Food Microbiology, 1997, **14**, 63–69

ORIGINAL ARTICLE

Efficacy of using a sponge sampling method to recover low levels of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and aerobic bacteria from beef carcass surface tissue

W. J. Dorsa*, G. R. Siragusa, C. N. Cutter, E. D. Berry and M. Koohmaraie

Recovery of aerobic bacterial populations, as well as low levels of Escherichia coli *O157:H7 and* Salmonella typhimurium *from beef carcasses was determined by sponge (SP) and excision (EX) sampling during the course of three separate studies. For the first study, samples were taken from three points on the processing line: (1) pre-wash, (2) post-wash, and (3) after 24 h in the chill cooler. SP recovered fewer total aerobic bacteria from beef carcasses on a processing line than EX; however, the difference between EX and SP was similar at all sampled process points. Both sampling methods recovered higher levels of total aerobic bacteria from carcasses after a 24-h-chill period. Both methods also recovered low levels of* E. coli *and coliforms on beef carcasses. In the second study, both SP and EX recovered antibiotic-marked strains of* E. coli *O157:H7 and* S. typhimurium *from beef carcass tissue surface at an inoculation level of approximately 1 cfu 100 cm^{-2}. Recoveries from pre- or post-24-h-chilled tissue were similar for both SP and EX. SP appears to be an adequate sampling method for recovery of low levels of pathogens from surfaces of beef carcasses regardless of location within the process. However, a third study demonstrated that the freezing and storage of SP samples at −20°C significantly decreased recovery of* S. typhimurium *from beef tissue when present at low levels (≤10 cfu 100 cm^{-2}).*

© 1997 Academic Press Limited

Received: 11 June 1996

United States Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, Nebraska 68933-0166, USA

Introduction

Excision sampling (EX) is capable of recovering the most representative counts of the microbial flora present on beef carcasses (Brown and Baird-Parker 1982, Ingram and Roberts 1976). When recovery of specific bacteria from carcass surfaces is required, this method is considered the most efficacious and is commonly used by researchers (Charlebois

*Corresponding author.
Mention of a trade name, proprietary product or specific equipment is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

0740-0020/97/010063+07 $25.00/0/fd960060
© 1997 Academic Press Limited

et al. 1991, Cutter and Siragusa 1994, FSIS 1994). The destructive nature of EX sampling, as well as limited sampling area, time and skill level requirements, makes it an unacceptable sampling method for on-line beef carcass sampling for hazard analysis and critical control point (HACCP) monitoring. It has previously been shown that sponge sampling (SP) is a method capable of rapidly recovering aerobic bacteria from beef carcasses, although to a lesser extent than EX (Dorsa et al. 1996a). Grau (1988) successfully used cellulose sponges to recover *Campylobacter* spp. from beef calves and cattle carcasses.

Beef processing facilities will soon be required to sample carcasses for specific pathogenic bacteria as part of a new HACCP driven federal inspection system. The U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) has proposed Biotype I *Escherichia coli* as an indicator of fecal contamination on beef carcasses during processing (Federal Register, 1995). Additionally, *Salmonella* spp. testing on finished carcasses is also proposed by FSIS as a means to gauge pathogen reduction. *E. coli* O157:H7 and *Salmonella* spp. are both pathogens of concern on red meat (Anon. 1993). Consequently, simple, rapid, reliable, and repeatable sampling methods for these pathogens from beef carcasses must be established. The objectives of this study were to: (1) determine the effectiveness of SP compared with EX sampling of beef carcasses in a processing facility immediately after slaughter and after 24 h of refrigerated storage, (2) determine the ability of SP and EX sampling to recover low levels of *E. coli* O157:H7 and *Salmonella typhimurium* from pre- and post-24 h chilled beef carcass tissue, and (3) determine whether freezing sponge samples affects recovery of *S. typhimurium*.

