

Incidence of *Salmonella* Spp. on Beef Cattle Hides and Carcasses in Eight Commercial Beef Slaughtering Facilities

R. T. Bacon, J. N. Sofos, K. E. Belk
and G. C. Smith

Center for Red Meat Safety
Department of Animal Sciences
Colorado State University
Fort Collins, CO 80523-1171

Key Words: *Salmonella* spp., Beef cattle hides, Beef carcasses, Incidence

SUMMARY

This study evaluated the incidence of *Salmonella* spp. on beef cattle hides and carcasses in eight commercial slaughtering facilities. Beef cattle hides and carcasses (N = 640) were evaluated by sponge swab sampling at two separate in-plant sampling locations. Beef cattle hides were sampled following stunning and exsanguination but before the dehiding process, and beef carcasses were sampled following dehiding and other slaughtering/dressing processes including the application of decontamination treatments but before carcass chilling. Overall, the incidence of *Salmonella* spp. on beef cattle hides was 14.7%, while the corresponding (same animal) incidence following the slaughtering/dressing process but before carcass chilling was 0.9%.

INTRODUCTION

It has been estimated that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year in the United States alone. Nontyphoidal strains of the genus *Salmonella* are estimated to be responsible for over 1.4 million illnesses and subsequently account for 9.7% of total foodborne illnesses, 25.6% of total hospitalizations, and 30.6% of deaths caused by known foodborne pathogens (Mead *et al.*, 1999). *Salmonella* are associated with the intestinal tracts of animals and humans and although they are occasionally associated with other vehicles of transmission, they are primarily (95%) a foodborne pathogen

causing a disease known as salmonellosis (Mead *et al.*, 1999). Poultry, poultry salads, meat and meat products, dairy products, egg products and other protein foods are some of the more common foods that have been implicated with salmonellosis (Reed, 1993).

Salmonella have been isolated at various stages throughout the beef slaughtering/dressing process (Stolle, 1981), and in 1996, pathogen reduction performance standards for *Salmonella* were established by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (FSIS-USDA, 1996b). These performance standards were based on current, baseline data, obtained using tissue excision, that indicated a 1.0% incidence rate for steer-heifer carcasses and a 2.7% incidence rate for bull-cow carcasses (FSIS-USDA, 1994; 1996a). Subsequently, FSIS constructed a two-class sampling plan with values of *c* (maximum number of positive samples) and *n* (total numbers of samples tested), and assigned values of 1 and 82, respectively, for steers-heifers and values of 2 and 58, respectively, for bulls-cows based on an 80% probability of passing if the facility was operating at the current baseline incidence (FSIS-USDA, 1994; 1996a; 1996b; Sofos *et al.*, 1999).

The objective of this study was to determine the current incidence of *Salmonella* spp. on beef cattle hides and carcasses.

MATERIALS AND METHODS

Samples (N = 640) were collected by sponge swabbing beef carcasses, at both hide-on and hide-off locations, in eight beef packing plants, of which five slaughtered mostly steers and heifers and the remaining three slaughtered cows and bulls. Decontamination intervention technologies, applied in sequence in some or all of the eight plants, included: (a) steam vacuuming (104-110°C, 138-345 kPa steam, negative 7 to 12 mm of Hg vacuum) of spot contamination, in a concentrated area

of the slaughtering sequence following hide removal; (b) pre-evisceration carcass washing (29-38°C water at 193-331 kPa, 6-8 sec) immediately following steam vacuuming; (c) pre-evisceration application of organic acid solution rinsing (1.6-2.6% lactic or acetic acid solution, 43-60°C, 317-324 kPa, 2-4 sec) following pre-evisceration washing; (d) thermal pasteurizing (71-77°C water, 69-228 kPa, 10-14 sec) following "Zero Tolerance" final rail inspection; (e) post-evisceration carcass washing (16-32°C water, 483-897 kPa, 10-14 sec) following thermal pasteurizing; and; (f) post-evisceration application of organic acid solution rinsing (1.6-2.6% lactic or acetic acid solution, 43-60°C, 317-324 kPa, 2-4 sec), following final washing and immediately before chilling.

