APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2001, p. 3964–3969 Vol. 67, No. 9
0099-2240/01/$04.00+0 DOI: 10.1128/AEM.67.9.3964–3969.2001

Effect of Turbulent-Flow Pasteurization on Survival of *Mycobacterium avium* subsp. *paratuberculosis* Added to Raw Milk

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Received 19 December 2000/Accepted 27 June 2001

A pilot-scale pasteurizer operating under validated turbulent flow (Reynolds number, 11,050) was used to study the heat sensitivity of *Mycobacterium avium* subsp. *paratuberculosis* added to raw milk. The ATCC 19698 type strain, ATCC 43015 (Linda, human isolate), and three bovine isolates were heated in raw whole milk for 15 s at 63, 66, 69, and 72°C in duplicate trials. No strains survived at 72°C for 15 s; and only one strain survived at 69°C. Means of pooled *D* values (decimal reduction times) at 63 and 66°C were 15.0 ± 2.8 s (95% confidence interval) and 5.9 ± 0.7 s (95% confidence interval), respectively. The mean extrapolated $D_{72°C}$ was <2.03 s. This was equivalent to a >7 $\log_{10}$ kill at 72°C for 15 s (95% confidence interval). The mean *Z* value (degrees required for the decimal reduction time to traverse one log cycle) was 8.6°C. These five strains showed similar survival whether recovery was on Herrold's egg yolk medium containing mycobactin or by a radiometric culture method (BACTEC). Milk was inoculated with fresh fecal material from a high-level fecal shedder with clinical Johne's disease. After heating at 72°C for 15 s, the minimum *M. avium* subsp. *paratuberculosis* kill was >4 $\log_{10}$. Properly maintained and operated equipment should ensure the absence of viable *M. avium* subsp. *paratuberculosis* in retail milk and other pasteurized dairy products. An additional safeguard is the widespread commercial practice of pasteurizing 1.5 to 2° above 72°C.

Mycobacterium avium subsp. *paratuberculosis* is the cause of Johne's disease, a chronic bowel disease of dairy cows and other ruminants that has a worldwide distribution. It has been suggested that this bacterium may also play a role in the etiology of Crohn's disease in humans (for a review, see reference 1). Under normal milking conditions, bulk raw milk from an infected herd will probably be contaminated with *M. avium* subsp. *paratuberculosis.* A major source is from fecal contamination of the udder. Animals with clinical Johne's disease excrete *M. avium* subsp. *paratuberculosis* at varying levels in their feces. Levels as high as 10^8 CFU/g have been reported (3). Direct excretion in the milk is likely to be less significant than fecal contamination. Aseptically removed milk from clinically affected cows may have low levels of *M. avium* subsp. *paratuberculosis.* Up to 8 CFU/50 ml have been reported, but only a proportion of the milk from clinically affected cows yielded positive cultures (27, 29, 30). Some asymptomatic animals can also shed *M. avium* subsp. *paratuberculosis* in their milk (29). The likely raw milk contamination level is not known with any certainty, with estimates ranging from <1 CFU/ml (11, 23), through over 250 CFU/ml (22), to more than 10^4 CFU/ml (8).

In 1993, Chiodini and Hermon-Taylor first reported the survival of *M. avium* subsp. *paratuberculosis* in laboratory experiments designed to simulate pasteurization at both 63°C for 30 min and 71.7°C for 15 s (2). Since then, six additional groups have published the results of heat inactivation studies on this bacterium (8, 12, 21, 26, 28; J. Keswani and J. F. Frank, Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996, abstr. P-89, p. 384, 1996). All these groups wished to reproduce as closely as possible the essential features of the pasteurization process. However, the methodology chosen and the equipment available varied between laboratories. Heating in test tubes (2), vials (28), capillaries (Keswani and Frank, Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996), laboratory-scale pasteurizers (8, 26), and pilot-scale pasteurizers (12) was performed. It is therefore not surprising that the results obtained showed marked variations. For example, the $\log_{10}$ kills recorded or predicted for cultures of the type strain ATCC 19698 at 71.7°C covered every decade from <1 $\log_{10}$ (28) to >6 $\log_{10}$ (26).

