABSTRACT

A recent dairy survey conducted in 1996 by the National Animal Health Monitoring System suggests between 20 and 40% of dairy herds in the United States have some level of Johne's disease. This figure will continue to increase unless producers implement management regimes that will help control the spread of this disease within their herds. The neonatal calf is the target for infection with *Mycobacterium paratuberculosis,* the causative agent of Johne's disease. Calves become infected via exposure to the bacterium through contaminated feces, bedding, colostrum, and milk. Shedding of viable *M. paratuberculosis* has been documented in the colostrum and milk of infected dams. This study evaluated the efficacy of on-farm pasteurization to destroy *M. paratuberculosis* in waste milk fed to calves to circumvent this mode of transmission. In three replicate experiments, waste milk was experimentally inoculated with *M. paratuberculosis* and heated at 65.5°C for 30 min. No viable bacteria were recovered after 28 wk of incubation. These results suggest that batch pasteurization of waste milk contaminated with *M. paratuberculosis* was effective at generating a clean product to feed to young calves.
(**Key words:** pasteurization, waste milk, *Mycobacterium paratuberculosis,* Johne's disease)

**Abbreviation key: BHI** = brain heart infusion, **HPC** = hexadecylpyridinium chloride, **HEYM** = Herrold's egg yolk medium.

## INTRODUCTION

Johne's disease is a chronic, progressive enteritis in ruminants caused by the bacterium, *Mycobacterium paratuberculosis* (7). Cows with this disease become infected as calves less than 1 mo of age, yet clinical signs of disease may not become apparent for 3 to 5 yr. Calves generally become infected through ingestion of fecal matter contaminated with the bacteria; however, viable *M. paratuberculosis* are also present in the colostrum and milk of infected dams (3). Because of the long latency period before clinical signs develop, producers may be unaware that individual animals are infected. Diagnosis of subclinical infection with *M. paratuberculosis* is difficult because animals usually do not have a measurable antibody titer in this stage and may only shed the organism intermittently in their feces, reducing the effectiveness of serologic or fecal culture tests for detection (4). Once the disease progresses to a more clinical state, then signs such as diarrhea, weight loss, and inappetence become apparent. Animals also shed high numbers of bacteria in their feces and become sero-positive at this time, so diagnosis is easier if the producer or herd veterinarian has the proper tools (4).

The majority of economic loss to the producer facing Johne's disease may be incurred by attempting to treat the disease before it is adequately diagnosed and culling animals showing signs of clinical disease. However, producers are also faced with economic loss from cows with subclinical disease through an increased incidence of mastitis, decreased milk production, and increased calving intervals (8). It is imperative that once Johne's disease is diagnosed in the herd, management techniques are employed to further prevent the spread of this disease. If the producer feeds colostrum or milk from infected dams to his calves, he risks increased contamination of his herd. Calves maintained in a known infected herd should be fed colostrum from noninfected dams or milk replacer (10). However, these recommendations come at considerable expense to the producer who must dump his waste milk because it may be a source of *M. paratuberculosis* and purchase these products from outside sources. One alternative is to pasteurize waste milk before feeding it to calves. It is not known whether on-farm pasteurization can destroy *M. paratuberculosis*. In the present study, we evaluated the effectiveness of a batch pasteurizer unit housed on a local dairy farm to kill *M. paratuberculosis* in waste milk fed to calves.

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## MATERIALS AND METHODS

### Milk Samples

Raw milk samples (waste milk) were obtained from a dairy herd with Johne's disease. Previous testing of the herd by serology indicated at least 20% infection rate in the herd. This dairy farm had a batch pasteurizer unit (Solar Milk Minder) on-site which was used for heat treatment of waste milk. Typically, milk is piped into a stainless steel holding tank of the pasteurizer unit and then subjected to a heating procedure of 65.5°C for 30 min before it is fed to the calves. During the heating regime, an agitator bar in the bottom of the holding tank moves the milk to achieve uniform heating of the product. Hot water jackets the holding tank and provides the heat needed for pasteurization. Since cows with known infection were contributing each day to the waste milk, it was important to determine if *M. paratuberculosis* could be isolated from the milk, before and after heat treatment. To determine this, we obtained waste milk samples (50 ml) before and after heat treatment on 4 consecutive days. Samples were frozen at –20°C in sterile, polypropylene tubes (Becton-Dickinson, Lincoln Park, NJ) and transported to the National Animal Disease Center, Ames, Iowa, for culture.