Materials and Methods

Experimental design, Study 1

A total of 30 beef carcasses were sampled (15 per day on 2 consecutive days) from a 450-animal-per-day cow/bull slaughter facility. On the flank/midline area of each of the 30 beef carcasses, six 10-cm×10-cm areas were marked using a sterile stainless steel template and edible ink. Both EX and SP samples were taken side-by-side from these areas at each of three points in the process: (1) pre-wash, (2) post-wash in the chill cooler, and (3) 24 h after entering the chill cooler. Least squares means (LSM) of population data were analyzed using the general linear model procedure (GLM) of SAS (SAS Institute, Cary, NC, USA) with a probability level of P=0·05 used as the level of significance unless otherwise indicated.

Sampling methods, Study 1

EX, 100 cm^2 samples, were cut 0·5 cm thick using sterile scalpels and forceps, then placed into a filtered Stomacher bag (Spiral Biotech, Bethesda, MD, USA) and immediately placed on ice for transport back to Roman L. Hruska U.S. Meat Animal Research Center (MARC; Clay Center, NE, USA). Sampling with SP was accomplished by pre-moistening a sterile virgin sponge (Speci-Sponge; NASCO, Fort Atkinson, WI, USA) in 25 ml of (w/v) 1% sterile buffered peptone water (Becton Dickinson and Co., Cockeysville, MD, USA) +0·1% (w/v) Tween 20 (BPW-T), adjusted to pH 7·2, donning a sterile rubber glove (Aladan Corp., Dothan, AL, USA), expressing all excess buffer, and firmly rubbing the sponge approximately 10 times in the horizontal and 10 times in the vertical direction over the marked 100-cm^2 area of the carcass. After sampling, the sponge was placed back into the Whirlpak™ bag containing the expressed buffer and placed on ice for the 30 min transport back to the laboratory.

Bacterial enumeration, Study 1

Upon arrival at MARC, 100 ml BPW-T was added to the Stomacher bags of EX samples and pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, OH, USA). Samples were then serially diluted in 2% (w/v) buffered peptone water (BPW; BBL), and spiral plated in duplicate on tryptic soy agar (BBL) using a Model D spiral plater (Spiral Systems Instruments, Bethesda, MD, USA).

Plates were incubated aerobically (37°C for 24 h) and number of colony forming units (cfu) per square centimeter determined with a CASBA IV computer-assisted colony image analyzer (Spiral Biotech, Inc., Bethesda, MD, USA) and converted to $\log_{10}$ values. Total coliforms and *E. coli* were determined using 3M Petrifilm™ *E. coli* count plates incubated aerobically at 35°C for 24 h and enumerated according to the manufacturer's instructions (3M, Inc., St. Paul, MN, USA).

Inoculum, Study 2 and 3

Feces were collected immediately after defecation from cattle held at the MARC feedlot and frozen until use. Thawed feces was diluted 1:10 using distilled H_2O and sterilized by autoclaving. The fecal slurry was transferred to a sterile filter-Stomacher bag, stomached for 1 min, and the filtrate used as inoculation menstruum. Eighteen-hour cultures of a streptomycin-resistant *E. coli* O157:H7 strain (Dorsa et al. 1996c) or a nalidixic acid-resistant *S. typhimurium* (resistant at 250 $\mu g\ ml^{-1}$) were used to inoculate separate sterile bovine feces to contain approximately 1, 10, 100 cfu ml^{-1} of the bacteria. Uninoculated sterile fecal slurry was used as a control.

