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How Effective is Sponge Sampling for Removing Bacteria from Beef Carcasses?

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ABSTRACT

Removal of bacteria by sponging carcass surfaces is a common, non-destructive sampling method. The proportion of bacteria removed by sponging was measured as a percentage of those on the sponge plus those on the excised carcass surface beneath the sponged area. For a total of ten experienced operators working at five abattoirs, the mean proportion of bacteria removed at each site was 39.1% (rump), 39.9% (flank) and 33.7% (brisket). The standard deviation at each site was relatively high (28.5, 21.3 and 17.4%, respectively), reflecting the wide variation of recovery among operators (2.3–93.1%). To determine the proportion that could be removed by continued sponging, numbers of bacteria were counted after each of five sequential spongings, together with those remaining on the excised site after homogenizing of tissue by stomaching. The proportion of bacteria recovered by sequential sponging varied between 11.1 and 97.4% and, with one exception, larger proportions were recovered from the first sponge. The present study demonstrates that sponge sampling, while convenient, is a highly variable means of removing bacteria for enumeration.

INTRODUCTION

The USA Pathogen Reduction Final Rule in 1996 *(1)* introduced the requirement for microbiological monitoring of carcasses at defined sites. The most common means of sampling involves abrading carcass sites with a rehydratable sponge, a method both convenient and non-destructive of the meat surface. Microbiological testing is an important verification tool for meat processing and, in Australia, data are collected by export meat slaughter establishments as part of the *E. coli* and *Salmonella* Monitoring (ESAM) program administered by the Australian Quarantine and Inspection Service.

Observation of the sponging technique at meat establishments indicated variability among operators, particularly in the pressure applied to, and therefore the degree of abrasion of, the site. Accordingly an investigation was carried out in which trained operators were required to sponge designated sites marked at the rump, flank and brisket of beef carcasses, after which the sponged area was excised. By counting bacteria removed by the sponge and those remaining on the excised area, the proportion which each operator removed by sponging could be

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calculated. The results of this study are presented in this report.

MATERIALS AND METHODS

Samples were collected in January and February 2008 from five abattoirs. Sampling was carried out on 14 beef carcasses that had been held under active refrigeration for 16 to 24 h. On each carcass side, an area measuring 10 cm × 10 cm (100 cm^2) was marked at each of the rump, flank and brisket sites, using a knife sanitized by immersion in water at 82°C.

One side of a polyurethane sponge (Whirlpak speci-sponge, NASCO, USA) moistened with Butterfield's solution (25 ml; bioMérieux) was used to sample each site. A total of 10 samplers were used, all of whom had undergone a training course in sponge sampling and were either industry/government inspectors or research staff.

The ability of each operator to sponge each site was assessed according to whether the operator conformed with the technique prescribed in the Microbiological Guidelines that accompany the Australian Standard for production and transportation of meat and meat products for human consumption *(2)*: *"Wipe the sponge over the sampling area (10 cm × 10 cm) approximately 10 times in the vertical and 10 times in the horizontal directions. The pressure of sponging is important and should be as if you are removing dried blood from the carcase. However, the pressure should not be so hard as to crumble or destroy the sponge."*

Using a knife sanitized by immersion in water at 82°C, the previously-sponged and demarcated area was carefully excised by slicing approximately 2 mm below the surface, and the sample was placed in a sterile Stomacher bag.

All samples were packed in insulated containers with chiller packs for transportation to the laboratory, a journey never longer than 3 h. At the laboratory, samples were held at 2–4°C until examination within 1 h of arrival.

After the sponge had been manually squeezed several times and the fluid had been stripped from it, serial dilutions of the fluid were prepared in 0.1% peptone water (Oxoid, Hampshire, England). Aliquots (1 ml) were transferred to APC Petrifilm® (3M, Sydney, Australia). To each excision sample, 30 ml of peptone water (0.1%) containing 2% (v/v) Tween 80 (Merck Pty Ltd, Victoria, Australia) was added and the tissue homogenized in a stomacher (Colworth Stomacher 400, A.J. Seward & Co. Ltd, London, UK) for 2 minutes. Aliquots were diluted and plated as already described. Duplicate Petrifilm® plates were incubated at 25°C for 72 h, after which time colonies were counted. The limit of detection for sponge samples was 0.25 CFU/cm^2 and for excised samples 0.33 CFU/cm^2.

The total number of bacteria recovered was defined as the number obtained by sponging plus the number from the excised tissue. All counts were converted to counts per square centimeter and analyzed using analysis of variance to test for differences between sites (rump, brisket, flank). Plant and Operator were also included in the model, so that the variability between plant and the variability between operators could be evaluated. The variability between operators within each plant was confounded with carcass, as each operator sponged only a single carcass. Only Operator 10 sponged carcasses at each of the five plants.

RESULTS AND DISCUSSION

Bacterial numbers recovered when ten experienced operators sponged sites of beef carcasses chilled overnight are presented in Table 1. At each establishment, operators sampled adjacent carcass sides, each sponging an area marked by Operator 10, who also excised each site after sponging was completed. The mean proportion of bacteria removed at each site was 39.1% (rump), 39.9% (flank) and 33.7% (brisket) and the standard deviation at each site was relatively high (28.5, 21.3 and 17.4%, respectively), reflecting the wide variation of recovery among operators (2.3 – 93.1%). There was no significant difference among sites, on average, for the proportion of bacteria removed (P = 0.67) or aerobic plate counts (APCs) (P = 0.19).

