# Prediction of Bacterial Penetration into Carcass Tissue during Washing

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SUMMARY: A method for predicting the depth of penetration of bacteria into various beef tissues was developed. Blue Lake, an insoluble dye, was used to simulate bacteria on the meat surface, because movement of the Blue Lake into the meat tissue during spray washing could be easily visualized. The beef tissue surfaces studied were 1) exterior lean, 2) exterior fat, 3) interior body cavity (peritoneum) and 4) smooth-cut tissue. The model indicated that many bacteria not removed during washing are driven into the beef tissue by the washing process. Interior body cavity tissue was most resistant to penetration followed by exterior lean and fat tissues which were about equal in resistance. Cut surfaces were the most susceptible to Blue Lake penetration.

INTRODUCTION: Tissues under the hide of healthy cattle are usually sterile (ELMONSSALAMI and WASSEF, 1971; GILL and PENNEY, 1979). The slaughtering process results in surface tissue contamination with counts of microorganisms ranging from $10^2$ to $10^4/cm^2$) (ANDERSON et al., 1980; DIXON et al., 1991; KEELEY, 1988). Washing, a necesssary step in the slaughtering process, can reduce this microbial population by about one log (ANDERSON et al., 1984) which may leave approximately $10^3$ organisms/$cm^2$. These organisms, some of which may be pathogenic (HOLMBERG et al., 1984), are distributed by the washing process within the surface tissues where they can cause spoilage or illness. Pathogenic bacteria from red meat carcasses have been associated with outbreaks of foodborne diseases (HOLMBERG et al., 1984). Sources of contamination and recommendations for control have been studied for many years (FAIN, 1990); however, the problem still persists. Studies have been conducted to optimize washing procedures (ANDERSON et al., 1975; CROUSE et al., 1988; ELMONSSALAMI and WASSEF, 1971). In all cases, the exact location of the microflora and whether bacteria penetrate the tissues during the different steps of slaughter are unknown. Several studies have shown that microorganisms are able to move within muscle tissue (GILL and PENNEY, 1977, 1982; MAXCY, 1981). In an attempt to answer the question of the effect of the washing process on penetration of bacteria into meat surface tissue, De Zuniga et al. (1991) developed a procedure using a Blue Lake, an insoluble dye, as a simulator of bacteria. Blue Lake serves as an excellent model because it is easily visualized and particle sizes are only slightly smaller than most bacteria. This study expands on that research. The objectives of this study were to further refine the procedure and to evaluate the penetration of Blue Lake into four types of beef surface tissue (exterior lean, exterior fat, interior body cavity, and smooth-cut surface) exposed to the washing process.

EXPERIMENTAL METHODS: The model Carcass Acquired Pathogen Elimination/Reduction (CAPER) system (DE ZUNIGA et al., 1991) was used to deliver wash water at pressures of 690, 2070, 4140 or 6200 kPa at volumes of 4.9, 8.3, 11.7 and 14.4 L/min, respectively. The nozzle was Spraying Systems Tee-Jet No. 5008, oscillated at 60 cycle per min. Chain speed was 5.5 m/min. Beef tissue samples of 1) exterior lean, 2) exterior fat, 3) interior body cavity and 4) smooth-cut lean surface tissue were obtained from a commercial slaughter plant, returned to the laboratory, trimmed to approximate sizes, placed in plastic bags and frozen for further use. Before a study, beef tissue samples were thawed in a refrigerator (4°C) and recut into narrow strips (20 cm long x 3 cm wide x 1.5 cm deep). Four strips of meat, one of each of the four types of tissue, were placed randomly on a polyethylene holding frame; surfaces were moistened with distilled water from a squeeze dispenser and coated with Blue Lake (mean diameter 0.6 microns). Next, the samples in their polyethylene holding frame were placed in the CAPER system, randomly assigned to one of the treatment pressures. After treatment, the strips of meat were frozen to facilitate cutting. The frozen strips were cut from the side opposite that coated with Blue Lake to prevent the knife carrying the Blue Lake into the tissue. Each strip was cut transversely into 8 pieces giving 32 pieces (2.5 x 3 x 1.5 cm) for each replication of each treatment. The surfaces were examined under a dissecting microscope. Depth of penetration was measured at intervals of 5 millimeters along the edge of the meat surface using a millimeter scale. Four replications were made of each treatment. Data were analyzed by Statistical Analysis System (SAS) (SAS INSTITUTE, INC., 1985) on an Amdahl 470 V/7 computer.

