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Research Note

Manual Squeezing as an Alternative to Mechanical Stomaching in Preparing Beef Carcass Sponge Samples for Microbiological Analysis

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ABSTRACT

The U.S. Department of Agriculture (USDA) requires beef abattoir operators to periodically analyze beef carcass sponge samples for levels of *Escherichia coli.* Additional beef carcass sponge sampling is commonly used by processors to evaluate the efficacy of beef abattoir antimicrobial intervention systems. The USDA sample preparation procedure requires that beef carcass sponge samples be mechanically stomached for 2 min before the sample fluid is squeezed out for analysis. When a large number of sponge samples must be analyzed, the stomaching step can limit throughput. In this study, we compared the USDA sample preparation procedure with repeatedly squeezing the sponge during a 10-s interval to expel the sample fluid. Separate sponge samples were obtained from each half of 100 chilled postintervention beef carcasses from a large-volume abattoir during a 4-month period. The USDA and squeezing treatments were randomly assigned to the halves of each carcass. All sponge samples were analyzed for *E. coli,* coliforms, *Enterobacteriaceae,* and aerobic mesophilic bacteria using Petrifilm methods. The sample preparation method had no significant effect (signed rank value > 0.05) on the results of any analytical test, although aerobic mesophilic bacteria counts tended to be higher after the USDA method than after manual squeezing alone. These results suggest that manual squeezing may be a simple and rapid alternative sample preparation method when gram-negative bacteria such as *E. coli,* coliforms, or *Enterobacteriaceae* are being enumerated from beef carcass sponge samples used to monitor operational abattoir hygiene.

In quantitative microbiological analyses, it is important to take representative samples and ensure that subsequent subsamples transferred to microbiological growth media also are representative. This principle is especially important when the sample is obtained by swabbing or sponging a surface. Factors that affect the representativeness of a sponge sample include whether and how consistently the sponge is wetted before sampling, the degree to which sponging removes microbes from the sampled surface, how consistently a defined surface area is sponged, and how completely the microbes are removed from the sponge for analysis. The present study was focused on this last factor by comparing two different techniques intended to remove microbes from the sponge sample.

The U.S. Department of Agriculture (USDA) currently requires operators of beef abattoirs to perform sponge sampling of chilled beef carcasses at a specified frequency *(6).* The sampling frequency differs with the size of the abattoir operation. Abattoirs defined as low-volume establishments (≤6,000 cattle per year) are required to sample 1 randomly selected carcass per week for 13 weeks beginning in June each year. Larger abattoirs are required to sample at least 1 randomly selected carcass of every 300 carcasses processed or at least 1 carcass per week. The sampling involves wetting a sponge with a defined amount of diluent, rubbing the wetted sponge a specified number of times over three 100-cm^2 areas of the carcass in a particular order, placing the sponge in a sample bag, and adding additional diluent. The sponge is then either refrigerated and shipped to the laboratory for analysis or analyzed immediately. Samples are analyzed for biotype I (so-called generic) *Escherichia coli.* Processors are required to use the results of the quantitative *E. coli* analysis to verify process control in their operations and to take corrective actions should the results indicate that variation outside of normal and acceptable limits has occurred *(7).* Beyond this required testing, beef carcass sponge sampling is commonly used by processors to evaluate the efficacy of beef abattoir antimicrobial intervention systems.

The USDA *Microbiology Laboratory Guidebook (8)* specifies that the sponge sample or an initial dilution thereof should be mechanically blended or stomached for 2 min before the sample fluid is squeezed out of the sponge for further dilution and/or plating. However, a procedure for squeezing the sponge is not specified. When a laboratory has a large number of sponge samples to analyze, this 2-min stomaching step can become a bottleneck and can reduce laboratory efficiency. In the present study, we com-

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pared two different methods for preparation of beef carcass sponge samples for plating. The first method was the USDA method and consisted of stomaching the sponge (containing 25 ml of diluent) for 2 min and then repeatedly squeezing the sponge by hand (through the sample bag) for 10 s to expel the sample fluid for plating. The test method omitted the stomaching step and consisted of repeatedly squeezing the sponge for 10 s to expel the sample fluid. We analyzed the sponge samples prepared each way for biotype I *E. coli* as required by USDA regulations and for aerobic mesophilic bacteria, coliforms, and *Enterobacteriaceae* because these analyses are commonly used as indicators for beef carcass hygiene and antipathogen intervention treatment efficacy.