Experimental design, Study 2

Twenty beef short plates were collected on each of 4 days, from a local cow/bull processing facility as described by Dorsa et al. (1996b). Upon return to the MARC laboratory, the short plates were placed on separate sterile plastic trays and each was marked using a sterile template and edible ink at four separate 10-cm×10-cm sampling areas. Two sets of 20 beef short plates were inoculated with *E. coli* O157:H7 and the additional two sets of 20 were inoculated with *S. typhimurium*. The inoculation procedure for each of the bacterium involved placing 1 ml of the appropriate inoculum on a 100-cm^2 marked area, then spreading it to cover the entire marked area using a sterile spoon. This procedure was repeated over each of the four marked areas on all beef short plates. The inoculum was allowed to stand for 15 min, then pre-24-h-chilled EX and SP samples were taken as described for Study 1. After sampling, the short plates were loosely tented with polyethylene bags and placed in a research, 5°C, walk-in cooler for 24 h. After the 24-h-chill period, the remaining two unsampled 100 cm^2 marked areas of each short plate were sampled by EX and SP.

For each study, EX was used to sample a total of 10 separate areas containing each inoculation level and SP was used to sample 10 additional separate areas of the same inoculation levels from the same short plates. A total of 160, 100-cm^2 areas were sampled by SP and EX. SP and EX samples were taken side by side, using the procedures described previously, from pre- and post-24-h-chilled areas on the same short plate.

Experimental design, Study 3

Twenty beef short plates, five per inoculation level, were collected, marked, and inoculated with *S. typhimurium* as described for Study 2. Plates were tented as described above and placed into a research, 5°C, walk-in refrigerator for 24 h. After the 24-h period, each of the four 100 cm^2 areas on each short plate was SP sampled separately. The entire contents of the sponge sample bag was then transferred to a Stomacher bag and homogenized by stomaching for 1 min. Without removing the sponge from the Stomacher bag, the sponge was expressed, and as much stomachate as possible (~15 ml) was drawn into a 25-ml pipet. The stomachate was then evenly divided between the Stomacher bag and the original Whirlpak™ bag the sponge had been in, taking care not to re-wet the sponge at this point. The sponge was then aseptically cut into even halves with sterile scissors. One of the halves was then aseptically placed into the original Whirlpak™ bag containing one half of the stomachate and the other into the Stomacher bag containing the other half of the stomachate. Salmonellae enrichment procedures, as described below, were started immediately on the sample half in the Stomacher bag. The matching half in the original Whirlpak™ bags was placed flat and separated from other bags on a plastic tray and allowed to freeze for 3 h in a

research walk-in freezer maintained at –20°C. The frozen samples were then placed into a single corrugated box and stored at –20°C. After 8 days of frozen storage, these samples were thawed and subjected to the salmonellae enrichment and identification procedures described below.

*Bacterial detection (*E. coli *O157:H7), Study 2*

Immediately after collection, SP and EX samples were each placed into 100 ml of *E. coli* broth (EC) (International Bioproducts, Redmond, WA, USA) containing 20 $\mu g\ ml^{-1}$ novobicin and incubated in a Lab-line Environ-shaker (Lab-line Instruments, Inc., Melrose Park, IL, USA) for 24 h at 100 r min^{-1} and 37°C. Following enrichment, samples were assayed with a rapid dipstick immunoassay test (Micro-Screen™; Neogen Corp., Lansing, MI, USA). In addition, 1 ml of each enrichment sample was divided and spread plated over four prepoured agar plates (0·25 ml $plate^{-1}$) of sorbitol MacConkey agar (SMAC) containing 250 $\mu g\ ml^{-1}$ streptomycin to determine the presence or absence of the antibiotic-resistant *E. coli* O157:H7. These plates were aerobically incubated at 37°C for 24 h, and typical *E. coli* O157:H7 isolates present on the plates were confirmed serologically (*E. coli* O157 Test kit, Unipath Ltd., Basingstoke, Hampshire, UK).

