

Potential for Growth of *Listeria monocytogenes* on Decontaminated Beef

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SUMMARY

Recent outbreaks of illness caused by meat contaminated with *Listeria monocytogenes* have emphasized the need for its control. The objective of this study was to evaluate survival/growth of *L. monocytogenes* inoculated onto fresh top rounds of beef subsequently treated with acid or nonacid solutions. Dipping of meat inoculated with *L. monocytogenes* into acetic or lactic acid solutions reduced and then inhibited growth of the pathogen during storage at 4 or 10°C, while dipping in water, especially hot water, allowed growth of the pathogen during storage, despite initial reductions in contamination.

Key Words: *Listeria monocytogenes*, decontamination, beef

INTRODUCTION

A recent multi-state outbreak of listeriosis associated with contaminated hot dogs and possibly other luncheon meats has increased awareness and concern about *Listeria monocytogenes* and food safety (CDC, 1999). In response to the outbreak, the President of the United States directed the U.S. Departments of Agriculture and Health and Human Services to take aggressive action for control and prevention of foodborne listeriosis. *Listeria monocytogenes* is ubiquitous in nature and is found in decaying vegetation, manure, soil, and water, as well as on the hide of livestock animals. Consequently, *L. monocytogenes* can be found in meat slaughtering and processing plant environments and the prevalence on fresh meat (beef, pork and lamb) can be as high as 68% and even higher in ground, raw and ready-to-eat meat products (Johnson et al., 1990). Decontamination technologies used extensively in the United States to reduce microbial contamination of fresh meat include spraying or rinsing of the

animal carcass with water, chemicals (e.g. acetic and lactic acid) or pressurized steam (Smulders and Greer, 1998; Sofos and Smith, 1998). The immediate effects of these treatments result in 1-3 log reductions in contaminants, but the long-term residual effects are not completely understood. The objective of this project was to evaluate the effects of various acid and nonacid wash treatments on meat contaminated with *L. monocytogenes* with regards to food safety.

MATERIALS AND METHODS

Preparation of meat. Fresh top rounds of beef were obtained from a commercial plant (Monfort Co.) or from the Meat Science Laboratory at Colorado State University. The meat was stored at 4°C and used within 72 h post-mortem. The outer surface of the meat was sliced off and the internal portion was cut into pieces approximately 2.5 cm x 5 cm x 1 cm (total surface area of 40 cm²).

Preparation of *Listeria monocytogenes*. A streptomycin-resistant (Sm^R) derivative of *L. monocytogenes* strain N-7144 (serotype 1/2b, meat isolate; provided by Dr. K. J. Moore, National Food Processors Association, Washington, D.C.) was grown on Trypticase Soy Agar supplemented with 0.6% yeast extract (TSAYE) and containing streptomycin (800 mg/l) at 30°C for 2 d. Trypticase Soy Broth with yeast extract (TSBYE) containing 0.25% glucose was inoculated with a single colony growing on TSAYE with streptomycin and incubated statically at 30°C for 24 h. Fifty microliters of culture was inoculated into 10 ml TSBYE without glucose and incubated statically at 30°C for 24 h. The cultures were centrifuged, the cells washed once and resuspended in 5 ml 0.1% buffered peptone water (PW). The washed organisms were used to inoculate the surface of beef slices and also diluted and plated in duplicate on TSAYE media for determination of colony forming units (CFU).

Inoculation of meat. The washed organisms were diluted to a concentration of 1.6 x 10⁶ CFU/ml and then 125 µl was inoculated onto the upper surface of each meat piece and spread with a sterile glass rod. The meat pieces were incubated at 4°C for 15 min. Next, the pieces were turned

over and the opposite sides were inoculated in the same manner as described above. The final concentration of *L. monocytogenes* applied to the surface of each meat piece was approximately 4.6-4.8 log CFU/cm². Uninoculated meat samples were used as controls.

Treatment of meat. Uninoculated and inoculated meat samples were left untreated or treated by dipping in the following solutions: 1) water (pH 6.7), 55°C; 2) water (pH 6.7), 75°C; 3) 2% lactic acid (pH 2.3), 55°C (DL lactic acid; Sigma); or 4) 2% acetic acid (pH 2.8), 55°C (glacial acetic acid; Mallinkrodt). Solutions were heated to either 55 or 75°C just prior to dipping of meat samples. Samples were dipped into 1 liter of heated solutions for 30 sec and then drained. Day-0 samples were immediately placed into stomacher bags and processed for CFU and pH determinations. All other samples were sealed in vacuum bags and stored at 4 or 10°C until analyzed.