In four of the steer/heifer facilities (designated as plants 1 through 4), the decontamination system consisted of: (a) steam vacuuming, (b) pre-evisceration carcass washing, (c) pre-evisceration acetic acid solution rinsing, (d) thermal pasteurizing, (e) post-evisceration carcass washing, and (f) post-evisceration acetic acid solution rinsing. In a fifth steer/heifer facility (designated as plant 5), the decontamination system was identical to that used in the other four steer/heifer facilities, except that carcasses were not rinsed with an acetic acid solution at either the pre-evisceration or post-evisceration sites.

In all three of the cow/bull slaughtering facilities (designated as plants 6 through 8), the decontamination system consisted of: (a) steam vacuuming, (b) thermal pasteurizing, (c) post-evisceration carcass washing, and (d) post-evisceration lactic acid solution rinsing.

Sample Collection. Sampling occurred during the slaughtering/dressing process at two separate in-plant locations, which were: (1) hide-on, or external hair sampling, (site 1), which occurred post-exsanguination but before hide opening and subsequent hide removal, and (2) after slaughtering/dressing and decontam-

ination treatment application (site 2), which occurred immediately following post-evisceration carcass washing and any organic acid solution rinsing, but before carcass chilling.

Sampling was performed by sponge swabbing and followed procedures described in the USDA/FSIS Meat and Poultry Inspection regulations (FSIS-USDA 1996). Immediately before sampling, sterile sponges (BioPro Enviro-Sponge Bags, International BioProducts, Redmond, WA) were hydrated with 10 ml of 0.1% sterile, buffered peptone water (BioPro, International BioProducts). Sampling of each carcass side was achieved using a 100 cm² disposable, sterile template (USDA Template, International BioProducts) at each of three anatomical locations for a total sampling area of 300 cm². Sponging at each anatomical carcass site, within the 100 cm² template area, consisted of 10 passes vertically (up-and-down being considered as 1 pass) and 10 passes horizontally (side-to-side being considered as 1 pass) with a pressure equivalent to that which would be used to remove dried blood.

The three anatomical carcass sites included: (a) flank, at a point where the medial border of the cutaneous flank muscle comes to within 7.62 cm of the midline, (b) brisket, at a point on the midline that is level with the elbow and (c) rump, at a point where a line from the posterior aspect of the aitch bone to the achilles tendon intersects the cut surface of the round (FSIS-USDA 1996). Samples were collected aseptically using sterile, latex gloves (BasicTM International BioProducts) which, in addition to the template, were changed between sampling of each carcass side. Following carcass sampling, an additional 15 ml of refrigerated 0.1% sterile, buffered peptone water (Difco Laboratories, Detroit, MI) was added to the sponge in order to bring the total volume of buffer to 25 ml. After excess air was expelled and the sponge bags were folded down, the samples were packed with icepacks and a cardboard pad, to prevent direct contact with the samples, into shipping

coolers for overnight delivery to the laboratory (Warren Analytical Laboratory, Greeley, CO) for analysis.

At both of the in-plant sampling locations (site 1 and site 2), 40 hide or carcass sides were sampled and tested for the presence of *Salmonella* spp. Hide-on sponge samples were collected at site 1 to determine the incidence of *Salmonella* spp. on the external (hide) surfaces of incoming cattle because this is the primary source of bacteria subsequently transferred to carcasses and because errors in slaughtering/dressing are the primary vehicles of beef carcass contamination (Castillo *et al.*, 1998; Kochevar *et al.*, 1997). For these reasons, Good Manufacturing Practices (GMP's) should be observed in order to minimize the amount of hide-to-carcass contamination occurring during the dehidng process.