Some of the groups studying the heat inactivation of *M. avium* subsp. *paratuberculosis* have discussed turbulent flow as an important feature of commercial pasteurization (see references 7, 12, and 26). The Reynolds number (Re) describes the type of flow. This parameter can be derived from the flow velocity, tube diameter, fluid viscosity, and fluid density. The Re indicates whether flow in a pipe is laminar (Re < 2,000) or turbulent (Re > 3,000) (15). When the milk flow in a holding tube is turbulent, the fastest flowing particle travels 1.1 times faster than the average particle. By contrast, with laminar flow the fastest flowing particle travels twice as fast as the average particle. Because the rate of heat inactivation of bacteria increases exponentially with time, the difference between these two types of flow during pasteurization can represent a difference of many orders of magnitude in inactivation. This distinction between different flow types is universally recognized in the regulations governing commercial high-temperature short-time (HTST) or continuous-flow pasteurization. In particular, "the holding tube must be such that the fastest flowing particle . . . will not traverse the holding tube in less than the required

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holding time" (31). A direct measure of the relation of the velocity of the fastest particle to the mean particle can be obtained by determining the residence time distribution (RTD). This technique measures the conductivity distribution of injected NaCl and can provide an independent validation of the type of flow.

The present work is the first report of the heat inactivation of *M. avium* subsp. *paratuberculosis* under validated conditions of turbulent flow using an experimental design that allowed kinetic data to be obtained. As both milk fat and clumping might favor the survival of the bacteria, the worst case scenario of nonhomogenized whole milk was used. Bacteria present in fecal samples from a cow with clinical Johne's disease were used in some portions of the study.

MATERIALS AND METHODS

Bacterial strains. *M. avium.* subsp. *paratuberculosis* ATCC 19698 (type strain), ATCC 43015 (Linda, human isolate), and strains A, B, C, and D isolated from New Zealand cattle were used for the kinetics experiments. Strains A, B, C, and D were used in the preliminary experiments. The two ATCC strains had been passaged less than five times since they were obtained from the culture collection. The New Zealand bovine isolates had also been passaged less than five times. Strains were characterized by Southern blotting using procedures described previously (4).

Preparation of cultures for spiking milk. Cultures for testing were inoculated into Middlebrook 7H9 broth (Difco, Detroit, Mich.) supplemented with 10% (vol/vol) albumin dextrose complex (ADC; Difco), 0.41% pyruvate, 0.05% Tween 80 (Sigma, St. Louis, Mo.), and 0.0002% (wt/vol) mycobactin J (Allied Monitor, Inc., Fayette, Mo.). Cultures were grown on a roller apparatus for up to 44 days at 37°C. Growth was monitored by measuring culture absorbance (A_{525}). Cultures were checked for purity and for acid-fast bacilli typical of *M. avium* subsp. *paratuberculosis* by the Ziehl-Neelsen stain. Cultures were centrifuged (3,500 × *g*) for 20 min at 5°C, reconstituted in 100 ml of sterile phosphate-buffered saline (PBS; 0.02 M, pH 6.8) and homogenized in a sonicating water bath for 2 min to reduce the degree of clumping. Sonicated culture (45 ml) was added to 120 liters of milk. Counts of each strain were determined following inoculation of appropriate dilutions onto triplicate slopes of Herrold's egg yolk medium supplemented with 2 μg of mycobactin J/g, 50 μg of amphotericin B/g, 200 U of polymyxin B/g, 100 μg of carbenicillin/g, and 7.5 μg of trimethoprim/g (HEYMM) (19). Slopes were incubated at 37°C.

Fecal samples. Fecal samples from cows with clinical Johne's disease were examined microscopically for clumps of acid-fast bacteria. Using these data, a cow with samples showing numerous clumps was selected. A further sample was then removed from the bowel and held chilled for no more than 24 h before use in the pasteurization trial. Feces (25 g) were mixed with 100 ml of milk and shaken vigorously for 15 min. This suspension was added to 120 liters of milk and thoroughly mixed.