Although all operators had received training some years previously in carcass sponging, conforming exactly to procedures set out in the Australian Standard, their technique in this 2008 study varied considerably. Important departures from the standard method were doubling-over the sponge (effectively halving the area available for removing bacteria) and sponging "lightly," differences which might be expected to reduce recovery of bacteria, or using more than 10 up-and-down strokes and exceeding the marked area, which might be expected to increase recovery. This is in agreement with a recent study in which total viable counts were also shown to be significantly different depending on the person sampling the carcass, as well as the animal species tested and the bacterial load *(7)*. However, unlike the present study, operator variability using sponging and excising methods on different carcass sites was compared.

To determine the proportion that could be removed by continued sponging, a single operator (Operator 10) undertook sponging and excision on two carcass sides. Numbers of bacteria recovered from each site after use of five separate sequential sponges are presented in Table 2, together with the numbers remaining on the sponged site measure by excision. The proportion of bacteria recovered by sequential sponging varied between 11.1 and 97.4% and, with one exception, larger proportions were recovered from the first sponge.

As indicated in Table 1, sponges were capable of removing relatively large numbers of bacteria, with 26.2 and 28.6% of sponges removing more than 50% of the surface load and containing more than 100,000 colony-forming units (CFU), respectively. In contrast, the sponge used by Operator 10 removed only 2.3% of the 1.1 million bacteria from a rump site, indicating that there may be other factors, apart from operator technique, that influence removal of bacteria. One factor, which was noted during the study, was fat cover, with the possibility that the pores of the sponge become occluded, thereby reducing removal of bacteria. This is in addition to variables such as rates of bacterial attachment to the meat surface, uneven distribution of bacteria on the carcass, whether carcasses are sampled "warm" or chilled, abrasiveness of the swab/sponge, and the vigor with which the sponge is applied to the site *(4, 5, 6, 9)*. In addition, it is well recognized that the sponge itself retains bacteria.

A further variable confounding comparison of non-destructive and excision sampling methods is that previous studies have compared bacterial populations on different sample sites, either on the same or different carcasses *(3, 4, 6, 7, 8, 9)*. By contrast, the present study limited the number of variables listed above, first because the sponged area was the

TABLE 1. Recovery of bacteria by sponging sites on chilled beef carcasses by ten experienced samplers

Operator(s) at each abattoir	Total number of bacteria removed by sponging the 100 cm^2 site		
	Rump	Flank	Brisket
Abattoir 1			
1	17,500 (82.0)[a]	2,738 (39.8)	775 (24.3)
2	2,763 (31.0)	1,088 (33.0)	5,463 (25.4)
3	5,700 (13.1)	2,238 (93.1)	1,413 (35.1)
10	2,375 (58.8)	1,238 (58.7)	3,250 (57.5)
Abattoir 2			
4	41,750 (93.0)	211,750 (48.6)	8,875 (68.0)
10	2,263 (25.2)	57,125 (59.8)	33,000 (57.4)
Abattoir 3			
5	113,625 (64.4)	196,000 (31.0)	263,500 (18.0)
10	23,375 (2.3)	191,125 (25.8)	197,125 (16.3)
Abattoir 4			
6	316,500 (68.6)	539,250 (41.4)	275,000 (31.3)
7	12,000 (15.5)	156,750 (37.9)	332,875 (34.4)
10	75,250 (21.4)	175,875 (42.9)	44,875 (20.3)
Abattoir 5			
8	1,213 (21.2)	3,988 (21.7)	1,788 (9.0)
9	1,850 (22.5)	8,125 (15.5)	11,500 (43.4)
10	4,525 (27.6)	8,625 (8.6)	6,950 (30.8)
Mean recovery (%)	39.1	39.9	33.7
SD % recovery	28.5	21.3	17.4

[a]Number in parentheses was calculated by dividing the number recovered by sponging by the sum of the number of bacteria recovered by sponging and by excision.

same as the excised area and second because a single sponging technique was used.

Although, as the present study indicates, sponge sampling is a convenient but variable means of removing bacteria for enumeration, it remains a valuable method of evaluating process hygiene of the carcass slaughter and dressing process, both at the individual plant and national industry level in Australia. In addition, a fuller understanding of the benefits of carcass sampling for process control can result from a larger data set that may reduce the effect of differences in sampling operator technique, bacterial distribution, and other variables *(6, 7)*. In this regard, each year in Australia around 23,000 new data points for beef carcasses are added to the national microbiological database, the *E. coli* and *Salmonella* Monitoring (ESAM) program.

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TABLE 2. Effect of repeated sponge sampling on number and proportion (%) of the total APC removed from the rump, flank and brisket sites of chilled and freshly-slaughtered beef carcasses

Sponge sample	Chilled carcass 1	Chilled carcass 2
Rump 1	10,250	1,862
Rump 2	2,850	486
Rump 3	1,475	262
Rump 4	925	75
Rump 5	1,387	37
CFU removed by sponging	16,887 (83.5)	2,725 (11.1)
CFU removed by excision	3,330 (16.5)	21,750 (88.9)
Flank 1	162	51,375
Flank 2	200	10,250
Flank 3	62	4,162
Flank 4	25	1,650
Flank 5	50	1,537
CFU removed by sponging	500 (66.2)	68,974 (93.0)
CFU removed by excision	255 (33.8)	12,906 (7.0)
Brisket 1	262	2,562
Brisket 2	125	450
Brisket 3	75	112
Brisket 4	112	187
Brisket 5	100	75
CFU removed by sponging	675 (75.0)	3,886 (97.4)
CFU removed by excision	225 (25.0)	90 (2.6)

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