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Inactivation of *Mycobacterium bovis* in Meat Products

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The time-temperature combinations necessary to destroy *Mycobacterium bovis* in meat products were determined. In any given time, *M. bovis* was destroyed at temperatures 6 to 7°C (ca. 12°F) lower than those necessary for destruction of members of the *Mycobacterium avium-Mycobacterium intracellulare* complex. Hence, any processing heat adequate to kill *M. avium-M. intracellulare*-complex organisms will provide a very large safety factor with respect to *M. bovis*. Benzalkonium chloride treatment of wiener specimens for cultural examination effectively destroyed the normal flora of wiener emulsion without reducing the numbers of *M. bovis*. Treatment with a phenolic disinfectant followed by formaldehyde vapor was effective in disinfecting equipment contaminated with meat emulsion containing *M. bovis*.

Recent studies have determined the time-temperature combinations necessary to destroy organisms of the *Mycobacterium avium-Mycobacterium intracellulare* (MAI) complex in meat products prepared for human consumption (1, 2). Although most tuberculosis of swine is caused by members of the MAI complex, most cases of bovine tuberculosis and some cases of swine tuberculosis are caused by *Mycobacterium bovis*. The purpose of this report is to describe the time-temperature combinations necessary to destroy *M. Bovis* in meat products.

MATERIALS AND METHODS

Source of organisms. Field isolants of bovine- and porcine-derived *M. bovis* were obtained from W. D. Richards, National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, U.S. Department of Agriculture. Large quantities of each type of *M. bovis* were grown at 37°C on egg yolk agar in Roux bottles and harvested as previously described (1). The harvested organisms were stored at −70°C in portions containing ca. 6×10^{11} viable units suspended in 15 ml of saline.

Meat. The preparation of emulsions of beef, pork, and pork fat containing *M. bovis* was the same as previously described for studies with MAI complex organisms (2). The finished preparations contained at least 10^7 viable units of *M. bovis* per g. The emulsions were stuffed into size 84 (22-mm diameter) wiener casings that were formed into links by tying with cord.

Decontamination. The effect on *M. bovis* of decontamination with benzalkonium chloride (Zephiran-Winthrop Laboratories, Sterling, Drug, Inc., New York, N.Y.) was determined by heating a double batch of wieners at 57.5°C, then macerating one wiener from each exposure time in 0.3% benzalkonium chloride solution and one in sterile water. Serial 10-fold dilutions of each were inoculated on the egg medium after standing for 1 h at room temperature.

Disinfection. Some equipment and surfaces to be used in the preparation of *M. bovis*-spiked products could not be autoclaved, and adequate methods for chemical disinfection of areas contaminated with meat emulsion containing these organisms were not known.

Saline and meat emulsion suspensions, each containing ca. 10^7 *M. bovis* per g, were spread on microscope slides. One set of meat emulsion slides was spread as thin as possible; another set was spread ca. 1 mm thick. After air drying for 3 h, slides from each group were exposed to 2% aqueous Amphyl (National Laboratories, Toledo, Ohio; contains 44% anhydrous soap, 15% *o*-phenylphenol, 6.3% *p-tert*-pentylphenol, 4.7% alcohol, and 30% inert ingredients) for 1, 2, or 4 min; to ultraviolet irradiation (10 $\mu W/cm^2$) for 1 h or 18 h; to formaldehyde vapor for 1 h or 18 h; or to paired combinations of these. For the formaldehyde vapor treatment, all surfaces within the hood were wet with water, the hood was sealed, and then paraformaldehyde (10 g/m^3) was vaporized in the hood over an electric hot plate.

After exposure, the slides were rinsed twice in water, and the emulsions were rubbed off into 0.3% benzalkonium chloride solution. Dilution, inoculation, and incubation were as for MAI complex organisms (2).

Processing of wieners. Death rates for *M. bovis* at various temperatures were determined by immersing the wieners in a water bath at 2.5°C intervals from 50 to 70°C. All internal temperatures were monitored by thermocouples inserted into the center of the wieners. When the prescribed internal temperature was reached, the wieners were removed at intervals of 0, 1, 2, 5, 10, 20, 40, 60, and 90 min. All wieners were chilled in an ice-water bath. The chilled wieners were weighed, macerated in 4 volumes of 0.3% benzalkonium chloride, and diluted, and the organisms were enumerated as before.

The effect of smoking under relatively low humidity was studied by holding one group of wieners in the smokehouse (dry bulb at 55°C, wet bulb at 35°C) at central internal temperature of 47°C for 2 h while another group of wieners from the same batch of

emulsion was held in a water bath at 47°C. Representatives of both groups then were heated in the water bath as described above to determine the rate of killing.