Pilot-scale milk pasteurizer. The pilot-scale indirect-heating milk pasteurizer comprised two 50-liter balance tanks with in-line filters (500 μm), a variable-speed feed pump, plate heat exchangers (PHEs) for heating and cooling, a tubular holding section, and instrumentation. A positive-displacement, nonpulsating feed pump (Mono SLF 5022105/C; Sydney, Australia) was used. The flow rate was adjusted by altering the pump motor speed. The milk flow rate (maximum capacity, 200 kg/h) was continuously measured by a calibrated mass flow meter (model D-SA220F; Micro Motion Meters, Boulder, Colo.) installed between the feed pump and the PHE. Milk was indirectly heated using a two-pass, countercurrent PHE (model U2-41-R-SS; APV, Kolding, Denmark) having a heat transfer area of 0.7 m^3 and a hold-up volume of 1 liter. The maximum temperature difference between the whole milk and the hot water was 1°C. The PHE was heated by recirculated hot water that was maintained at the required temperature by injected steam. Steam condensate was continuously removed from the recirculated hot water loop and discarded via a pressure relief valve. The temperature of the milk leaving the holding tube (i.e., the pasteurizing temperature) was maintained at the required level by automatically controlling the temperature of the recirculated hot water. The temperatures away from the PHE and away from the holding tube and the temperature of the hot water entering the PHE were continuously measured with platinum resistance thermometers with transmitters (PT 100, three-wire class A; Servotech, Auckland, New Zealand) and captured by data-logging equipment. Temperature probes were calibrated according to the international temperature scale ITS-90 (24). The precision of the platinum resistance thermometer was ±0.1°C. After heating for the required time and at the required temperature, chilled water at 6°C was used to cool the milk to 26°C in a single-pass, countercurrent PHE (APV, model T4RV) with a hold-up volume of 0.5 liter.

Holding tube. The holding tube was an insulated section of 304 stainless steel tube, internal diameter (ID) 7.7 mm. The tube assembly was 11.7 m long, giving a holding time of 16.5 s (average particle) and 15 s (fastest particle) for whole milk pasteurized at 74°C and a flow rate of 120 liters/h. These conditions were calculated to give turbulent milk flow (Re, >11,000).

Pilot plant cleaning. The pasteurizer equipment was cleaned in place before and after each experiment and as required. The plant was rinsed with cold water, circulated with 1% (wt/vol) NaOH (75°C, 30 min), and rinsed with warm soft water (75°C, 15 min). Nitric acid (0.7% [wt/vol], 70°C) was then circulated for 15 min at the maximum pump rate of 200 kg/h. The pasteurizer was then sterilized by recirculating 90°C hot water for 5 min before each trial.

Determination of the RTD in the holding tube. The RTD was determined by injecting whole milk at 74°C at a flow rate of 120 liter/h with a small volume of 20% NaCl just prior to the 7.7-mm (ID) holding tube. The plant was allowed to reach steady state for 15 min before RTD measurement began. The electrically conductive tracer was injected (0.3 ml/stroke) according to a pseudorandom binary sequence by a solenoid-driven diaphragm pump (Gamma/4 1002; Prominent Fluid Controls, Ashby-de-la-Zouch, United Kingdom) triggered by a computer output signal. A static mixer was placed immediately after the injector to ensure good mixing between the NaCl and the milk. A second static mixer was placed immediately after the holding tube. Successive small samples of the output flow were quantitatively analyzed for the tracer at 1-s intervals by an in-line conductivity cell using a cross-correlation technique (13, 14). The conductivity signal was recorded as a computer file for further analysis. Runs were for 5 min to enable sufficient data to be collected for RTD calculations.

Pilot plant operation in pasteurization trials. Raw whole milk (4.26% fat, 3.4% protein) was used in the trials. For microbiological safety reasons the trials were carried out in the milk reception (noncritical hygiene) area of the New Zealand Dairy Research Institute dairy processing facilities. Prior to each trial, 120 liters of raw milk was pumped to a holding vat for control sampling and culture addition. The inoculated milk was then pumped to one of the pasteurizer balance tanks. Excess milk was held in sterilized milk cans. The pasteurizer was initially set at the highest temperature (72°C) by adjusting the hot water temperature as described above. After passing through the holding tube, the milk was cooled to 26°C and aseptically sampled after at least 1 min of equilibration at the set pasteurization temperature. The milk was pumped though the pasteurizer without interruption while the rate of steam injection was reduced and the plant was equilibrated at the next (lower) set temperature.

Preparation of milk for analysis. For each strain at each temperature, one 50-ml milk sample was collected, and after being held on ice (generally up to 2 h) the sample was centrifuged at 7,000 × *g* for 10 min at 5°C. The supernatant and the cream layer were discarded and the pellet was treated with 1% cetylpyridinium chloride to inactivate extraneous microorganisms. The pellet was resuspended by vortexing. The tube was decontaminated at room temperature for 50 min, centrifuged at 7,000 × *g* for 10 min at 15°C, and the supernatant was discarded. The pellet was resuspended in 0.75 ml of PBS.

