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Research Note

Location of Bung Bagging during Beef Slaughter Influences the Potential for Spreading Pathogen Contamination on Beef Carcasses

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ABSTRACT

Preevisceration carcass washing prior to bung bagging during beef slaughter may allow pooling of wash water in the rectal area and consequent spread of potential pathogens. The objective of this study was to compare protocols for bung bagging after preevisceration washing with an alternative method for bung bagging before preevisceration washing for the potential to spread enterohemorrhagic *Escherichia coli, E. coli* O157:H7, and *Salmonella* on carcass surfaces. The study evaluated incidence rates of pathogens in preevisceration wash water (10 ml) samples ($n = 120$) and on surface (100 cm^2) sponge samples ($n = 120$) in the immediate bung region when bagging occurred before (prewash bagging) and after (postwash bagging) preevisceration washing. Surface sampling from postwash bagging yielded incidence rates of 58.3, 5, and 8.3%, whereas wash water sampling yielded 28.3, 1.7, and 5% for enterohemorrhagic *Escherichia coli, E. coli* O157:H7, and *Salmonella*, respectively. Surface sampling from prewash bagging yielded incidence rates of 35, 1.7, and 0%, whereas wash water sampling yielded 18.3, 0, and 8.3% for enterohemorrhagic *Escherichia coli, E. coli* O157:H7, and *Salmonella*, respectively. Results of this research indicate that the rectal area is a significant source of pathogen contamination on carcasses and that wash water is an important mechanism for potential transfer of pathogen contamination from the rectal area. Results from this study suggest that bung bagging, as proposed in this study, before (prewash bagging) rather than after (postwash bagging) preevisceration washing was generally more effective in controlling pathogen contamination and potential spread from the rectal area of carcasses.

Cattle have been identified as a primary reservoir of *Escherichia coli* O157:H7 *(6)*, a widespread enteric pathogen and major cause of both sporadic cases and outbreaks of bloody diarrhea *(7)*. Although cattle have not been historically regarded as a major source of *Salmonella* infection in humans, recent studies have suggested incidence rates for *Salmonella* of 6.8 and 1.3% on beef cattle presented at slaughter and on carcasses, respectively *(3)*. Barkocy-Gallagher et al. *(2)* reported *Salmonella* present on 71% of hide and 12.7% on carcass surfaces before preevisceration wash procedures. *E. coli* O157:H7 was found on 60 to 76% of animal hides entering meat processing plants *(1, 2)* and on 26.7% of carcasses before the preevisceration wash *(2)*. Contamination of carcass material with pathogenic microorganisms like *E. coli* O157:H7 or *Salmonella* may occur during slaughter through fecal contamination originating from the rectal area. Removal of intact viscera minimizes spreading of contamination on carcasses during slaughter and is complemented by prevention of contamination from the rectum by tying it off with a plastic bag (bung bagging) typically after the preevisceration wash. McEvoy et al. *(5)* reported that common methods of bung bagging (after preevisceration wash) did not prevent spread of contamination but rather contributed to *E. coli* contamination on the carcass that remained during subsequent washing, chilling, and boning operations. An increase in total plate counts from 2.9 to 3.9 log after washing the carcass near the bung area was also observed *(6)*. The terminal rectum, i.e., rectoanal junction, is considered to be a principal site of *E. coli* O157:H7 carriage in cattle *(4, 8)*, and simple intervention strategies contribute to reducing the spread of pathogens from this location.

With this knowledge, we formed the hypothesis that the common method of bung bagging after preevisceration wash allows the physical effect of washing to remove and displace bacteria from the open and exposed rectal area, especially water pooled in this area. This may subsequently spread organisms in the immediate bung area and result in consequential contamination of carcass surfaces. The objective of this study was to compare an alternative protocol of bung bagging before preevisceration (prewash bagging) washing with common procedures of bung bagging after preevisceration washing (postwash bagging). This objective is based on the premise that if the bung is bagged before the preevisceration wash, it would recede to its natural location, thereby forming a plug that prevents water contact with the open rectum and consequently would minimize spread of pathogenic contamination from the animal's digestive tract.