RESULTS

Strain selection. No measurable difference in heat tolerance was found between porcine- and bovine-derived isolants of *M. bovis*. A porcine-derived isolant was selected for further studies.

Disinfection. Ultraviolet irradiation, Amphyl, and formaldehyde vapor separately or in any combination destroyed the *M. bovis* on slides from saline suspension and from thin meat emulsion smears, but some organisms from the thick meat emulsion slides survived each of the individual treatments. However, all treatments with 2% Amphyl (1, 2, or 4 min) followed by formaldehyde vapor for 1 or 18 h provided sterile cultures. Hence, after each use all exposed surfaces and all equipment that could not be autoclaved were bathed with 2% Amphyl followed, for scheduling convenience, by formaldehyde vapor for 18 h.

Decontamination. The 0.3% benzalkonium chloride solution efficiently eliminated the normal flora of wiener emulsion. Many of the flasks inoculated with wiener specimens treated with water were overgrown with contaminants, but among those in which the dilution factor was high enough to eliminate contaminants, no measurable difference in survival of *M. bovis* existed between those treated with water and those decontaminated with 0.3% benzalkonium chloride.

Smoking. The kill rates of *M. bovis* were the same for wieners presmoked in a smokehouse at 47°C and wieners kept in a water bath at 47°C for the same time period.

Death rates. No measurable reduction in viable units of *M. bovis* was found in wieners kept at 50°C for up to 90 min. The death rates of *M. bovis* in wieners kept at 50 to 60°C are illustrated in Fig. 1. The number of viable units remaining was greatly decreased in reaching 57.5 or 60°C. Above 60°C, no viable units remained by the time the wieners reached the designated temperatures.

The killing of *M. bovis* under various time-temperature conditions (Fig. 2) was determined by extrapolation from survival curves.

DISCUSSION

Benzalkonium chloride decontamination of field specimens for the cultivation of mycobacteria has been a routine procedure at this laboratory and was used for the processing of wieners containing MAI complex organisms (2), but the effect on *M. bovis* after heat shock in meat emulsion was unknown. We have found that a 1-h treatment of specimens with 0.3% benzalkonium chloride did not reduce the number of viable units of *M. bovis* after sufficient heat to induce logarithmic killing.

The difficulty of destroying large numbers of *M. bovis* chemically in emulsified meat products was demonstrated by the failure of each single method to kill all the organisms incorporated. The advantage of using the combined Amphyl-formadehyde vapor method apparently resides in the detergent activity of the Amphyl solution and the additive bactericidal activity of the Amphyl and the formaldehyde vapor.

M. bovis no longer is found with great fre-

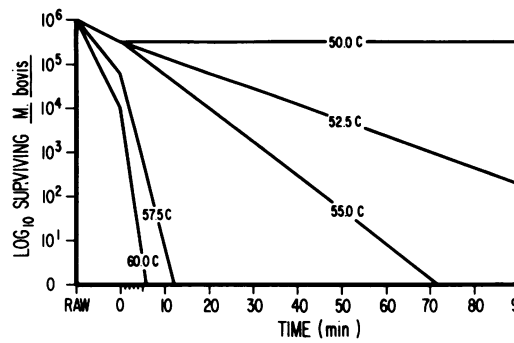

FIG. 1. *Survival of M. bovis in wieners submerged in a water bath at various temperatures.*

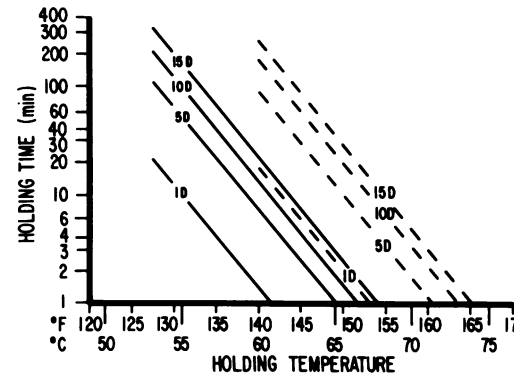

FIG. 2. *Relative holding times at various temperatures to accomplish different D-values. The D-value, or decimal reduction time, is the time at a given temperature required to reduce the population 90%, or a 1-log reduction. The D-values were calculated from survival curves for water bath-heated wieners. The times shown for M. avium were reported previously (2).* (———) *M. bovis;* (- - -) *MAI complex, serotype 10.*

quency in meat animals in this country, but it is the most universal pathogen among the mycobacteria and produces progressive disease in humans and in a variety of domestic animals. However, as illustrated in Fig. 2, if meat is processed with heat adequate to kill members of the MAI complex, a very large margin of safety in terms of *M. bovis* will be present.

LITERATURE CITED

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