*Bacterial detection (*S. typhimurium*), Study 2 and 3*

Immediately after collection, all SP and EX samples were pre-enriched in BPW and incubated in a Lab-line Environ-shaker for 24 h at 37°C and 100 r min^{-1}. Following pre-enrichment, 0·5 ml of sample from BPW was placed into 10 ml of tetrathionate broth (TT; Acumedia Manufacturers, Inc., Baltimore, MD, USA) and an additional 0·5 ml into 10 ml selenite cystine broth (SC; Difco, Detroit, MI, USA), then incubated for an additional 24-h period at 37°C. After incubation, samples from TT and SC were streaked for isolation on brilliant green agar containing sulfadiazine (BGS; BBL, Cockeysville, MD, USA) and 250 $\mu g\ ml^{-1}$ nalidixic acid (NA; Sigma, St. Louis, MO, USA) for presence or absence of the antibiotic-resistant *S. typhimurium*. Typical *S. typhimurium* colonies present on BGS+NA agar plates were verified serologically (Oxoid Salmonella latex test, Unipath Ltd., Basingstoke, Hampshire, UK) and by characteristic growth reaction on triple sugar iron agar (TSI; Difco).

Results and Discussion

Study 1

For all samples regardless of sampling site and time, SP recovered significantly fewer (P<0·01) total aerobic bacteria (1·6 $\log_{10}$ cfu cm^{-2}) from beef carcasses on a processing line than EX (2·1 $\log_{10}$ cfu cm^{-2}); however, the level of difference between EX and SP was similar at all process points sampled (Table 1). Thus, SP counts are relatable to EX counts, indicating SP is a practicable sampling method for estimation of aerobic bacterial populations on beef carcasses during the slaughter process.

Both methods recovered higher levels of total aerobic bacteria from carcasses after a 24-h chill period (Table 1). The average numbers of aerobic bacteria recovered using both methods were significantly different (P<0·01) at pre-wash (1·4 $\log_{10}$ cfu cm^{-2}), post-wash (1·9 $\log_{10}$ cfu cm^{-2}), and 24 h chill (2·3 $\log_{10}$ cfu cm^{-2}) points in the process. Because a 24-h-chilled carcass has experienced a degree of surface tissue desiccation, this result was not

Table 1. Least squares means of total aerobic plate counts ($\log_{10}$ cfu cm^{-2}) from excision and sponge sampling of 100 cm^2 areas of cow carcass flanks by the point in process

Point in process[d]	Method	
	Excision[a]	Sponging[b]
Pre-wash[c]	1·6	1·3
Post-wash[b]	2·1	1·6
24-h chill[a]	2·5	2·1

[a,b]Methods lacking common superscript letter differ (P<0·05).[a,b,c]Points in process lacking common superscript letter differ (P<0·05).[d]n=30 for each point in process sampling location. *P=0·792 for the overall interaction between the points in process and sampling methods.

expected. Investigations by Anderson et al. (1987) and Firstenberg-Eden (1981) indicated that moist carcass surfaces yield superior swab recovery of total aerobic bacteria. If carcass moisture is a factor in the ability of SP to recover bacteria from a carcass surface, the remoistening of the surface that occurs during the SP procedure appears to be adequate for 24-h-chilled carcasses.

E. coli and coliforms were recovered from more samples by the EX method than SP for all three process locations and times (Table 2). SP yielded more coliform positive samples at the post-wash site than at the 24-h chill site (Table 2). When *E. coli* or coliforms were detected, they were in low numbers, <6 and 16 cfu cm^{-2}, respectively. Countable SP samples for *E. coli* or coliforms did not necessarily yield countable samples by EX, the inverse also being true. For example SP samples from carcasses chilled for 24 h yielded seven countable samples, but only four of the seven were countable by EX.

Study 2

Both SP and EX successfully recovered the antibiotic-resistant *E. coli* O157:H7 and *S. typhimurium* from beef carcass tissue surface at an inoculation level of 1 cfu 100 cm^{-2} as indicated by growth on the culture medium (Table 3 and 4). Recoveries were similar from pre- or post-24-h chilled tissue. SP appears to be a satisfactory sampling method for recovery of *E. coli* O157:H7 and *S. typhimurium*, even at low levels, from beef carcasses.

