

Post-Chilling Application of a Commercial Steam Vacuum Unit to Fresh Beef Adipose Tissue Inoculated with *Salmonella* Spp.

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SUMMARY

This study determined the efficacy of a commercial steam vacuum unit in reducing *Salmonella* spp. populations on beef adipose tissue following chilling. Tissue samples were separated into four treatment groups: (1) inoculated, with steam vacuum application; (2) inoculated, without steam vacuum; (3) uninoculated, with steam vacuum application; and (4) uninoculated, without steam vacuum application. Following inoculation, chilling and steam application, samples were sponge swabbed and excised; the latter proved most effective in inoculum recovery. *Salmonella* counts were reduced ($P < 0.05$) to populations of 3.3 to 3.6 log colony-forming-units (CFU)/cm², following steam vacuum application, as compared to untreated control populations of 4.0 to 4.2 log CFU/cm².

INTRODUCTION

Steam-vacuuming involves the application of hot water, steam or the combination of hot water and steam followed by the uptake of condensate and associated contamination as a means for spot decontamination. In April 1996, this steam vacuuming application was approved by the United States Department of Agriculture (USDA) as an alternative to knife-trimming for removal of feces, ingesta and other contamination, provided the spot of contamination is not in excess of 2.54 cm² (FSIS-USDA, 1995, 1996a; Sofos and Smith, 1998). Subsequently, the application of steam vacuuming has been found to be at least as effective as knife-

trimming and more consistent in its effectiveness than water washes in carcass cleaning/decontamination (Dorsa *et al.*, 1996; Kochevar *et al.*, 1997).

The majority of studies evaluating the efficacy of steam vacuuming as a spot decontamination treatment have concluded that application of steam, solely or in combination with other decontamination interventions, effectively reduces microbial populations on beef carcass surfaces, thereby improving microbiological quality and reducing risk to public health. However, there has not been extensive data compiled on the efficacy of steam vacuuming when applied to beef carcasses following chilling. The objective of this study was to mimic post-dehiding, carcass contamination with *Salmonella* spp. and determine the efficacy of steam vacuuming when applied following a chilling period that was long enough to allow adequate time for attachment and tissue penetration of the microorganisms.

MATERIALS AND METHODS

Samples (N = 70) of beef carcass adipose tissue (300 – 400 cm²) were collected from the brisket and rib regions immediately prior to chilling. Following collection, the samples were placed in coolers and transported (about 30 miles, in about 45 minutes) to Colorado State University. Tissue samples were laid flat onto trays and separated into four different treatment groups: (1) inoculated, with steam vacuum application (n = 40); (2) inoculated, without steam vacuum application (n = 10); (3) uninoculated, with steam vacuum application (n = 10); and (4) uninoculated, without steam vacuum application (n = 10). Treatment 2 was considered an inoculated, untreated control, and was necessary for comparison to treatment 1 to determine the efficacy of steam vacuuming in reducing the inoculated pathogenic organism. The uninoculated control with steam vacuuming (Treatment 3) and uninoculated control without steam vacuum application (Treatment 4), were necessary to determine the

efficacy of steam vacuuming on uninoculated samples, in addition to the extent of background interference or streptomycin-resistant bacteria already present on the tissue samples. For all of the samples, a sterile, 100 cm² disposable template (USDA Template, International BioProducts, Redmond, WA) was placed onto the adipose tissue and the perimeter was traced with a scalpel making it possible to sample the same area following chilling.

Inoculum. An environmental *Salmonella* isolate, demonstrating resistance to streptomycin at a concentration of 600 µg/ml, was used to inoculate samples in treatment groups 1 (inoculated, with steam vacuum application) and 2 (inoculated, without steam vacuum application). The *Salmonella* strain was grown in trypticase soy broth + streptomycin (600 µg/ml) and a spectrophotometer (Spectronic 20, Bausch and Lomb Inc., Rochester, NY) was used to develop a growth curve based on optical density. Adipose tissue samples were inoculated with 2ml of inoculum (c.a. 7.2 x 10⁶ CFU/ml) resulting in the application of 1.44 x 10⁷ CFU, which was uniformly spread over 100 cm² (1.44 x 10⁵ CFU/cm² or 5.2 log CFU/cm²) using a sterile glass rod. This recognizably high level of *Salmonella* was necessary in order to facilitate the detection of treatment differences following chilling. Inoculated and uninoculated samples were chilled for 24 ± 2 h at 2.6° C in an attempt to duplicate commercial slaughter plant chilling time and temperature.

Steam Vacuum Application.