Analysis of samples. Following incubation at 4 or 10°C for the appropriate time period (7, 14, 21 and 28 d), each meat sample was placed in a stomacher bag with 100 g of PW and homogenized by shaking 30 times. Ten-fold serial dilutions were made with PW and plated in duplicate onto PALCAM media for CFU determination. Spiral plating and analysis (Spiral Systems, Spiral Biotech, Inc., Bethesda, MD) were used to determine numbers of CFU growing on TSAYE and TSAYE with streptomycin. All plates were incubated at 30°C for 2 d before counting of colonies. The pH of the homogenized meat was also determined. Six meat samples were analyzed for each condition from two separate experiments (three samples each).

RESULTS

Survival and growth of *L. monocytogenes* from treated and untreated meat is shown in Figures 1A and B. *Listeria monocytogenes* was not found on uninoculated meat throughout the duration of the experiment. Since *L. monocytogenes* N-7144 Sm^R was recovered from inoculated meat in comparable numbers on PALCAM or TSAYE with streptomycin, only results from PALCAM are shown. *Listeria monocytogenes* survived on inoculated fresh meat that was not treated (NT) but

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vacuum-packaged and incubated at 10 or 4°C for 28 d (Figures 1A and B). Dipping of meat inoculated with *L. monocytogenes* in 55°C water (W), 75°C water (HW), lactic acid (LA) or acetic acid (AA) resulted in immediate decreases of approximately 0.9, 1.6, 1.9 and 2.1 log CFU/cm², respectively (day-0; Figures 1A and B). After storage at 10°C for 28 d, populations of *L. monocytogenes* on 55°C water-treated meat increased approximately 1.8 log CFU/cm² (Figure 1A). The pathogen on acid-treated meat survived but did not grow, whereas populations on 75°C water-treated meat increased rapidly 3.7 log CFU/cm² by day-14 and maintained a high level of organisms at the end of the study (Figure 1A). The number of *L. monocytogenes* recovered from 75°C water-treated meat at day-28 was greater (>1 log CFU/cm²) than those found on untreated and 55°C water-treated meat (Figure 1A). During storage at 4°C, there was no growth of the pathogen for 28 d in 55°C water-treated or acid-treated samples, but growth occurred after 21 d in 75°C water-treated samples (Figure 1B). The normal bacterial flora on untreated or treated meat (not inoculated with *L. monocytogenes*) grew 2.5-3.7 log CFU/cm² when stored at 10°C, but <1 log CFU/cm² when stored at 4°C (Figures 1C and D). In samples which *L. monocytogenes* did not overgrow the normal flora, the normal flora grew as well or better than in uninoculated samples (data not shown). The initial pH of the meat homogenates were 6.44, 6.56, 6.65, 5.70 and 5.52 for the untreated, 55°C water-treated, 75°C water-treated, lactic acid-treated and acetic acid-treated samples, respectively. At day-28, the pH of untreated and water-treated samples decreased 0.56-0.74 units, whereas acid-treated samples increased 0.05-0.15 units.

IMPLICATIONS

Results of this study indicate that application of organic acids (acetic or lactic acid) to meat can reduce and then inhibit growth of *L. monocytogenes* during storage at 4 or 10°C. Although water, especially hot water, also decreased the initial population of *L. monocytogenes* on meat, growth of the pathogen occurred following storage. This study emphasizes the importance

of long-term residual effects of acid and water decontamination treatments and storage temperatures on meat safety.

