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Evaluation of six sampling methods for recovery of bacteria from beef carcass surfaces

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W.J. DORSA, C.N. CUTTER AND G.R. SIRAGUSA. 1996. Six bacterial sampling methods that might be used for rapid sampling of beef carcasses were evaluated in two separate studies. In Study 1, bacterial recovery from uninoculated beef rounds was 2·6, 2·3, 2·1 and 1·3 $\log_{10}$ cfu cm^{-2}, respectively for excision (EX), and swabbing with cheesecloth (CC), sponge (SP) and cotton-tipped wooden swabs (CS). For Study 2, beef tissue was inoculated with bovine faeces at different levels and the mean recovery was 3·7, 3·0, 3·1 and 3·1 $\log_{10}$ cfu cm^{-2}, respectively for EX, and swabbing with SP, griddle screen (GS) and 3M mesh (M). For both studies EX was determined to be the most consistently effective method while the initial study determined swabbing with CS was the least effective of the methods used. In both studies the most abrasive materials approached the effectiveness of EX even at low inoculation levels. As the inoculation levels increased, the additional effect of abrasiveness was lessened. When the carcasses were contaminated with bovine faeces, the bacterial populations that were rapidly recoverable from beef tissue using SP, GS or M were not significantly lower than those recovered using EX. Consequently SP, GS or M are an adequate method of beef carcass sampling for rapid, in-plant process monitoring to detect faecal contamination.

INTRODUCTION

Microbiologists have been attempting to develop and improve red meat carcass sampling methods for decades. It was not until the 1930s that the development and improvement of carcass surface sampling methods began in earnest (Nortje *et al.* 1982). Since that time many methods have been developed and evaluated (Clark 1965; Williams 1967; Davidson *et al.* 1978; Nortje *et al.* 1982). Non-destructive sampling methods include: adhesive contact tape, swabbing, rinsing, direct agar contact, scraping, and vacuuming (Lee and Fung 1986). Various materials for swabbing of carcass surfaces have been extensively considered (Angelotti *et al.* 1958; Roberts *et al.* 1984; Anderson *et al.* 1987). None of these methods yield 100% recovery of the bacteria present on a carcass surface, when compared to excision.

Excision is considered the most effective bacterial carcass sampling method (Ingram and Roberts 1976; Rivas *et al.* 1993), but in red meat processing facilities excision is neither practical nor acceptable. Consequently, a more practical, non-destructive, and rapid method for carcass bacterial sampling must be validated. These factors should be accomplished without significantly affecting the sum total of recovered bacteria. Any improvement in the proficiency of sampling methods presently available would have immediate impact. The recent emphasis on rapid microbial testing of animal carcasses by the US Food Safety and Inspection Service requires rapid sampling methods. This study evaluated several non-destructive, fast and practical microbial sampling methods that could be used to sample red meat animal carcasses.

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MATERIALS AND METHODS

Sampling, Study 1

Randomly selected areas on the round, near the anus of beef carcass halves from animals slaughtered at the Roman L. Hruska US Meat Animal Research Center abattoir, were marked within 20 min of slaughter by impressing a sterile 5 cm × 5 cm stainless steel template firmly against the carcass surface. One sample for each of four sampling methods was taken from each carcass half. The sampling materials employed were: swabbing with sterile cotton tipped wooden swabs (CS; Hardwood Products, Co., Guilford, MN), sterile cheesecloth cut into 6·5 cm × 3 cm squares, eight layers thick (CC), sterile virgin sponges (Speci-Sponge) cut into 3·5 cm × 4 cm squares (SP; NASCO, Fort Atkinson, WI) and excision (EX). For EX, 25 cm^2 samples were cut 0·5 mm thick using sterile scalpels then placed into a stomacher bag containing 25 ml BPW-T, consisting of 1% buffered peptone water (Becton Dickinson and Co., Cockeysville, MD) plus 0·1% (w/v) Tween 20 adjusted to pH 7·8 (Fisher Scientific, St Louis, MO). Sampling with CC and SP was accomplished by pre-moistening these materials in 25 ml of BPW-T, donning a sterile glove (Aladan Corp., Dothan, AL), expressing all excess buffer, and firmly rubbing the material approximately 10 times in multiple directions over the marked 25 cm^2 area of the carcass. After sampling, CC and SP were placed back into the 25 ml of buffer and transferred to stomacher bags. After sampling with CS, they were placed into 2 ml of BPW-T and expressed.

Sampling, Study 2

Lean and adipose surface tissues were cut from the surface of beef carcasses brisket/mid-line area within 15 min of slaughter at a local cow/bull beef processing facility and transported to lab on ice within 1 h. The sample tissues were aseptically cut into 7·5 cm × 7·5 cm pieces, surface sterilized with u.v. light (Cutter and Siragusa 1994) and used within 3 h of animal slaughter.

Faeces were collected immediately after defaecation from at least three cattle held at a feedlot on a corn/silage diet, combined into a composite sample, and hand mixed in a sterile 4 l plastic container. The faecal composite was diluted 1 : 10, 1 : 100 and 1 : 1000 (high, medium and low inoculation, respectively) in sterile distilled water, and 10 ml was aliquoted into sterile weigh boats. A control set of sterile distilled water was also aliquoted into 13 cm × 13 cm × 2 cm weigh boats. Lean and adipose beef carcass tissues were placed surface side down into the weigh boats and exposed for 15 min, allowed to drain for 30 s, and placed on a sterilized plastic cutting board. A sterile 5 cm × 5 cm stainless steel template was used to mark sampling areas. Four different sampling methods were used to sample each tissue type for each faecal dilution.