Samples were thawed and split into two aliquots each. One sample was cultured as received, and the other sample was inoculated with $1 \times 10^5$ cfu *M. paratuberculosis*/ml of milk as a positive control for culture sensitivity. Samples were prepared for culture by centrifugation of each sample at 2000 rpm for 30 min. The whey was decanted, and the pellet and cream were decontaminated overnight in 0.9% hexadecylpyridinium chloride (**HPC**; Sigma Chemical Co., St. Louis, MO) in 1.9% brain heart infusion broth (**BHI**; Becton-Dickinson) at 37°C. The following day samples were centrifuged at 2500 rpm for 20 min, and the pellet was resuspended in 1 ml of antibiotic mixture consisting of (per milliliter) 100 $\mu$g of vancomycin, 100 $\mu$g of naladixic acid, and 50 $\mu$g of fungizone (Sigma Chemical Co.) in BHI. Samples (200 $\mu$l/tube) were inoculated onto three tubes of Herrold's egg yolk medium (**HEYM**) containing 2 mg of mycobactin/L J (Allied Monitor, Fayette, MO). Samples were incubated at 37°C in a horizontal position for 1 wk with loose caps to permit evaporation of residual moisture on the surface of the medium. Caps were then tightened, tubes were returned to the upright position, and incubation of samples continued for a total of 24 wk. We examined tubes every 4 wk using a dissecting microscope at 25× magnification, and samples were recorded as plus or minus depending on the presence of colonies on the slants.

### Experimental Procedure

In three separate experiments, waste milk was inoculated on site at the dairy farm with *M. paratuberculosis* (strain 19698-1974; NADC) at a concentration of 1 to $5 \times 10^3$ cfu/ml of milk. Before inoculation with the bacteria, a negative control sample (50 ml) was obtained in a sterile tube. Aliquots of milk (50 ml) were also obtained in sterile tubes at 0, 10, 20, and 30 min during the period before the pasteurizer unit reached 60°F. A sample was taken once the unit reached 60°C. During the heat treatment period in which the unit was maintained at 65.5°C, milk samples were taken at 0, 15, and 30 min. A final milk sample was obtained once the temperature reached 71.1°C. All experimental milk samples were obtained in triplicate. Samples were transported on the same day back to the NADC and set up for culture by the method previously described, except milk samples were serially diluted $10^1$ to $10^5$ before culture to allow enumeration of bacterial colonies. The bacterial stock was also serially diluted and plated out to verify number of viable *M. paratuberculosis* that were inoculated into the milk. Tubes were examined every 4 wk during incubation with a dissecting microscope at 25× magnification, and colonies were enumerated and recorded as colony-forming units per milliliter of milk. Data from experiments 2 and 3 were averaged and presented as one value (mean ± SEM). Data from experiment 1 were excluded due to a dilution error in the initial bacterial inoculum.

## RESULTS

No viable *M. paratuberculosis* were recovered from waste milk samples after incubation on HEYM for 28 wk regardless of heat treatment (Table 1). Samples taken before heat treatment were significantly contaminated with fungi, resulting in overgrowth on the agar slants. In contrast, samples obtained after the regular heat treatment at 65.5°C for 30 min were relatively free of contamination. Viable bacteria were recovered from all milk samples spiked with *M. paratuberculosis* before culture as shown in Table 1.