At tissue inoculation levels ≥100 cfu ml^{-1} *E. coli* O157:H7, all SP and EX samples were

Table 2. Numbers of samples positive for *Escherichia coli* and coliforms from excision and sponge sampling of 100-cm^2 areas of cow carcass flanks

Sampling method	Pre-wash[a]		Post-wash[a]		24-h Chill[a]	
	E. coli	coliforms	*E. coli*	coliforms	*E. coli*	coliforms
Excision	4	8	6	11	4	11
Sponging	1	2	2	9	3	7

[a] *n*=30 for each point in process sampling location.

Table 3. Recovery of a marked *Escherichia coli* O157:H7 strain inoculated onto beef from 100-cm^2 areas of beef short plate surface tissue, pre- and post-24-h chill (5°C) sampled by sponging or excision and tested by a rapid dipstick immunoassay or culture method

Inoculum level (cfu 100 cm^{-2})	Sponge				Excision			
	Pre-24-h chill		Post-24-h chill		Pre-24-h chill		Post-24-h chill	
	Rapid	*Culture*	*Rapid*	*Culture*	*Rapid*	*Culture*	*Rapid*	*Culture*
0	0[a]	0	0	0	0[a]	0	0	0
1	50	100	50	90	40	100	80	100
10	100	100	90	100	80	100	100	100
100	100	100	100	100	100	100	100	100

[a]Percentage of samples positive for *E. coli* O157:H7 (*n*=10).

Table 4. Recovery of a marked *Salmonella typhimurium* strain inoculated onto beef from 100-cm^2 areas of beef short plate surface tissue, pre- and post-24-h chill, after 5°C refrigerated storage

Inoculum level (cfu 100 cm^{-2})	Sponge		Excision	
	Pre-24-h chill	Post-24-h chill	Pre-24-h chill	Post-24-h chill
0	0[a]	0	0	0
1	100	100	100	90
10	100	100	100	100
100	100	100	100	100

[a]Percentage of samples positive for *S. typhimurium* (*n*=10).

positive by the rapid immunoassay (Micro-Screen™), regardless of whether sampled before or after chilling (Table 3). Detection of *E. coli* O157:H7 with the rapid immunoassay only slightly decreased at the 10 cfu 100 cm^{-2} inoculation level. Numbers adequate for the rapid immunoassay detection of the bacterium were apparently most variable at the lowest level of inoculation, between 40–80% of the samples yielding positive results (Table 3). Because the bacterium was present in the enrichment medium at these inoculation levels, as indicated by its countable presence on the culture medium, other more sensitive rapid detection methods may have yielded more favorable detection results.

Study 3

Consistent with Study 2, SP samples from beef carcass tissue at as low an inoculation level as one *S. typhimurium* cfu 100 cm^{-2} yielded positive results (Tables 4 and 5). However, freezing and storage of samples for 8 days at –20°C dramatically impaired recovery of the bacterium at lower levels of inoculation (1 and 10 cfu 100 cm^{-2}). This was not unexpected because it is well documented that freezing of salmonellae cells will cause permanent damage (Brown and Baird-Parker 1982, Smith 1995), probably due to ice crystal formation both outside of and within the cells (Mazur 1966). Beef baseline data collected by FSIS (1994) have indicated low incidence (1%) and population levels (≤0·3 cfu cm^{-2} of positive samples) of *Salmonella* spp. on beef carcasses. Because *Salmonella* spp. may be present at low levels, these data sets indicate that samples from beef carcasses at processing facilities should not be frozen using the described freezing method before being tested for *Salmonella* spp.

Table 5. The effect of 8 days frozen storage (–20°C) on the recovery of *S. typhimurium* from sponge samples taken from 100-cm^2 areas of post-24-h, 5°C chilled beef short plate surfaces

Inoculum level (cfu 100 cm^2)	Immediately after sampling	8-Day frozen samples
0	0[a]	0
1	100	35
10	100	40
100	100	100

[a]Percentage of samples positive for *S. typhimurium* (n=20).