Enumeration methods. Appropriate dilutions (0.1 ml) of decontaminated sample were inoculated onto each of three slopes of HEYMM. The slopes were incubated for 20 weeks at 37°C. The remaining material (0.45 ml) was also evaluated for viable *M. avium* subsp. *paratuberculosis* organisms by the BACTEC radiometric culture method. BACTEC vials (Becton Dickinson Microbiologic Systems, Sparks, Md.) were each supplemented with 0.1 ml of PANTA plus (Becton Dickinson), 1.0 ml of fresh egg yolk, and 8 μg of mycobactin J.

Confirmation of culture identity and relationships. Acid-fast staining, mycobactin dependence, and the presence of IS*900* were the criteria used to identify organisms as *M. avium* subsp. *paratuberculosis.* Mycobactin dependence was determined by subculturing onto HEYMM or non-mycobactin-supplemented Lowenstein-Jensen medium. DNA for the IS*900* PCR was extracted from bacterial colonies by two cycles of boiling and cooling. The primers used were 5′-GATCGGAACGTCGGCTGGCAGG-3′ and 5′-GATCGCCTTGCTCATC GCTGCCG-3′ (5), using the conditions described by Wards et al. (32).

Data analysis. The thermal inactivation data were analyzed using the first-order kinetic model (16) as follows:

$$\frac{N(t)}{N_0} = e^{-kt} \quad (1)$$

where t is time. k is usually calculated by ordinary least squares from the slope of

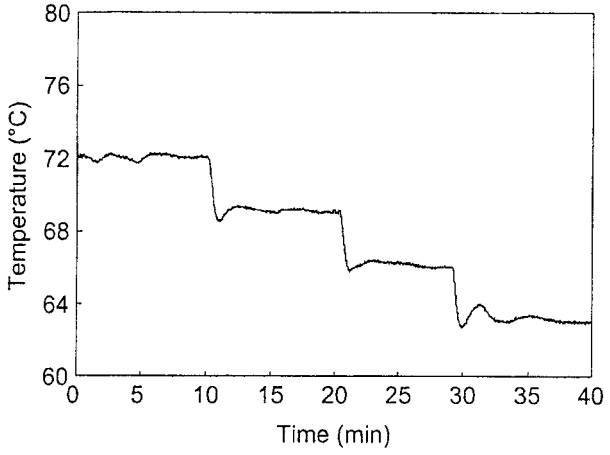

FIG. 1. Temperature control during a continuous pasteurizer run of whole milk spiked with *M. avium* subsp. *paratuberculosis*. Samples for enumeration were taken after the temperature had equilibrated for at least 1 min at each selected temperature.

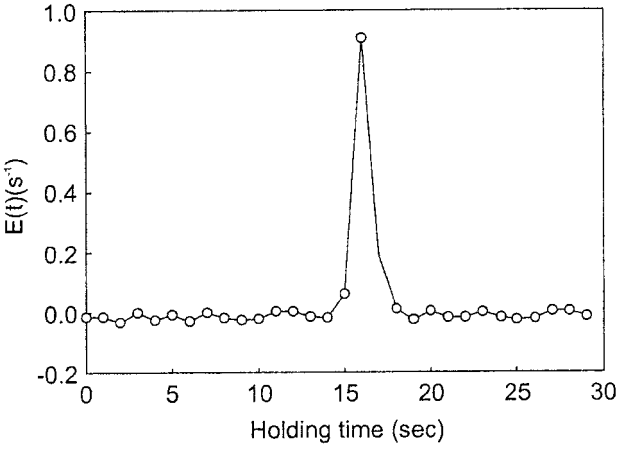

FIG. 2. RTD $E(t)$ of whole milk at 74°C after pumping at 120 liters/h through a 15 s holding tube (ID, 7.7 mm; Re, 11,050).