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MATERIALS AND METHODS

Bung bagging and preevisceration washing. This study was conducted in a commercial slaughtering facility in the Midwest. Bung bagging was performed manually by a plant employee using gloved hands to secure a plastic bag around the exposed rectal opening with string. In the case of prewash bagging, the bagged bung was dropped inside the body cavity and allowed to recede to its natural location. Preevisceration washing of carcasses occurred in a commercial spray cabinet applying potable water in the range of 27 to 38°C.

Sponge sampling. Carcass surface sponge ($n = 120$) samples were collected around the rectal area after the preevisceration wash, with prior bung bagging (prewash bagging; $n = 60$), and after the preevisceration wash, but before bung bagging (postwash bagging; $n = 60$). Sponge sampling followed procedures described in the U.S. Department of Agriculture, Food Safety and Inspection Service Meat and Poultry Inspection regulations for *E. coli* (biotype I) carcass surface sampling *(9).* Sterile specimen sponges from sterile Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) were aseptically hydrated with 10 ml of sterile 0.1% buffered peptone water (Fisher Scientific, Houston, Tex.). Sponges were squeezed to remove excess buffer from the sponge. The moistened sponge was aseptically removed from the Whirl-Pak bags and approximately 100 cm^2 of carcass surface area was sampled. Sponging within the area consisted of 10 passes vertically (up and down was considered one pass) and 10 passes horizontally (side to side was considered one pass) with a pressure equivalent to that required to remove dried blood from the carcass surface. The sponge was carefully placed back into the Whirl-Pak bag and an additional 15 ml of buffered peptone water was added (resulting in a 25-ml total volume). Excess air was expelled from the bag before it was closed and delivered to the analytical laboratory on ice for PCR analysis.

Carcass surface wash water collection. Carcass surface wash water ($n = 120$) samples consisted of liquid pooled in the rectal area of the carcass after passing through a preevisceration wash cabinet. Sterile pipettes and disposable sample tubes (Fisher Scientific, Pittsburgh, Pa.) were used to aseptically collect approximately 5- to 10-ml liquid samples in sterile 15-ml Falcon tubes (Corning Inc., N.Y.). Sampling was performed near the rectal area immediately after the preevisceration wash, with prior bung bagging (prewash bagging; $n = 60$), and after the preevisceration wash, but before bung bagging (postwash bagging; $n = 60$). Sampling of postwash bagging did not evaluate the effect of bung tying because the inherent nature of the operation would have allowed the pooled water to enter the body cavity, thereby creating a condition that would not accurately represent the actual pathogen profile in the pooled water at this stage of the operation. The relative contribution of tying off the bung before versus after the wash on pathogen profiles was not compared; rather the effect of bung bagging location on pathogen profiles in the pooled water and rectal carcass surface was compared. Samples were transported to the analytical laboratory on ice for PCR analysis.

Enrichment of sample sponges for PCR analysis. Sponge samples were enriched for PCR analysis by adding an equal volume (25 ml) of prewarmed (42°C) 2× Trypticase soy broth (TSB; Becton, Dickinson and Company, Cockeysville, Md.) to each Whirl-Pak bag (Nasco). All samples were pummeled in an IUL Masticator (IUL, S.A., Barcelona, Spain) for 60 s. The TSB enrichment was incubated for 12 h at 37°C with agitation.

Enrichment of carcass surface wash water for PCR analysis. Liquid samples (pre- and postwash bagging) were aseptically transferred from the 15-ml transport containers (Corning) to sterile 50-ml centrifuge tubes (VWR Scientific, West Chester, Pa.) with a Drummond Pipet-Aid (Fisher Scientific) and sterile single-use 25-ml serological pipets (Fisher Scientific). To this initial sample volume, an equal amount (5 to 10 ml by weight) of prewarmed (42°C) 2× TSB (Becton, Dickinson and Company) was added, and the sample tube was agitated to thoroughly mix the solution. Carcass wash water enrichment tubes were incubated for 12 h at 37°C with agitation.