Following chilling (24 ± 2 h; 2.6° C), a two-drop, commercial steam vacuum system (BFD Corporation, Aurora, CO) was used to decontaminate the samples of Treatment 1 (inoculation, with steam vacuum application) and Treatment 3 (uninoculated, with steam vacuum application). Steam (1.72 bar; 130°C) was applied to the chilled adipose tissue in four vertical passes with the application nozzle, and the subsequent condensate was vacuumed (-0.24 bar) and collected in a 125

gallon receiving tank. Sterile gloves were used to handle each individual sample while steam vacuuming was performed in order to prevent cross-contamination.

Sample Collection and Enumeration.

Sponge and excision sampling methods were used for the enumeration of the inoculated and uninoculated tissue samples. Immediately before sampling, sterile sponges (BioPro Enviro-Sponge Bags, International BioProducts) were hydrated with 10 ml of sterile, 0.1% buffered peptone water (BioPro, International BioProducts). Sponge sampling of each tissue sample occurred within a 100 cm² disposable, sterile template (USDA Template, International BioProducts) and 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, as described in the USDA/FSIS Meat and Poultry Inspection regulations (FSIS-USDA, 1996b). Swabbing with sponges was done aseptically using sterile, latex gloves (International BioProducts) which, in addition to the template, were changed between samples. Following sponging, an additional 15 ml of sterile, 0.1% buffered peptone water (Difco Laboratories, Detroit, MI) was added to the sponge bag, bringing the total volume of buffer to 25 ml.

The sponged tissue was then excised using a sterile scalpel blade and forceps. Excision of the tissue was done aseptically using sterile forceps, which, in addition to the template and scalpel blade, were changed between samples. The excised tissue was placed into a sample bag where it was weighed and diluted (10⁻¹) using sterile, 0.1% buffered peptone water. The sponged tissue was further excised and evaluated to determine the microbiological population remaining on the 100 cm² surface inasmuch as the time (24 ± 2 h) between inoculation and sampling, although occurring at refrigeration temperature, may allow for increased attachment

and tissue penetration of the microorganisms thereby reducing bacterial enumeration accuracy of the sponge sampling method.

Both sponge and excised samples were pummeled (Masticator, IUL Instruments, Barcelona, Spain) for one minute and serial dilutions were made using sterile, 0.1% buffered peptone water (Difco). For each appropriate dilution, 0.1 ml of diluent was plated onto each of trypticase soy agar, xylose lysine tergitol 4 agar, trypticase soy agar + streptomycin at a concentration of 600 µg/ml, and xylose lysine tergitol 4 agar + streptomycin at a concentration of 600 µg/ml. Trypticase soy agar (TSA) was used to determine the collective bacterial population, streptomycin resistant or not, since it is non-selective. Conversely, xylose lysine tergitol 4 (XLT₄) agar was used since it is a selective agar for *Salmonella* and would enumerate only those organisms, streptomycin resistant or not. TSA + streptomycin and XLT₄ + streptomycin were used to determine the population of general, streptomycin-resistant bacteria and to select for *Salmonella* spp. that are streptomycin resistant, respectively. After plating, the plates were inverted and incubated for 24 ± 2 h at 35° C at which point the plates were removed and colonies were manually counted using a Quebec Darkfield Counter (AO Scientific Instruments).

Statistical Analysis. After counting the sponge sample plates and reporting the data as CFU/ml of diluent plated, the data were multiplied by 25 (because 25 ml of total buffer volume was used) and divided by 100 (because the area sampled was 100 cm²) to yield data expressed as CFU/cm². Likewise, after enumerating the excision samples and reporting the results as CFU/g sample analyzed, the data were multiplied by the total excised sample weight and divided by 100 (representing the 100 cm² surface area sampled) to yield data expressed as CFU/cm². The data (CFU/cm²) were transformed to log₁₀ CFU/cm² for statistical analyses. Minimum detection limits for sponge

sampling, excision sampling, and total enumeration (sponging plus excision) were 1.8, c.a. 2.5, and c.a. 2.7 log CFU/cm², respectively, based on the maximum sensitivity of the tests with no further dilution of the samples beyond the original buffer volume of 25 ml for the sponge samples and 10⁻¹ dilution for the excision samples. Sponge and excision samples falling below the minimum detection limit were entered as 1.8 and 2.5 log CFU/cm², respectively, so that statistical analysis could be performed. Values for the mean log (\bar{X}) and standard deviation (s) of each enumerated set were calculated on the assumption of a log-normal distribution of microorganisms (Brown and Baird-Parker, 1982; Gill *et al.*, 1996; 1997; Kilsby and Pugh, 1981).