the $\ln(N/N_0)$-versus-time plot. However, least squares cannot be used when only two time points (initial and final) are available. By rearranging equation 1, the following equation is produced:

$$k = \frac{-\log(N/N_0)}{2.3026t} \quad (2)$$

where $t = 15$ s. The decimal reduction time D is found directly from k as follows in equation 3:

$$D = \ln(10)/k = 2.3026/k \quad (3)$$

The relationship between D and $\log_{10}$ kill is, in turn, derived directly from equations 1 and 3, as follows:

$$\log_{10} \text{kill} = t/D \quad (4)$$

The Z value is the degrees required for the decimal reduction time to traverse one log cycle. Z is usually calculated from the results obtained from inactivation by using a range of time and temperature combinations. An Arrhenius relationship between temperature (T) and k is assumed (16), as follows:

$$k(T) = e^{\frac{-E_a}{RT}} \quad (5)$$

Under this model, the equivalent temperature-time combinations lie in straight lines when $\log_{10} t$ is plotted against temperature. These straight lines have a slope of $1/Z$. Kessler (16) showed that the slope of $\log_{10} k$ versus T is approximately $1/Z$ over a narrow range, e.g., 60 to 80°C. The form of equation 3 shows that the relationship between $\log_{10} D$ and T will also be linear. Hence, least squares can be used to obtain an estimate of Z from the slope of the $\log_{10} D$-versus-T graph. Least squares can simultaneously provide 95% confidence intervals for predicted D values. These can then be used, with equation 4, to give 95% confidence intervals for $\log_{10}$ kill.

RESULTS

Performance of the pasteurizer. The temperatures of milk exiting the holding tube in the 2 min prior to sample collection were recorded in a typical pasteurizer run (Fig. 1). Temperature ranges were as follows: set at 72°C, mean 72.04°C (71.94 to 72.11°C); set at 69°C, mean 69.02°C (68.91 to 69.19°C); set at 66°C, mean 66.02°C (65.94 to 66.06°C); set at 63°C, mean 63.03°C (62.96 to 63.08°C). A schematic diagram of the RTD determination is shown in Fig. 2. RTD is normally represented by the function $E(t)$, which can be normalized so that the area under the curve is unity (17). From Fig. 2, the calculated RTD curve had a mean residence time of 16.2 s, a minimum residence time of 15 s, and a variance (σ^2) of 0.629. The fastest particle thus traveled 8% faster than the mean, a value within the theoretical 10% for fully turbulent flow (Re, 11,050). The dilution effect of the injection of NaCl (1 liter/h) into the milk flow rate (120 liters/h) had no significant effect on the RTD.

Preliminary experiments. Because of the wide variations in published heat inactivation data, a preliminary set of investigations was carried out to establish cleaning and sterilization protocols for the pasteurization plant and to determine over what range of temperatures spiked milk samples should be examined in order to obtain kinetic data. As part of the preliminary experiments, feces from a cow selected as a high-level shedder of acid-fast bacteria (enumerated at 1.3×10^7 CFU/g) were spiked into the raw whole milk used in pasteurization trials at 3.25×10^3 CFU/ml. Duplicate runs were carried out at 72, 74, and 76°C and 50-ml samples were processed and plated on HEYMM slopes. No survivors were detected at any temperature, in either run, indicating a >4 $\log_{10}$ kill. Raw milk controls, tested before culture addition or heating, were always negative.

Survival after heating. Prior to pasteurization, the level of *M. avium* subsp. *paratuberculosis* in milk samples spiked with cultures ranged from 0.7×10^3 to 16×10^3 CFU/ml. *M. avium* subsp. *paratuberculosis* ATCC 19698, ATCC 43015, and bovine strains A, B, and D were heated in raw whole milk for 15 s at 72, 69, 66, and 63°C. Each strain and temperature combination was repeated on a subsequent day. No strains survived at 72°C for 15 s. Only one strain had survivors at 69°C for 15 s. Small numbers (1.5 CFU/ml) of ATCC 43015 were isolated from milk treated at this temperature in one of the two trials. Duplicate heat inactivation experiments were also carried out with milk spiked (20 to 32 CFU/ml) with feces from a cow that was a moderate-level shedder. In one of the two trials, *M. avium* subsp. *paratuberculosis* was isolated (0.4 CFU/ml) after 69°C for 15 s.

The data were analyzed according to the formulas derived above. Temperature inactivation can be expressed as the relationship between $\log k$ and temperature (Fig. 3) or in terms of log kill and temperature (Fig. 4). The data from the two runs