MATERIALS AND METHODS

Beef carcass sponge samples. For each of 10 weeks during February through May 2008, personnel at a cooperating high-volume beef abattoir randomly selected 10 chilled beef carcasses for sampling. Using the USDA-mandated sampling procedure, separate sponge samples were obtained from each carcass half. This sampling procedure consisted of wetting a standard sponge (Nasco, Fort Atkinson, WI) with 10 ml of Butterfield's phosphate diluent (BPD; Nelson Jameson, Marshfield, WI) and swabbing 100-cm^2 areas at three carcass sites as described by the USDA for conducting biotype I *E. coli* testing *(6, 7).* One side of the moistened sponge was used to rub 10 times horizontally and 10 times vertically over areas (10 by 10 cm) on the flank and brisket in sequence. The sponge was turned over, and the unused side was used to rub an area (10 by 10 cm) on the rump. The sample sponge was then returned to the sampling bag, and approximately 15 ml of BPD was added. The sponge samples were then placed in insulated boxes with refrigerant packs and shipped to the laboratory, where they were received the next day and analyzed within 8 h.

Analysis of sponge samples. For the sponge sample from the right half of each carcass, a random number generator was used to randomly assign whether the sponge would be stomached for 2 min at 230 rpm (Seward lab blender, Fisher Scientific, Itasca, IL) and then repeatedly squeezed by hand for 10 s or just repeatedly squeezed by hand for 10 s to expel the sample fluid. The 10-s squeezing time was chosen for ease of memory by personnel and because it was longer than the sample mixing time recommended elsewhere *(3).* Whichever treatment was not used to prepare the sponge sample from the right half of the carcass was used to prepare the sponge sample from the left half of the same carcass. From the expressed sample fluid, 1.0 ml was transferred to individual Petrifilm aerobic count, *Enterobacteriaceae,* and coliform–*E. coli* plates (3M Microbiology Products, St. Paul, MN), and 1.0 ml was added to 9.0 ml of BPD to make a 10^{-1} dilution. From this initial dilution, 1.0 ml was transferred to an additional Petrifilm plate of each type, and further BPD dilution and plating were done as needed. For the expressed sample fluid and each subsequent dilution, a single plate was prepared for each analysis. Preparing multiple rather than single plates for the expressed sample fluid and summing rather than averaging colony counts was done to effectively decrease the detection limit of the analysis. Aerobic count plates were incubated for 48 h at 35°C, and all other plates were incubated for 24 h at 35°C. For each type of analysis, colonies were counted on plates containing 15 to 150 colonies unless the least dilute plate contained $<$15 colonies, in which case all colonies on the that plate were counted. On aerobic count plates, all red colonies were counted. On the *Enterobacteriaceae* plates, all red colonies that produced gas and/or acid were counted. On the coliform–*E. coli* plates, all red and all blue colonies with associated gas were counted to obtain the coliform count, but only the blue colonies with associated gas were counted to obtain the *E. coli* count. When no colonies were present on the plate for the lowest dilution, an arbitrary value of 0.5 CFU was assigned.

Statistical analysis. Log-transformed counts were calculated for each analysis for each sponge sample. When the arbitrary value of 0.5 CFU was assigned, a value of -1.38 log CFU/cm^2 was obtained. Results were paired by carcass, and the PROC UNIVARIATE command in SAS 9.1 (SAS Institute, Cary, NC) was used for the Wilcoxon signed rank test. This nonparametric test does not depend upon any assumptions about the distribution of the data and was used to determine whether the two sponge sample preparation methods differed in terms of numbers of aerobic mesophilic bacteria, *Enterobacteriaceae,* coliforms, or *E. coli.* A significance level of 0.05 was used.

RESULTS AND DISCUSSION

As indicated by the standard deviations for each analysis, the two sample preparation methods did not appear to affect the variability of the analytical results (Table 1). The mean counts for aerobic mesophilic bacteria and *Enterobacteriaceae* were lower after manual squeezing than after mechanical stomaching and manual squeezing, by 0.4 and 0.1 log CFU/cm^2, respectively, whereas the manual squeezing method produced very slightly higher mean counts for coliforms and *E. coli*: 0.1 log CFU/cm^2 for each. None of the differences between sample preparation methods were significant, however, with signed rank values ranging from 0.07 for aerobic mesophilic counts to 0.83 for coliform counts. These results suggest that the two sample preparation methods differ little in how well gram-negative enteric bacteria, i.e., *E. coli,* coliforms, and *Enterobacteriaceae,* are recovered, but stomaching may be more likely to recover a higher number of total aerobic mesophilic bacteria. The bacteria counted as aerobic mesophiles would likely include gram-positive bacteria and a broader range of gram-negative bacteria than those counted in the other analyses.