Carcasses sampled at site 1 were visually tracked until completion of the dehidng process, at which point one of the two sides of each carcass was tagged for ease of identification at sampling site 2. The previously tagged, unsampled side was then sponge swabbed at site 2 to determine the incidence of *Salmonella* spp. on the carcasses following the slaughtering/dressing process and application of decontamination treatments.

Microbiological analysis. Following arrival of samples to the laboratory, the sponges and associated buffer were pummeled in a stomacher (Seward Model 400, Tekmar Company, Cincinnati, OH) for 1 min, at which point sterile lactose broth (LB) (Mikrobiologie, EM Science, Gibbstown, NJ) was added to the homogenate to create a 10:1 lactose broth to sample weight ratio. Following sample enrichment for 24 h at 35°C +/- 2°C, 1 ml of the sample was added to each of 9ml of cysteine selenite broth (CSB) (Difco Laboratories) and tetrathionite broth (TTB) (Difco Laboratories) to which 0.1 ml of 1% Brilliant Green solution and 0.2 ml iodine solution had been added in order to increase selectivity.

Inoculated CSB tubes were incubated at 35°C +/- 2°C for 24 h, while TTB tubes were held at 42°C +/- 0.5°C for 24 h in a water bath. Following selective enrichment for samples retrieved from plants 1 through 7, 1 ml of inoculated TTB and CSB were combined and added to 9 ml of M Broth (Difco Laboratories) to which 1 ml of 0.0001% Novobiocin solution had been added. Following a second enrichment at 42°C +/- 0.5°C for 5 h in a water bath (VWR Scientific Products), the samples were vortexed and an enzyme-linked immunosorbent assay (ELISA) test was used to screen for the presence of *Salmonella* species (Salmonella-TekTM, Organon Teknika Corporation, Durham, NC).

After selective enrichment for samples derived from plant 8, 1 ml of TTB and 1 ml of CSB were combined and added to 9 ml of M Broth (Difco). Following a second enrichment at 42°C +/- .5°C for 6 h in a water bath (VWR Scientific Products), the samples were vortexed and an ELISA test was used to screen for the presence of *Salmonella* spp. (Biocontrol@ Assurance EIA, BioControl Systems, Inc., Bothell, WA). Positive ELISA results based upon sample absorbency were interpreted as presumed positive for the presence of *Salmonella* spp. A negative result was considered conclusive, as the sample did not contain detectable levels of *Salmonella* antigen. All samples categorized as presumptive-positive by means of the ELISA test were biochemically confirmed using an API 20E test strip (API Analytab Products, Planinview, NY).

Statistical Analysis. The chi-square goodness-of-fit test was used to test for statistical differences (P < 0.05) in confirmed *Salmonella* spp. Frequencies between in-plant sampling sites (Table 1) (SAS, 1995).

RESULTS AND APPLICATION

The microbiological loads carried by incoming cattle are important because the exterior of the hide is the primary source of contamination that is eventually transferred to the

underlying, sterile carcass tissue (Castillo *et al.*, 1998; Kochevar *et al.*, 1997). At plants 3 and 5, there were no *Salmonella* spp. positive samples taken from either of the sampling sites. The remaining plants (1, 2, 4, 6, 7 and 8) all produced *Salmonella* spp. positive samples at site 1 (post-exsanguination but before dehiding), with plant 1 possessing by far the highest incidence at 47.5% or a total of 19 positive samples (Table 1). Following the slaughtering/dressing processes and application of decontamination interventions, the incidence of *Salmonella* spp. positive samples decreased ($P < 0.05$) in all of the facilities except for plants 3 and 5, where there were no positive samples at either sampling sites. Plants 1, 2, 4, 6, 7 and 8 demonstrated reductions in the incidence of *Salmonella* spp. positive samples from 47.5, 10.0, 23.1, 10.0, 17.5 and 15.0% at site 1, respectively, to an incidence of 7.5% for plant 1, 2.5% for plant 8, and 0.0% for the four remaining plants following the slaughtering/dressing processes and application of decontamination treatments. The current pathogen reduction performance standards allow for one positive sample in 82 total samples taken and two positive samples in 58 total samples taken, for steer-heifer and bull-cow plants, respectively (FSIS-USDA, 1996b). By these standards, at least for the 40 samples taken, all of the plants would be passing except for plant 1 where three positive samples were identified (Table 1). However, caution should be taken when making comparisons to the baseline data used to create the two-class sampling plan because that data was obtained by the tissue excision method as opposed to sponge sampling (FSIS-USDA, 1994; 1996a).