Conclusions

SP appears to be a reliable method for sampling beef carcasses on-line for total aerobic bacteria, regardless of location in the slaughter process. It is also very effective for recovering *E. coli* O157:H7 and *S. typhimurium* from beef carcass tissue, even when present at very low levels (1 cfu 100 cm^{-2}). Thus, SP is an effective way to sample carcasses in a processing facility for the presence of either bacteria using standard culture methods and has the potential for use as a sampling method for rapid immunoassay tests. Additionally, if low levels of *S. typhimurium* are expected, samples should not be frozen and held at –20°C prior to culturing.

Acknowledgments

The authors wish to thank Mrs Dawn Wiseman, Mrs Carole Smith, and Mrs Jane Long for their expert technical assistance, Mr Kenneth Ostdiek for beef sampling, and Mr James Wray for statistical analyses.

References

Anderson, M. E., Huff, H. E., Naumann, H. D., Marshall, R. T., Damare, J., Johnston, R. and Pratt, M. (1987) Evaluation of swab and tissue excision methods for recovering micro-organisms from washed and sanitized beef carcasses. *J. Food Protect.* **50,** 741–743.

Anonymous. (1993) *Healthy People 2000 Review 1992.* U.S. Dept of Health and Human Services. Hyattsville, MD. DHHS Pub. No. (PHS) 93-1232-1 pp. 73–74.

Brown, M. H. and Baird-Parker, A. C. (1982) The microbiological examination of meat. In *Meat Microbiology* (Ed. Brown, M. H.), pp. 423–520. New York, Applied Science Publishers Ltd.

Charlebois, R., Trudel, R. and Messier, S. (1991) Surface contamination of beef carcasses by fecal coliforms. *J. Food Protect.* **54,** 950–956.

Cutter, C. N. and Siragusa, G. R. (1994) Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot

scale model carcass washer. *J. Food Protect.* **57,** 97–103.
Dorsa, W. J., Cutter, C. N. and Siragusa, G. R. (1996a) Evaluation of six beef carcass surface bacterial sampling methods. *Lett. Appl. Microbiol.* **22,** 39–41.
Dorsa, W. J., Cutter, C. N., Siragusa, G. R. and Koohmaraie, M. (1996b) Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and steam-vacuum sanitizer. *J. Food Protect.* **59,** 127–135.
Dorsa, W. J., Cutter, C. N. and Siragusa, G. R. (1996c) Effects of steam-vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes. J. Food Protect.* (accepted 6/96).
Federal Register. 1995. *Proposed rule. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems*. USDA-FSIS. Vol. 60, No. 23. Office of the Federal Register. Washington, D.C.
Firstenberg-Eden, R. (1981) Attachment of bacteria to meat surfaces: A review. *J. Food Protect.* **44,** 602–607.
FSIS. (1994) *Nationwide Beef Microbiological Baseline Data Collection Program: Steers and Heifers*. U.S. Dept. of Agric. Food Safety Insp. Serv., Washington, DC.
Grau, F. H. (1988) *Campylobacter jejuni* and *Campylobacter hyointestinalis* in the intestinal tract and on the carcasses of calves and cattle. *J. Food Protect.* **51,** 857–861.
Ingram, M. and Roberts, T. A. (1976) The microbiology of the red meat carcass and the slaughterhouse. *R. Soc. Health J.* **96,** 270–276.
Mazur, P. (1966) Physical and chemical basis of injury in single-celled microorganisms subjected to freezing and thawing. In *Cryobiology* (Ed. Meryman, H. T.), pp. 213–315. San Diego, Academic Press.
Smith, M. G. (1995) Survival of *E. coli* and Salmonella after chilling and freezing in liquid media. *J. Food Sci.* **60,** 509–512.