The distribution of results for the aerobic mesophilic bacteria analysis and the closeness of the signed rank value for this analysis to the 0.05 significance boundary suggest that stomaching tended to provide better recovery of total aerobic mesophilic bacteria. Twenty-three samples prepared by manual squeezing yielded aerobic mesophilic bacterial counts of -1.38 (detection limit) to -0.38 CFU/cm^2 (inclusive), whereas only 11 samples prepared by stomaching yielded results in this range.

If microbiological analysis of beef carcass sponge samples is mainly being used to detect changes in levels of bacteria considered hygiene indicators, such as *E. coli,* coliforms, or *Enterobacteriaceae,* our results suggest that there is little risk of obtaining faulty information when samples are prepared by squeezing rather than by stomaching. There appears to be a greater risk of error if samples are being analyzed to compare aerobic mesophilic bacterial levels with a numerical standard.

Although our results suggest that manual squeezing could be substituted for mechanical stomaching when pre-

TABLE 1. *Populations of aerobic mesophilic bacteria (AC), Enterobacteriaceae (EB), coliforms, and biotype I* Escherichia coli *(EC) on beef carcasses as determined by different sponge sampling methods*[a]

	No. of samples							
	AC		EB		Coliforms		EC	
Population (log CFU/cm^2)	ST	SQ	ST	SQ	ST	SQ	ST	SQ
None detected	3	3	41	54	52	55	70	71
Less than −1.38 to −0.38	8	20	16	15	15	13	17	18
−0.37–0.38	30	24	17	5	13	6	5	1
0.39–1.38	16	17	6	5	3	9	7	6
1.39–2.38	10	9	14	16	13	15	0	3
2.39–3.38	16	17	6	5	4	2	1	1
3.39–4.39	17	10	0	0	0	0	0	0
Mean (SD) population (n = 100)	1.4 (1.7)	1.0 (1.6)	−0.3 (1.3)	−0.4 (1.4)	−0.5 (1.3)	−0.4 (1.3)	−1.0 (0.8)	−0.9 (0.9)

[a] ST, preparation of sponge sample by stomaching and then manual squeezing; SQ, preparation of sponge sample by manual squeezing only. Sample fluid was plated on Petrifilm aerobic count, *Enterobacteriaceae,* and coliform–*E. coli* plates.

paring beef carcass sponge samples for *E. coli,* coliform, and *Enterobacteriaceae* analyses, care must be taken before this substitution is done. The sample size in the present study was small, and samples were obtained from only one abattoir. Seasonal effects on beef carcass aerobic mesophilic bacterial and *Enterobacteriaceae* counts have been well documented *(4, 5).* Before changing methods, it may be advisable for the abattoir involved to continue this study and obtain a larger number of samples over a longer period of time. In doing so, the effects of any additional seasonal variation in beef carcass microflora on sample preparation could be determined. Other abattoirs interested in substituting manual squeezing for mechanical stomaching also should conduct comparison studies. Dorsa et al. *(1)* suggested that sponge sampling of chilled beef carcasses instead of excision sampling is likely to result in lower numbers of total aerobic bacteria and less frequent detection of *E. coli* and coliforms; therefore, sample collection method may further affect any minor differences resulting from the sample preparation methods evaluated in the present study. It is not known whether sample preparation techniques affect the performance of microbiological techniques other than those used in the present study. Sample preparation method is just one of many variables that can affect microbiological results from beef carcasses.

The similarity of results from manual squeezing and mechanical stomaching when preparing samples for *E. coli* analysis was not observed in a previous study in which manual shaking was compared with mechanical stomaching as a method for preparing ground meat samples for biotype I *E. coli* analysis *(2).* In that study, we found that mechanical stomaching yielded slightly but significantly higher levels of *E. coli* from ground beef and chicken ($P = 0.006$) compared with manual shaking. The differences in the findings of these two studies may reflect a tighter attachment of *E. coli* to meat than to the sampling sponge.

These results suggest that when gram-negative bacteria such as *E. coli,* coliforms, or *Enterobacteriaceae* are being enumerated from beef carcass sponge samples to monitor operational abattoir hygiene, then manual squeezing may be useful as a more rapid and cost-effective alternative to mechanical stomaching. A more cost-effective alternative may be particularly important when there is only a small number of samples to analyze.

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