For each sampling method--sponging, excision and a combined total enumeration--data were evaluated with analysis of variance (AOV) using the model $y = a + x_1 + x_2 + x_1x_2$ and least squares means were computed for *Salmonella* counts by Treatment (inoculated, with steam vacuum application; inoculated, without steam vacuum application; uninoculated, with steam vacuum application, and; uninoculated, without steam vacuum application) (x_1), media used for recovery (TSA, XLT₄, TSA+ Streptomycin, and XLT₄+ Streptomycin) (x_2), and treatment x media used for recovery fixed effects using the General Linear Models procedure of SAS® (SAS, 1995). Due to the significant treatment x media interaction for the excision sampling method (P = 0.0064), and for the total combined enumeration (P = 0.0042), only interaction subclass least-squares means are reported (Tables 1-4). When AOV detected effects (P ≤ 0.05), least-squares means were separated using Fisher's protected LSD (SAS, 1995).

RESULTS AND APPLICATION

Enumeration of sponge samples on trypticase soy agar (TSA), xylose lysine tergitol 4 (XLT₄) agar, trypticase soy agar + streptomycin at a concentration of 600 µg/ml

(TSA+Streptomycin), and xylose lysine tergitol 4 agar + streptomycin at a concentration of 600 µg/ml (XLT₄+Streptomycin) showed no differences ($P > 0.05$) between the inoculated treatment with steam vacuum application and the inoculated treatment without steam vacuum application (Tables 1-4). However, excision data across all four media types reflected reductions ($P < 0.05$) in microbial populations of 0.4, 0.5, 0.6 and 0.7 log CFU/cm² for TSA, XLT₄, TSA+Streptomycin, and XLT₄+Streptomycin, respectively, following the application of steam vacuuming (Tables 1-4). Additionally, there was no detectable evidence of background growth or interference on plates containing streptomycin, although TSA and XLT₄ plates resulted in an average of 4.7 and 3.5 log CFU/cm², respectively, for the uninoculated without steam vacuum application treatment and 4.7 and 3.4 log CFU/cm², respectively, for the uninoculated with steam vacuum treatment (Tables 1 and 2).

Sponge sampling results on TSA indicated that the uninoculated treatment with steam vacuum application had slightly higher ($P < 0.05$) microbial populations than the uninoculated treatment without steam vacuum application; but there were no differences ($P > 0.05$) between the sponge sampled, uninoculated treatment means recovered on XLT₄, TSA+Streptomycin, or XLT₄+Streptomycin (Tables 1-4). Additionally, excision data reflected no differences ($P > 0.05$) between the uninoculated treatments regardless of media used for recovery (Tables 1-4). However, it should be recognized that, with the exception of sponge samples plated on TSA and excision samples plated on TSA and XLT₄, the majority (97 out of 100 sponge and excision samples analyzed) of uninoculated treatment means were not detected, therefore indicating that populations were below the detection limit. None of the uninoculated sponge or excision samples recovered on media containing streptomycin were enumeratable (Tables 3 and 4).

Overall, sponge sampling was inferior to excision sampling in recovering and accurately enumerating microbial populations following chilling. Excision sampling detected differences in inoculated tissue treatments that were not reflected by sponge sampling. Furthermore, the sum of the sponge sampling and excision sampling populations closely mirrors those derived by excision solely (Tables 1-4). It is possible that the 24 h period of time following inoculation may have allowed for firmer microbial attachment and penetration, thereby decreasing cell recovery during sampling and subsequently reducing the accuracy of the sponge sampling method.

Overall, steam vacuuming reduced microbial populations on inoculated beef tissue by 0.5 to 0.7 log CFU/cm² (Tables 1-4), leaving 3.3 to 5.0 log CFU/cm² on the treated tissue surfaces. While previous researchers have demonstrated reductions in total coliform counts of 1.67 log CFU/cm² (Kochevar *et al.*, 1997) and of 4.0 log CFU/cm² (Dorsa *et al.*, 1996) on inoculated beef tissue resulting from use of steam vacuum units, in both of those studies, steam vacuuming was performed on hot (non-chilled) beef tissues leaving the bacteria with less time to attach and penetrate. Results of the present study suggest that, while steam vacuuming of chilled beef tissue surfaces does reduce *Salmonella* counts, it will not—used singularly—remove enough *Salmonella* at the current inoculated population to provide an end-product of suitable microbiological quality for use in further processing; reduction of *Salmonella* populations at lower levels is unknown. Furthermore, it should be kept in mind that steam vacuum was designed to be a spot decontamination treatment and results following whole carcass application may prove to be inconsistent.

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Table 1. Least squares means (\bar{X}) and standard deviations (s) of the log values for *Salmonella* counts (CFU/cm²), and the number of samples (n⁺) in which *Salmonella* counts were too numerous to count (TNTC^a) for sponging, excision, and sponging + excision sampling methods following recovery on trypticase soy agar (TSA).