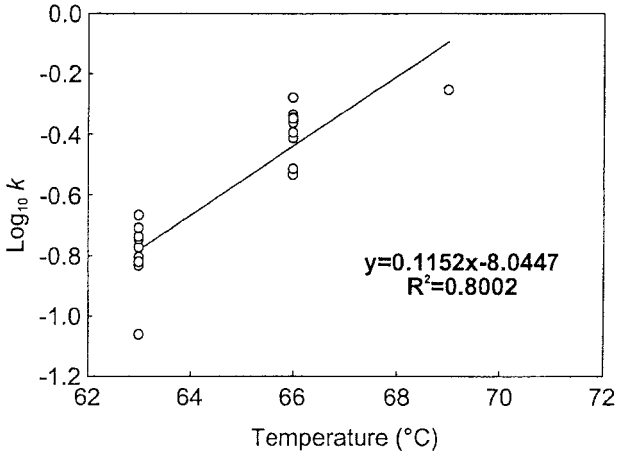

FIG. 3. Heat inactivation of *M. avium* subsp. *paratuberculosis*. A $\log_{10} k$ plot of strains ATCC 19698, Linda, A, B, and D at 63, 66, 69, and 72°C is shown. Data are from duplicated runs at 63 and 66°C (10 data points each) and at 69°C (1 data point). The absence of data points at 69 and 72°C indicates no survivors.

at the same temperature were pooled, as the variation between individual strains was not significantly different from the variation between replicated runs with the same strain ($P = 0.504$). *D* values were derived (Table 1). Means of the pooled *D* values at 63 and 66°C were 15.0 ± 2.8 s (95% confidence interval) and 5.9 ± 0.7 s (95% confidence interval), respectively. The mean extrapolated $D_{72°C}$ was <2.03 s. This was equivalent to a >7 $\log_{10}$ kill at 72°C for 15 s (95% confidence interval). The mean *Z* value was 8.6°C.

DISCUSSION

The present study was planned to reproduce as closely as possible the pasteurization conditions used in commercial dairy plants. For this reason, raw milk was used and turbulent flow conditions were achieved and validated in a pilot plant pasteurizer processing 120 liters/h. Although the most obvious difference between batch and commercial HTST pasteurization is turbulent flow, other factors are likely to be involved. These include shear forces and other physical stresses (25). A full clean-in-place (CIP) sterilization between runs proved effective in preventing cross-contamination between runs.

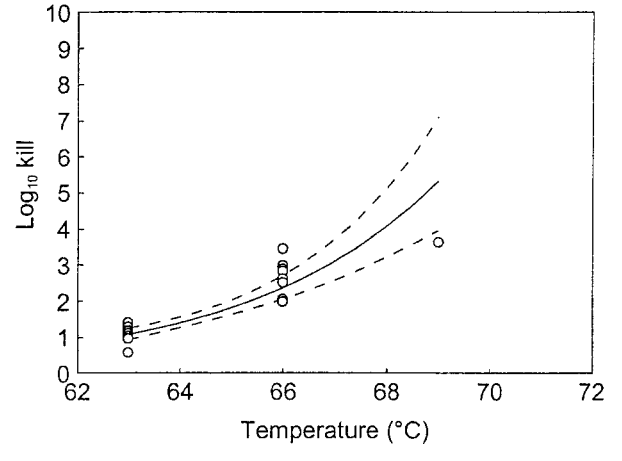

FIG. 4. Heat inactivation of *M. avium* subsp. *paratuberculosis*. A $\log_{10}$ kill plot of strains ATCC 19698, Linda, A, B, and D, each pasteurized at 63, 66, 69, and 72°C for 15 s, is shown. Data are the same as for Fig. 3. The absence of data points at 69 and 72°C indicates that the kill exceeded the >4 $\log_{10}$ to 5 $\log_{10}$ detection limit. Solid line, mean kill; dashed lines, upper and lower 95% confidence intervals.

Bacterial *D* values are traditionally determined from the best-fit line when the $\log_{10}$ of survivors at a given temperature is plotted against the exposure time. *D* values derived at different temperatures can then be used to calculate the *Z* value. If exposure times had been used as a variable in the determination of *D* values in the present study, then the full CIP sterilization adopted procedure would have been necessary each time the holding tube was changed. The time taken for this full sterilization cycle would have meant an excess of 90 min between taking samples for two points on a single *D* curve. It would thus have taken many weeks to test multiple strains at multiple temperatures. Under these conditions, there would have been variations in the raw whole milk used as the heating medium and in the preparation conditions for the inoculum. The alternative option of varying the holding times by changing the pumping rate was not adopted, as this would have varied the Reynolds number.

An examination of the equations governing the derivation of *D* and *Z* values suggested the novel approach of generating these values from survival data at different temperatures without the necessity of varying the exposure time. Single batches of seeded raw milk could then be processed in continuous uninterrupted flows, with temperatures being adjusted stepwise from the highest to the lowest value. In this manner, the experimental work could be accomplished more rapidly and the pasteurization trials were able to include as many features of normal commercial operation as was practical.