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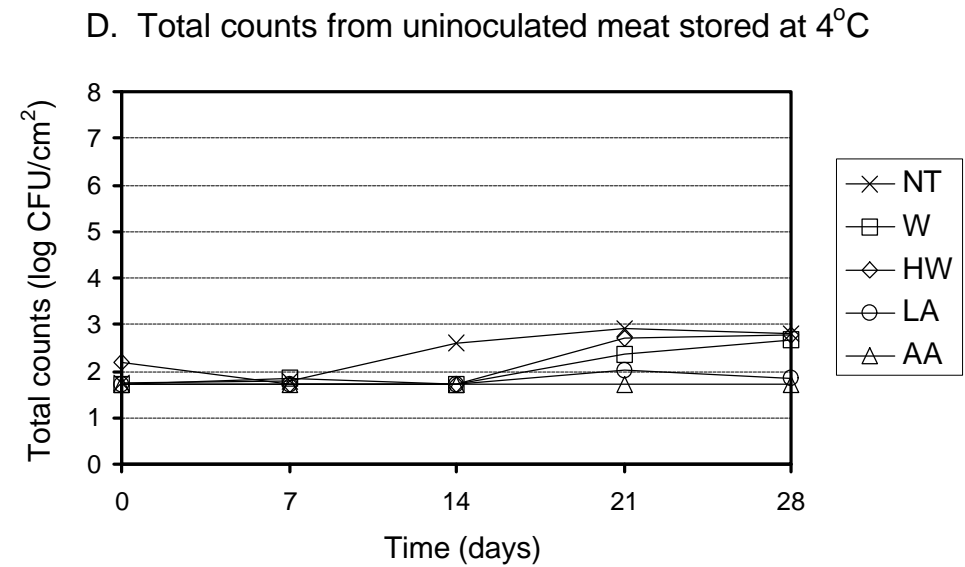
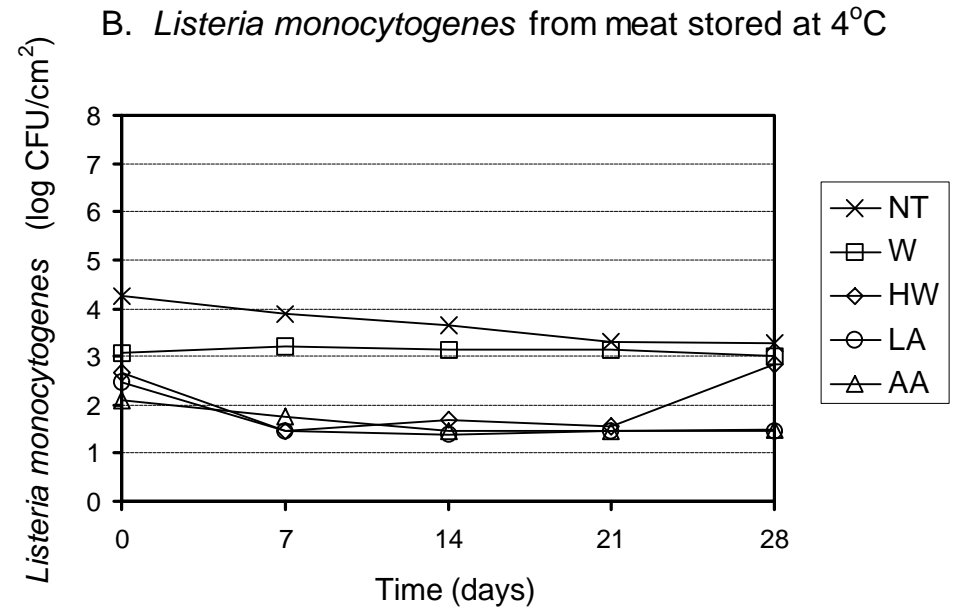
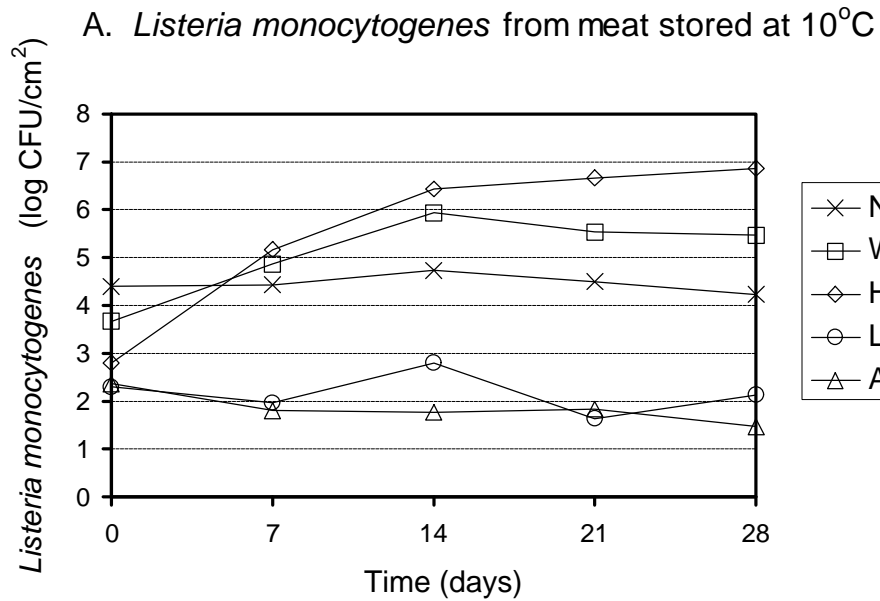


Figure 1. Survival and growth of *Listeria monocytogenes* (A and B) and total aerobic bacteria (C and D) from meat not treated (NT) or treated with water 55 C (W), hot water 75 C (HW), lactic acid (LA) or acetic acid (AA). Meat was stored at 10 C (A and C) or 4 C (B and D). Limit of detection is <1.4 log CFU/cm² for A and B; <1.7 log CFU/cm² for C and D.