A plot of sampling time versus temperature of milk in the pasteurizer unit is depicted in Figure 1. The basal temperature of the milk before heating and bacterial inoculation was 17°C. The temperature of the milk increased approximately 17°C per 10-min interval until it reached 50°C and began to increase more slowly. A temperature of 60°C was reached after 40 min of heating time, but another 20 min was required before the target temperature of 65.5°C was obtained. The target temperature was maintained consistently by the pasteurization unit, averaging 66.5 ± .21°C over the 30-min treatment period. The temperature of the milk

**Table 1**. Isolation of *Mycobacterium paratuberculosis* (Mpt) from raw waste milk pre- and post pasteurization.[1]

| | Culture time | | | | |
|---|---|---|---|---|---|
| Samples | 8 wk | 12 wk | 16 wk | 20 wk | 28 wk |
| 1-Pre | NG | NG | NG | NG | NG |
| 1-Post | NG | NG | NG | NG | NG |
| 2-Pre | NG | NG | NG | NG | NG |
| 2-Post | NG | NG | NG | NG | NG |
| 3-Pre | NG | NG | NG | NG | NG |
| 3-Post | NG | NG | NG | NG | NG |
| 4-Pre | NG | NG | NG | NG | NG |
| 4-Post | NG | NG | NG | NG | NG |
| 1-Pre w/Mpt | + | + | + | + | + |
| 1-Post w/Mpt | + | + | + | + | + |
| 2-Pre w/Mpt | + | + | + | + | + |
| 2-Post w/Mpt | + | + | + | + | + |
| 3-Pre w/Mpt | + | + | + | + | + |
| 3-Post w/Mpt | + | + | + | + | + |
| 4-Pre w/Mpt | + | + | + | + | + |
| 4-Post w/Mpt | + | + | + | + | + |

[1]Samples of waste milk were obtained pre- and post pasteurization. Each sample was split into two aliquots with one aliquot cultured as received and the other spiked with $1 \times 10^5$ cfu Mpt/ml and then cultured to determine efficiency of recovery of viable Mpt. NG = no growth on agar slants; + = positive identification of Mpt colonies on agar slants.

reached the final sampling temperature of 71.1°C after 110 min.

Recovery of viable *M. paratuberculosis* before and after pasteurization is shown in Figure 2. After adding the *M. paratuberculosis* inoculum to the waste milk and mixing with the agitator bar for 10 min, samples of milk were taken from the holding tank to obtain a pre-pasteurization viable bacteria count. An average of 2.5 $\times 10^3$ cfu/ml of milk were recovered before heating for

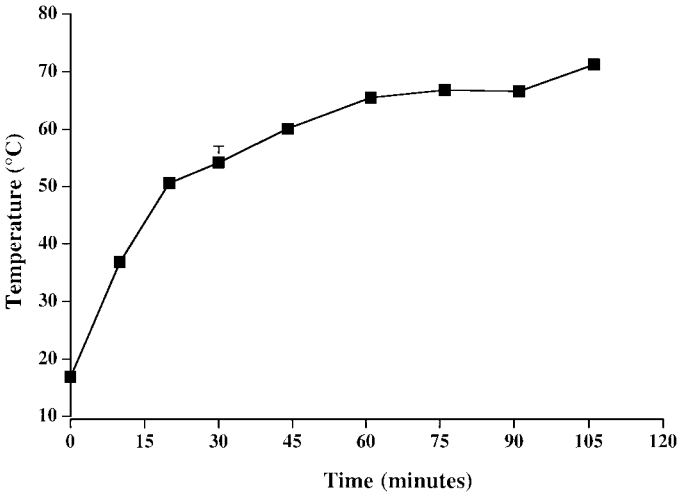


**Figure 1**. Sampling time versus temperature of the milk in the batch pasteurizer unit. Values are means ± SEM for two replicate experiments.