RESULTS AND DISCUSSION: The F-test analysis (Table 1) shows that effects of both line spray pressure and type of beef tissue were significantly different at $P \leq 0.0001$. A significant interaction was observed between water pressure and type of meat tissue. No differences were noted among the four replications of the study. The interactive effect of spray pressure and type of meat tissue on the penetration of Blue Lake was clearly demonstrated (Figure 1). The penetration of Blue Lake into the tissues that were not sprayed was significantly less ($P<0.0001$) than penetration into tissues sprayed at 690 to 6200 kPa for all types of meat tissue. The data for all types of unsprayed tissue show that Blue Lake was able to enter small crevices in the meat to a depth of about 0.25 mm. With this depth of penetration only part of the organisms would be detected if the meat were sampled using the swab method instead of the core method. These data indicate that bacteria might be able to penetrate into all types of meat tissue studied even without any washing treatment.

Cut tissue was more susceptible to penetration of Blue Lake than either of the three other types of tissue at the three highest pressures (2070-6200 kPa). Some observations showed very deep dye penetration (3-5 mm), thus greatly increasing the overall mean depth of penetration for cut tissue. This would be expected because cut tissue has no membraneous surface to hold its cells together. However, at a pressure of 690 kPa no differences were noted among exterior lean, fat or cut tissue. This suggests that at this lower pressure the spray did not have enough force to drive the Blue Lake into the tissue.

No significant differences in penetration were noted between exterior lean and exterior fat tissue surfaces except at 4140 kPa. Exterior lean and fat tissue are exposed to the same type of processing at slaughter, resulting in similar surface cuts and tears. These data show a slightly higher penetration at the lower pressures than reported in a previous study using beef plate meat (DE ZUNIGA et al., 1991). In the previous study no attempt was made to separate lean and fat tissue. Surfaces of carcasses may vary in characteristics due to differences in the slaughtering process.

Interior body tissue was very resistant to the penetration of Blue Lake with no significant differences noted at any of the spray pressures. The interior body lining protected the meat from penetration of Blue Lake as long as the tissue was not disrupted. Once the protective inner lining was broken, Blue Lake penetration was observed. This is shown in Figure 2 where the top piece of interior tissue has a hole in the protective lining. This hole permitted the Blue Lake to penetrate into the tissue. The protective lining of the bottom piece of meat is intact, thus no penetration of Blue Lake occurred. Therefore, no penetration of bacteria would be expected. This demonstrates the need to follow slaughtering procedures that will prevent breaking this interlining.

A representative piece of cut tissue (Figure 3) shows the penetration of the Blue Lake due to spraying at 2070 kPa. In this case, the cut tissue was structurally broken due to the force of the spray treatment. Any bacteria on the surface would have been driven into the tissue increasing the probability of spoilage. The following equations describe the mean depth (D) to which the Blue Lake penetrated the different types of meat tissue as a function of line pressure at the nozzle:

EXTERIOR LEAN: $D = 0.262 + 0.014\ (X) - 6.525 \times 10^{-5}\ (X^2) + 1.040 \times 10^{-1}\ (X^3) - 5.322 \times 10^{-11}\ (X^4)$. $R^2 = 0.80$

EXTERIOR FAT: $D = 0.25 + 0.015\ (X) - 7.156 \times 10^{-5}\ (X^2) + 1.224 \times 10^{-1}\ (X^3) - 6.695 \times 10^{-11}\ (X^4)$. $R^2 = 0.89$

SMOOTH-CUT SURFACE: $D = 0.25 + 0.017\ (X) - 6.015 \times 10^{-5}\ (X^2)$. $R^2 = 0.80$

INTERIOR BODY CAVITY: $D + 0.25 + 0.009\ (X) - 4.61 \times 10^{-5}\ (X^2) + 8.184 \times 10^{-8}\ (X^3) - 4.52 \times 10^{-11}\ (X^9)$. $R^2 = .44$

Where D = depth of penetration into surfaces of beef tissue, millimeters; X = line spray pressure, kPa.