Overall, the incidence of *Salmonella* spp. on the external surfaces (hides) of cattle was 15.4% (49/319), but following dehiding and other slaughtering/dressing processes, and the application of decontamination treatments, the incidence was reduced ($P < 0.05$) to 1.3% (4/320) (Table 1).

REFERENCES

- Castillo, A. L., Dickson, J. S., Clayton, R. P., Lucia, L. M., and Acuff, G. R. 1998. Chemical dehairing of bovine skin to reduce pathogenic bacteria and bacteria of fecal origin. *J. Food Prot.* 61:623-625.
- FSIS-USDA. 1994. Nationwide Beef Microbiological Baseline Data Collection Program: steers and heifers, October 1992-September 1993. Food Safety and Inspection Service, Science and Technology, Microbiology Division, U.S. Department of Agriculture, Washington, D.C.
- FSIS-USDA. 1996a. Nationwide Beef Microbiological Baseline Data Collection Program: cows and bulls, December 1993-November 1994. Food Safety and Inspection Service, Science and Technology, Microbiology Division, U.S. Department of Agriculture, Washington, D.C.
- FSIS-USDA. 1996b. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) systems; Final Rule. *Federal Register.* 61:38805-38989.
- Kochevar, S. L., Sofos, J. N., Bolin, R. R., Reagan, J. O. and Smith, G. C. 1997. Steam-vacuuming as a pre-evisceration intervention to decontaminate beef carcasses. *J. Food Prot.* 60:107-113.
- Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607-625.
- Reed, G. H. 1993. Foodborne illness (part 2): Salmonellosis. *Dairy Food Environ. Sanitarian* 13:706.
- SAS. 1995. SAS® System under Microsoft Windows, Release 6.12. SAS Institute Inc., Cary, NC.
- Sofos, J. N., S. L. Kochevar, J. O. Reagan and G. C. Smith. Incidence of *Salmonella* on beef carcasses relating to the U. S. meat and poultry inspection regulations. *J. Food Prot.* 62:467-473.
- Stolle, A. 1981. Spreading of *Salmonellas* during cattle slaughtering. *J. Appl. Bacteriol.* 50:239-245.

Table 1. Incidence of confirmed *Salmonella* spp. on beef animal hides and carcasses in eight commercial beef slaughtering facilities.

Plant	In-Plant Sampling Location			
	Hide-On		After Slaughtering/Dressing and Decontamination Treatment Application	
	<i>Salmonella</i> spp. Positive Samples	Incidence (%)	<i>Salmonella</i> spp. Positive Samples	Incidence (%)
1	19	47.5 ^a	3	7.5 ^b
2	4	10.0 ^a	0	0.0 ^b
3	0	0.0	0	0.0
4	9	23.1 ^a	0	0.0 ^b
5	0	0.0	0	0.0
6	4	10.0 ^a	0	0.0 ^b
7	7	17.5 ^a	0	0.0 ^b
8	6	15.0 ^a	1	2.5 ^b
Total	49	15.4 ^a	3	1.3 ^b

Number of samples analyzed at each location in each plant: 40. Plant 4 had 39 samples at the hide-on sampling location due to laboratory errors during analysis.

^{ab} Incidences (%) in the same row with different superscript letters are different (P < 0.05).