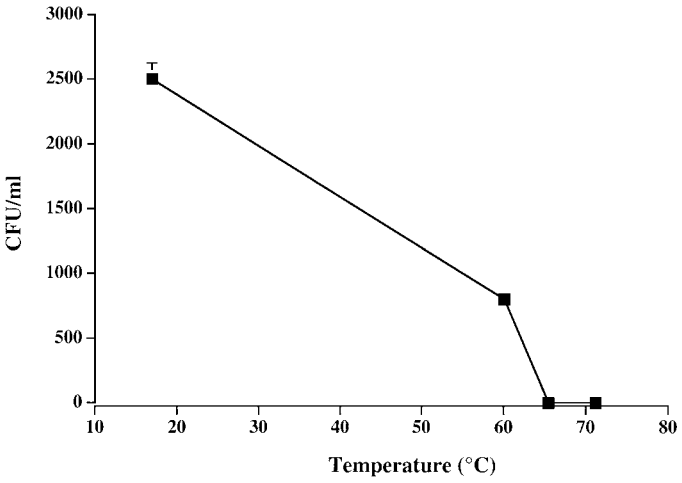


**Figure 2**. Recovery of viable *Mycobacterium paratuberculosis* in milk before and after heat treatment in the batch pasteurizer unit. Values are means ± SEM for two replicate experiments.

the two experiments. The recovery of *M. paratuberculosis* was reduced to $1 \times 10^3$ fu/ml of milk after heating the milk to 60.1°C, although the number of viable counts was still significant and comparable to prepasteurization levels. However, no viable *M. paratuberculosis* were isolated from milk samples when the target temperature of 65.5°C was reached at either 0, 15, or 30 min. A further increase in temperature to 71.2°C also resulted in negative recovery of *M. paratuberculosis*.

## DISCUSSION

Based on information obtained from the National Animal Health Monitoring System dairy survey conducted in 1996, economic losses in the United States from Johne's disease in dairy herds exceeds $200 million (15). This figure represents financial losses from culling or death of clinically affected cows, reduced reproductive efficiency, reduced feed efficiency, and decreased milk production in subclinically infected cows. Subclinical infection may result in a reduction of 15 to 16% in milk production (1, 2). Cows beyond second lactation that are infected with *M. paratuberculosis* have demonstrated losses of 590 to 1270 kg of milk per lactation (17). Treatment of *M. paratuberculosis* infection with antimicrobials is not considered an option because it is expensive, requires extensive treatment periods, and is not totally effective (6,11). Vaccination of animals in the herd at calfhood is an alternative method of management that may allay the progression of subclinical disease to a more clinical stage in some animals and reduce economic losses, but it does not prevent infection (5, 16). Therefore, producers need to be informed of management tools that can lessen the degree of expo-

sure of neonates to the bacterium. Removing the calf from the dam immediately after birth reduces the risk of transmission of the bacterium through contaminated fecal matter present in the maternity pen but also reduces the chance of infection from colostrum that may contain viable *M. paratuberculosis*.

Viable *M. paratuberculosis* are present in the colostrum and milk of cows with Johne's disease (12, 13, 14). Cows with clinical disease or asymptomatic cows with heavy fecal shedding may shed 5 to 8 cfu of *M. paratuberculosis*/50 ml of milk (13). Although the natural shedding of organism into milk may be low, it has been suggested (9) that fecal contamination of colostrum and milk from cows that are shedding high numbers of *M. paratuberculosis* may be a significant risk of transmission to the young calf. Feeding colostrum from known noninfected cows is a recommended management tool to prevent this route of transmission while still providing the calf with the passive immunity essential for it to remain healthy. However, subsequent feeding of raw waste milk to calves may be a significant source of *M. paratuberculosis* infection within the herd. Pasteurization of waste milk appears to be a likely alternative to feeding milk replacer and results in a considerable savings to the producer. The present study indicates that holding milk at 65.5°C for 30 min is more than adequate to achieve total destruction of *M. paratuberculosis*. The total time to achieve pasteurization using a batch pasteurizer on site was 90 min and required no special technical expertise. In addition, after heat treatment, agar slants were free of contamination with fungi and other non-acid fast microorganisms that may be present in milk fed to calves. Therefore, in addition to reducing infection with *M. paratuberculosis*, pasteurization could reduce the risk from other pathogenic microorganisms as well.

## CONCLUSIONS

It is recommended that producers who have identified Johne's disease within their herd implement stringent management tools to reduce transmission of the infection to young calves. Feeding colostrum from noninfected dams and pasteurizing waste milk are two essential recommendations for promoting a healthy, productive herd.

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