EX was accomplished as described in Study 1. The other three materials used to collect samples from the surface tissues were sterile 4 cm × 4 cm pieces of Scotch-Brite™ No. 88 Extra Heavy Duty Scouring Pad (M; 3M, Inc., St Paul, MN), 3M No. 200 Griddle Screen (GS) and SP. Sampling was accomplished as described for CC and SP in Study 1. Physiological saline plus 0·05% w/v Tween 20 was used as a buffer instead of BPW-T. After sampling, materials or excised tissues were placed back into the 25 ml of buffer and transferred to stomacher bags.

Bacterial enumeration

With the exception of CS samples that were expressed into 2 ml of BPW-T, all samples were pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, OH), serially diluted in 2% BPW, and spiral plated in duplicate on Tryptic Soy agar (BBL) using a Model D spiral plater (Spiral Systems Instruments, Bethesda, MD). Plates were incubated aerobically (35°C for 48 h), enumerated using a CASBA II laser colony scanner Model 500A (Spiral Biotech, Inc., Bethesda, MD), and plate counts reported as $\log_{10}$ cfu cm^{-2}.

Experimental design and statistical analyses

For each sampling method in Study 1, 64 samples were taken from 64 carcass halves over a 3 d period. In Study 2, four samples were taken in duplicate for each sampling method, for three replications. This yielded a study total of 24 samples per sampling method. The least square means of bacterial populations were calculated using General Linear Model (GLM) procedure of SAS (version 6.06.01, 1989, SAS Institute, Inc., Cary, NC). The probability level was $P < 0·05$ unless otherwise specified.

RESULTS AND DISCUSSION

The results from Study 1, indicated the somewhat abrasive material, CC, gave $\log_{10}$ cfu cm^{-2} results that were not significantly lower than EX (Table 1). While fewer bacteria were

Table 1 Bacterial recovery from uninoculated beef carcass rounds ($n = 64$), using various sampling methods (Study 1)

	$\log_{10}$ cfu cm^{-2}			
	Excision	Cheesecloth	Sponge	Swab
Uninoculated	$2·6^{a}$	$2·3^{ab}$	$2·1^{b}$	$1·3^{c}$

Different superscripts denote statistical differences ($P < 0·05$).

recovered by SP than by EX, sampling by SP was statistically similar to CC. The least effective material used for sampling bacterial populations on beef carcasses was CS. EX recovered 1·3 $\log_{10}$ cfu cm^{-2} more bacteria from the sample site than CS. Although swabbing with cotton tipped swabs is an accepted sample collection method for doing sanitation checks in processing facilities, it does not appear to be a very desirable way to sample carcasses.

Since the more abrasive materials were the most effective for sampling uncontaminated carcasses when compared to EX, additional abrasive materials were evaluated on artificially contaminated beef tissue in Study 2. Effectiveness of the sampling materials was not altered by tissue type (lean/adipose) so these data sets were pooled.

Though there was no significant difference exhibited between sampling materials on inoculated tissue, as the inoculation levels increased the more abrasive sampling materials did yield bacterial populations closer to that of EX (Table 2). As observed in Study 1 for CC, the more abrasive materials used in Study 2, GS and M, recovered lower bacterial populations than EX, but not significantly lower. SP recovered significantly lower bacterial populations than EX only on the uninoculated samples. For uninoculated tissue the difference between sample population means for SP, GS and M when compared to EX was 1·2, 0·5 and 0·7 $\log_{10}$ cfu cm^{-2}, respectively. These differences were reduced, however, when tissue was contaminated with the high faecal inoculum, and were 0·5, 0·2 and 0·1 $\log_{10}$ cfu cm^{-2}, respectively for SP, GS and M.

EX is the most effective method for sampling beef carcasses. However, good EX sampling of beef carcasses requires a certain amount of both time and proficiency. As a result, it is unlikely that EX will ever be a practical sampling method for a processing plant quality control monitoring program that is attempting to collect duplicable samples for rapid microbial tests from a moving processing line. For sampling beef carcasses that have been faecally contaminated, this study indicates that SP is capable of bacterial recovery proportional to that of EX. SP is available commercially in an easy to use form while the other materials are not. Consequently, SP is an adequate method of beef carcass sampling for rapid, process monitoring to detect faecal contamination. Since it appears that an increase in sampling material abrasiveness improves bacterial recovery from beef carcasses, especially at lower contamination levels, any increase in abrasiveness which can be afforded to a SP method in the future might improve its sampling abilities.

Table 2 Bacterial recovery from beef lean and adipose tissues ($n = 24$) inoculated with bovine faeces, using various sampling methods (Study 2)

	$\log_{10}$ cfu cm^{-2}			
Inoculation level	Excision	Sponge	Griddle screen	3M Mesh
Uninoculated	$3·3^{a}$	$2·1^{b}$	$2·9^{ab}$	$2·7^{ab}$
Low inoculum	$3·2^{a}$	$2·6^{a}$	$2·3^{a}$	$2·2^{a}$
Medium inoculum	$3·5^{a}$	$3·2^{a}$	$2·8^{a}$	$3·0^{a}$
High inoculum	$4·7^{a}$	$4·2^{a}$	$4·5^{a}$	$4·6^{a}$

Different superscripts within rows denote statistical difference ($P < 0·05$) only among sampling methods within a given inoculation level.

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