In summary, water sprayed at high pressures tends to drive particles the size of Blue Lake into meat surfaces. The most susceptible surfaces are those that have had the covering membrane removed. Interior body cavity tissue is the most resistant to penetration of the Blue Lake followed by exterior lean and fat with cut surface tissue being the most susceptible to penetration. Penetration by the Blue Lake indicated that bacteria on the surface of meat might be able to penetrate to a depth of 0.25 mm without any treatment. It is postulated that this may be the depth of normal crevices in the meat tissue.

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Table 1. Analysis of variance table showing mean squares, F values and probability of F.

| Source | Mean square | F value | PR > F |
|---|---|---|---|
| Pressure | 3.04 | 46.32 | .0001 |
| Meat type | 2.48 | 37.77 | .0001 |
| Rep | 0.07 | 1.07 | .3682 |
| Pressure*Meat | 0.22 | 3.35 | .0009 |

Figure 1. Penetration of Blue Lake into four different types of meat tissue (interior body cavity, exterior lean, exterior fat, and smooth-cut) as affected by five different pressures (0, 690, 2070, 4140, and 6200 kPa) during washing with a No. 5008 flat fan Spraying Systems nozzle.

[a-g]Values with different superscripts are significantly different at $P \leq 0.05$.

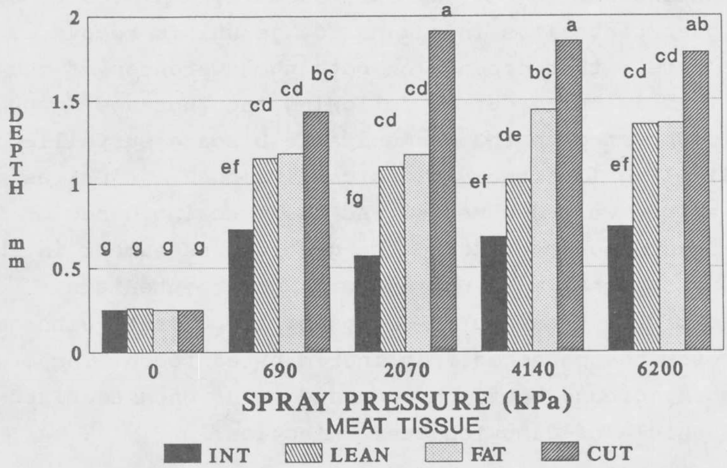


Figure 2. Penetration of Blue Lake through a break in the interior body cavity tissue (top piece of meat). The bottom piece of meat shows the interior body cavity tissue intact. Spray pressure was 4140 kPa.

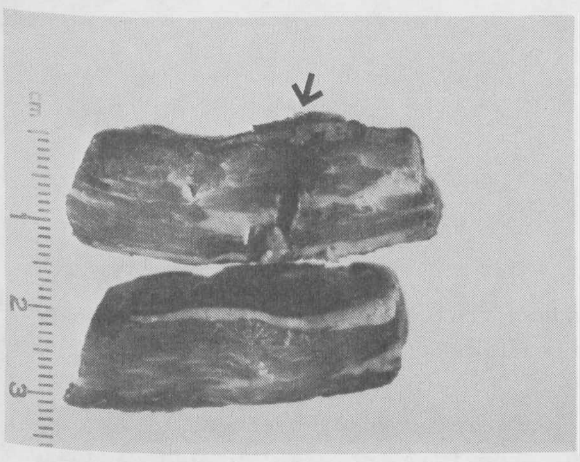

Figure 3. Penetration of Blue Lake into cut tissue. Penetration depth was 5 to 6 mm. Spray pressure was 2070 kPa.