A number of different microbiological factors might affect the outcome of experiments investigating the thermal resistance of *M. avium* subsp. *paratuberculosis.* Initial studies by Chiodini and Hermon-Taylor (2) suggested that strains of *M. avium* subsp. *paratuberculosis* isolated from Crohn's disease patients were more heat resistant than those isolated from cattle. In our experiments no major difference in the heat resistance was observed in the different strains that were examined, including an isolate from a Crohn's disease patient. This result was not surprising given that Southern blotting revealed that their restriction fragment polymorphisms were identical when hybridized to an IS*900* probe. This has been shown to be a sensitive method for distinguishing between different strains of *M. avium* subsp. *paratuberculosis* (4). The thermal resistance of *M. avium* subsp. *paratuberculosis* in feces appeared to be similar to that of laboratory cultures. We were unable to obtain a high-level shedder, to follow up on the preliminary experiments and obtain good kinetic data. The moderate-level fecal shedder that was used gave milk inoculated with 18- to 300-fold-lower CFU of *M. avium* subsp. *paratuberculosis* than was the case with laboratory-grown cultures. In one of the replicated trials, very low survival was found at 69°C for 15 s. Further experiments will be needed to examine whether this level of survival is significant.

One of the characteristics of *M. avium* subsp. *paratuberculosis* is its propensity to form clumps, which undoubtedly causes

TABLE 1. *D* values (s) for *M. avium* subsp. *paratuberculosis* strains heated at 63, 66, and 69°C for 15 s

Strain	*D* value (s)					
	63°C		66°C		69°C[a]	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
ATCC 19698	10.75	13.73	4.38	5.76	NS	NS
Linda	15.40	12.68	5.12	5.17	4.13	NS
A	11.83	12.95	7.56	5.01	NS	NS
B	26.62	14.77	5.13	7.59	NS	NS
D	15.53	15.74	7.29	6.02	NS	NS

[a] NS, no survivors.

difficulties in counting this organism accurately. It is possible that the degree of clumping of *M. avium* subsp. *paratuberculosis* may also affect its thermal resistance. Although there has been criticism that declumping methods may affect thermal resistance, Sung and Collins (28) found no difference in the viability between mildly sonicated and unsonicated cultures of *M. avium* subsp. *paratuberculosis.* Other possible factors that may affect the results of thermal resistance studies are the culture procedure, including the use of low levels of decontaminants, the incorporation of antibiotics in culture media, and the use of low levels of sonication. With the commercially produced raw milk used in our experiments and with heating as low as 63°C for 15 s, the prevention of culture overgrowth had been shown to require the use of some decontamination in addition to antibiotics. We cannot exclude the possibility that this treatment adversely affected recovery. The necessity of using decontamination is thus a point of difference between our study and the laboratory studies cited earlier (8, 21, 26, 28; Keswani and Frank, Abstr. 96th Gen. Meet. Am. Soc. Microbiol. 1996). These studies, with one exception (28), also omitted the use of antibiotics. The decontamination procedure used in the present study, however, was very mild compared with the double-incubation procedure recommended for culturing fecal samples for *M. avium* subsp. *paratuberculosis* (33). It was also less stringent than procedures that have been used in some studies for culturing *M. avium* subsp. *paratuberculosis* from nonheated raw milk (for a review, see reference 10).

Although there have been claims that radiometric liquid culture procedures are more sensitive than standard culture procedures (9), no difference in sensitivity between the two methods was observed in this study.

Mycobacteria obtained directly from lesions have been claimed to be less heat resistant than the same strain grown in vitro (20). It is thus possible that the *M. avium* subsp. *paratuberculosis* organisms excreted by infected cows differ in this or some other relevant feature(s) from laboratory-maintained and -grown cultures. When fresh fecal material was seeded directly into the milk before heating, the >4 $\log_{10}$ kill achieved at 72°C for 15 s was consistent with that found for the five laboratory strains. Several papers have highlighted the desirability of determining whether there are significant differences in the rates of destruction between the two types of culture origin (7, 11, 23). A definitive answer will require data suitable for kinetic analysis.