Treatment	n ^b	Method of Sampling							
		Sponging			Excision			Sponging + Excision	
		\bar{X}	s	n ⁺	\bar{X}	s	n ⁺	\bar{X}	s
Inoculated	10	4.1 ^{xy}	0.95	0	5.4 ^x	0.37	4	5.5 ^x	0.41
Inoculated + Steam	40	4.1 ^x	0.63	1	5.0 ^y	0.45	3	5.0 ^y	0.46
Uninoculated	10	3.4 ^z	0.38	1	4.7 ^y	0.06	10	4.7 ^z	0.05
Uninoculated + Steam	10	3.8 ^y	0.07	8	4.7 ^y	0.04	8	4.7 ^z	0.03

^a Inoculated sponge samples were TNTC above 5.8 log CFU/cm², while uninoculated sponge samples were TNTC above 3.8 log CFU/cm².

^a Inoculated excision samples were TNTC above c.a. 5.5 log CFU/cm², while uninoculated excision samples were TNTC above c.a. 4.5 log CFU/cm².

^b Number of samples (n) analyzed for each treatment.

^{xyz} Means in the same column with different superscript letters are different (P < 0.05).

Table 2. Least squares means (\bar{X}) and standard deviations (s) of the log values for *Salmonella* counts (CFU/cm²), and the number of samples (n⁻) taken in which *Salmonella* counts were not detected at a detection limit of 1.8, c.a. 2.5 log, and c.a. 2.7 log CFU/cm² for sponging, excision, and sponging + excision sampling methods, respectively, following recovery on xylose lysine tergitol 4 (XLT₄) agar.

Treatment	n ^a	Method of Sampling							
		Sponging			Excision			Sponging + Excision	
		\bar{X}	s	n ⁻	\bar{X}	s	n ⁻	\bar{X}	s
Inoculated	10	2.4 ^y	0.65	1	4.2 ^y	0.52	0	4.2 ^y	0.41
Inoculated + Steam	40	2.4 ^y	0.43	3	3.7 ^z	0.57	4	3.7 ^z	0.46
Uninoculated	10	1.8 ^z	0.16	9	3.5 ^z	0.59	2	3.5 ^z	0.05
Uninoculated + Steam	10	1.9 ^z	0.24	8	3.4 ^z	0.50	1	3.4 ^z	0.03

^a Number of samples (n) analyzed for each treatment.

^{yz} Means in the same column with different superscript letters are different (P < 0.05).

Table 3. Least squares means (\bar{X}) and standard deviations (s) of the log values for *Salmonella* counts (CFU/cm²), and the number of samples (n⁻) taken in which *Salmonella* counts were not detected at a detection limit of 1.8, c.a. 2.5 log, and c.a. 2.7 log CFU/cm² for sponging, excision, and sponging + excision sampling methods, respectively, following recovery on trypticase soy agar containing streptomycin at a concentration of 600 µg/ml (TSA + Strep).

Treatment	n ^a	Method of Sampling							
		Sponging			Excision			Sponging + Excision	
		\bar{X}	s	n ⁻	\bar{X}	s	n ⁻	\bar{X}	s
Inoculated	10	3.0 ^x	0.57	0	4.1 ^x	0.26	0	4.2 ^x	0.41
Inoculated + Steam	40	2.7 ^x	0.33	0	3.5 ^y	0.41	1	3.6 ^y	0.46
Uninoculated	10	1.8 ^y	0.00	10	2.7 ^z	0.06	10	2.8 ^z	0.05
Uninoculated + Steam	10	1.8 ^y	0.00	10	2.7 ^z	0.05	10	2.8 ^z	0.03

^a Number of samples (n) analyzed for each treatment.

^{xyz} Means in the same column with different superscript letters are different (P < 0.05).

Table 4. Least squares means (\bar{X}) and standard deviations (s) of the log values for *Salmonella* counts (CFU/cm²), and the number of samples (n⁻) taken in which *Salmonella* counts were not detected at a detection limit of 1.8, c.a. 2.5 log, and c.a. 2.7 log CFU/cm² for sponging, excision, and sponging + excision sampling methods, respectively, following recovery on xylose lysine tergitol 4 agar containing streptomycin at a concentration of 600 µg/ml (XLT₄ + Strep).

Treatment	n ^a	Method of Sampling							
		Sponging			Excision			Sponging + Excision	
		\bar{X}	s	n ⁻	\bar{X}	s	n ⁻	\bar{X}	s
Inoculated	10	2.6 ^x	0.65	1	3.9 ^x	0.33	0	4.0 ^x	0.34
Inoculated + Steam	40	2.3 ^x	0.34	5	3.2 ^y	0.36	6	3.3 ^y	0.34
Uninoculated	10	1.8 ^y	0.00	10	2.7 ^z	0.06	10	2.8 ^z	0.05
Uninoculated + Steam	10	1.9 ^y	0.00	10	2.7 ^z	0.05	10	2.8 ^z	0.04

^a Number of samples (n) analyzed for each treatment.

^{xyz} Means in the same column with different superscript letters are different (P < 0.05).