Letter to the Editor

Does Mycobacterium paratuberculosis Survive Current Pasteurization Conditions?

In a recent article, Stabel, Steadham, and Bolin reported that Mycobacterium paratuberculosis did not survive high-temperature, short-time (HTST) pasteurization simulated with an Armfield HTST laboratory pasteurizer (7). The authors effectively dismissed the considerable data generated by previous studies (1–5) which indicated that current HTST pasteurization conditions may not be effective in killing M. paratuberculosis and chose to conclude that turbulent flow of milk during pasteurization was essential for complete killing of contaminating M. paratuberculosis. However, Hope et al. (4) also employed small-scale continuous-flow pasteurizing equipment and reported that viable M. paratuberculosis cells were isolated from HTST-pasteurized milk initially containing 10⁴ M. paratuberculosis CFU/ml. These findings would tend to invalidate the argument regarding turbulent versus static heating put forward by Stabel et al. to explain the results of their study. In my opinion there are a number of other factors which may explain their inability to isolate viable M. paratuberculosis during their recent study.

My primary concern is that on the one hand Stabel et al., keen to simulate commercial HTST pasteurization conditions as closely as possible, used an Armfield HTST laboratory pasteurizer but on the other hand proceeded to use an inoculum which had been both frozen and sonicated before addition to the raw milk. M. paratuberculosis cells naturally present in infected milk would be subject to neither of these treatments prior to commercial pasteurization, and none of the previous studies of M. paratuberculosis and pasteurization have taken this approach. The detrimental effect of freezing at −80°C on the viability of M. paratuberculosis has been reported (6), and sonication applied to cells already injured by freeze-thawing may also have affected cell viability. The reason given by Stabel et al. for the use of sonication is fully appreciated. However, recent studies by Sung et al. (8) and ourselves (unpublished data) have shown that declumped M. paratuberculosis cells are much less heat resistant than clumped cells. Consequently, by incorporating a sonication step before heating, Stabel et al. were effectively increasing the chances of inactivating M. paratuberculosis during pasteurization. Further sonication of M. paratuberculosis cells in the pasteurized milk before enumeration may also have contributed to their inability to detect viable but sublethally injured M. paratuberculosis, if any existed, in the pasteurized product. In my experience, heat-treated M. paratuberculosis cells are not recovered by BACTEC culture if PANTA antibiotic supplement is added to the BACTEC medium, whereas they are recoverable if the PANTA is omitted. This observation clearly illustrates the sublethally injured status of M. paratuberculosis cells after heating and the adverse effect that additional stress can have on the viability of the organism.

In my opinion, Stabel et al. studied the effect of HTST pasteurization on potentially injured M. paratuberculosis cells which were likely to be more heat sensitive from the outset. I would contend that the methodology employed by these authors less accurately reflected the condition of M. paratuberculosis cells occurring in naturally infected milk than that used in previous studies, and consequently, the results of this study must be considered in light of this fact.

REFERENCES


Author’s Reply

This letter is written in response to a Letter to the Editor written by Dr. Irene Grant regarding the recently published article, “Heat Inactivation of Mycobacterium paratuberculosis in Raw Milk: Are Current Pasteurization Conditions Effective?” (6). The first point that Dr. Grant makes is that we “effectively dismissed” previous work in this area (1, 2, 5). I will allow that discussion of previous data was brief, but this was due to the restrictions of publishing in the format of a short-form paper rather than a full-length one. We did, however, cite all of the pertinent published work that had appeared in referred journals. The glaring difference between the previous studies and our study was the methodology used for heat treatment of the milk samples, and so this was the main point of our discussion.

We did not cite the abstract published by Hope et al. in the Proceedings of the Fifth International Colloquium on Paratuberculosis (3). Dr. Grant suggests in her letter that although Hope et al. utilized a small-scale, continuous-flow pasteurizer to conduct their studies they were able to isolate viable M. paratuberculosis cells after heat treatment. What Dr. Grant does not discuss is that the unit that they used had a linear holding tube. Commercial pasteurization units have curved holding tubes, which generate turbulent flow rather than laminar flow. Indeed, the authors state quite succinctly in their abstract that it was necessary to adjust the conditions of HTST to 72 to 73°C for 25 to 35 s in order to compensate for the laminar flow in the
holding tube. When experiments were conducted at these conditions, no viable bacteria were recovered from raw milk inoculated with $10^4$ M. paratuberculosis CFU/ml. Only when heat treatment of milk was reduced to 15 s at 72 to 73°C were any viable organisms recovered.

Dr. Grant’s next point of concern is the use of frozen and sonicated M. paratuberculosis cells to seed the raw milk prior to heat treatment in our experiments. We grew up various strains of M. paratuberculosis in broth medium and froze aliquots of each batch for use in these experiments. This methodology has been used by others in their experiments, and the NADC has provided some frozen stock cultures of M. paratuberculosis to these laboratories to conduct pasteurization studies (2). The reason for using frozen stocks was simply to maintain the integrity of each bacterial strain from experiment to experiment. Bacterial characteristics can change markedly if passed in vitro repeatedly, and freezing was one way of ensuring a static physiological state for the bacteria. Since freezing can reduce the number of viable bacteria, we examined serial dilution curves of each strain of thawed and sonicated M. paratuberculosis cells during each experiment to check viability of cultures. The brief sonication procedure that we used to declump the bacteria (35 W, 15 s) was not enough to reduce viability of the organisms since we always checked this by examination of the aforementioned serial dilution curves. We were always able to recover the appropriate amount of bacteria for each dilution tube upon culture. Another study has demonstrated that clumping and declumping of M. paratuberculosis did not affect its susceptibility to heat treatment (4). In addition, if any sublethally injured M. paratuberculosis cells existed we were unable to recover them after 6 months of incubation on solid medium. Finally, we were able to recover viable organisms (even after freezing and sonication) after heat treatment by using the holder test tube method in this study, which again points to the differences in methodology as the key to survival of the organism.

Concern for a human health link between M. paratuberculosis and Crohn’s disease in human beings makes interpretation of studies evaluating pasteurization of dairy products particularly important. In our opinion, the laboratory-scale pasteurizer unit utilized in our laboratory simulates commercial pasteurizer units more closely than any other methodology employed to date. Results from our studies with the laboratory-scale pasteurizer indicate that HTST pasteurization effectively kills all M. paratuberculosis cells experimentally inoculated into raw milk.

REFERENCES


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