A major characteristic of commercial pasteurization is turbulent flow. In practice, if the flow cannot be demonstrated as turbulent, then regulatory authorities normally require a holding time of twice that of the average particle. The pilot plant used in this work met both the theoretical and experimental criteria for turbulent flow. The residence time of the fastest particle in each instance was $<10\%$ shorter than the mean time. The holding tube had been constructed 10% longer than calculated for the mean flow to take this factor into account. That is, there was an assurance that every particle would receive at least 15 s of heating at the set temperature.

The mean extrapolated $D_{72°C}$ for the five strains of *M. avium* subsp. *paratuberculosis* examined was <2.03 s, representing >7 $\log_{10}$ kill at the 95% confidence interval. This figure is at the upper limit of those previously reported for this bacterium. The huge range of experimental conditions that have been used in previous heat inactivation studies make further comparisons unlikely to be helpful. Considering the public health significance of pasteurization, there are surprisingly few published data on the survival of any bacterium under validated conditions of turbulent flow. One such paper has been published recently from a laboratory with extensive experience in characterizing pasteurization conditions (25). Using data obtained with a pilot-scale HTST pasteurizer under commercial conditions of turbulent flow, Piyasena et al. (25) predicted at least an 11 $\log_{10}$ kill (95% confidence interval) of *Listeria monocytogenes* at 72°C for 15 s. This is in marked contrast to the approximately 5 $\log_{10}$ kill predicted by Mackey and Bratchell (18) from a 1989 analysis of all the published heat inactivation data for *L. monocytogenes* at the same temperature. There is a further important factor that must be included when deriving the actual microbial kill during commercial pasteurization. The pasteurizer operating temperature is always set 1 to 2°C above the pasteurization standard, in order to guarantee the absolute regulatory requirement that the pasteurization temperature never falls below the 72°C minimum. In New Zealand, the set temperature is generally 73.5 to 74°C. A similar situation has been described in Canada (6).

The final concentration of a viable bacterial species in milk after pasteurization is determined by the initial concentration of that organism in the milk and the decimal reduction resulting from pasteurization. The initial concentration of *M. avium* subsp. *paratuberculosis* in milk is in turn predominantly based on the extent to which the milk is contaminated with fecal material. The contribution of mycobacteria that are secreted directly into the udder is likely to be minor. None of these factors is known with any precision. It is possible that there will be large fluctuations in the number of *M. avium* subsp. *paratuberculosis* in the milk from any one infected farm, simply because of the random nature of milk contamination during

milking. Population spikes resulting from these fluctuations will in turn tend to be damped down when the milk is bulked in the holding silos at the processing site. If a midpoint in the earlier discussed estimates (e.g., 100 CFU/ml) is taken as a maximum silo level, the major contribution is likely to be from fecal contamination. If pasteurization then imposes a minimum 7 $\log_{10}$ reduction, then viable *M. avium* subsp. *paratuberculosis* organisms will be absent in 100 liters.

Pasteurization has traditionally been carried out at either 63°C for 30 min or 72°C for 15 s. These two sets of conditions do not necessarily give the same level of heat inactivation for a given bacterial population. The relative destructive effect of these two conditions depends on the *Z* value of the bacterium concerned. If *Z* is >4.3°C, then there is greater destruction at the lower temperature. The mean *Z* value of 8.6°C for the strains used in this work thus indicates that the kill at 63°C for 30 min can be expected to significantly exceed that at 72°C for 15 s.

It has been suggested that viable *M. avium* subsp. *paratuberculosis* is present in commercial pasteurized milk sold in retail markets in the United Kingdom (22). Pending publication of the results of a more comprehensive study, the following general observations can be made. Bacteria isolated from pasteurized milk can have several possible origins. In the first instance, it is essential to establish (by temperature recording) whether the correct pasteurizing temperature was reached and maintained for the milk concerned. Then, leaks that could allow contamination of the pasteurized stream by raw milk must be excluded. A reliable test for alkaline phosphatase can exclude gross, but not low level (<0.1%), contamination. There are other potential sources of postpasteurization contamination, such as at the filling station. These factors must also be considered when examining commercially pasteurized milk for the presence of viable bacteria.

The results described above demonstrate that, in properly pasteurized commercial milk or in dairy products made from such milk, viable *M. avium* subsp. *paratuberculosis* microorganisms are highly unlikely to be present.

ACKNOWLEDGMENTS

We thank Shane Harvey and Ian Milner for their technical assistance, Hugh Bentall for obtaining the clinical samples, and our colleagues for helpful criticism of the manuscript.

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