Multiplex PCR screening for pathogen incidence. Following incubation, 1 μl of each TSB enrichment was transferred into two separate reaction tubes containing 50 μl of lysis reagent for enterohemorrhagic *Escherichia coli* (EHEC) and *E. coli* O157:H7 (EC4 buffer, Molecular Epidemiology, Inc., Seattle, Wash.) and *Salmonella* (S3 buffer, Molecular Epidemiology, Inc.) analysis. Negative controls were performed by substituting 1μl of deionized water for sample enrichment in lysis reagent for EHEC, *E. coli* O157:H7, and *Salmonella* analysis. Positive controls consisted of 1 μl of control *E. coli* O157:H7 and *Salmonella* DNA to ensure that appropriate conditions and visualization procedures were performed. All lysis reactions were incubated for 20 min at 37°C, followed by 10 min at 95°C, according to manufacturer protocols (Technical Specifications for EC4 and S3; Molecular Epidemiology, Inc.).

Reaction tubes were immediately placed at 4°C following the lysis step until commercially available multiplex PCR assays for EHEC and *E. coli* O157:H7 (EC4, Molecular Epidemiology, Inc.) and *Salmonella* (S3, Molecular Epidemiology, Inc.) were used to test for pathogen incidence (sensitivity >99% and specificity >99% for both assays; from data provided by American Proficiency Institute, Traverse City, Mich.). The EC4 PCR assay for *E. coli* O157:H7 amplifies four specific genomic regions of the following sizes: 180, 255, 384, and 985 bp (Technical Specifications for EC4, Molecular Epidemiology, Inc.). The S3 PCR assay for *Salmonella* amplifies three specific genomic regions of the following sizes: 218, 382, and 803 bp (Technical Specifications for S3, Molecular Epidemiology, Inc.). Amplification was performed by cycling tubes in an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany) for 33 cycles of a 95°C, 10-s denaturation followed by annealing temperature-time combination of 65°C for 30 s or 72°C for 20 s. Amplicons were resolved by 2% low electroendosmodis (EEO) agarose (Fisher Scientific) gel electrophoresis (Bio-Rad Laboratories sub-cell model 192, Hercules, Calif.) at 240 V for 35 min and stained with ethidium bromide (Fisher Scientific). Banding patterns were visualized with an Epi Chemi II Darkroom UV transilluminator (UVP, Inc., Upland, Calif.).

Confirmation of PCR pathogen-positive signals. Samples yielding presumptive positive PCR results for *E. coli* O157:H7 were streaked onto sorbitol MacConkey agar (Becton, Dickinson and Company) with cefixime-tellurite (Dynal Inc., Lake Success, N.Y.) and incubated for 18 to 24 h at 37°C. Following incubation, suspect colonies were picked from sorbitol MacConkey agar with cefixime-tellurite, streaked onto tryptic soy agar (TSA) with 5% sheep blood (Hardy Diagnostics, Santa Maria, Calif.), and incubated for 18 to 24 h at 37°C. Colonies on 5% sheep blood agar were picked and tested for the presence of O157 antigen with the Oxoid latex agglutination kit (Oxoid Ltd., Basingstoke, UK) following the manufacturer's guidelines. Samples generating positive latex agglutination were considered *E. coli* O157:H7–positive results.

TABLE 1. *Incidence of pathogens on carcass surfaces and in wash water on carcass surfaces as a result of bung bagging before (prewash bagging) or after (postwash bagging) preevisceration carcass washing*[a]

Sample type ($n = 120$)	Pathogen	No. (%) of positives by bung bagging location ($n = 60$)	
		Prewash bagging	Postwash bagging
Carcass surface	EHEC	21 (35) B	35 (58.3) A
	E. coli O157:H7	1 (1.7) A	3 (5) A
	Salmonella	0 (0) B	5 (8.3) A
Carcass surface wash water	EHEC	11 (18.3) B	17 (28.3) A
	E. coli O157:H7	0 (0) A	1 (1.7) A
	Salmonella	5 (8.3) A	3 (5) A

[a] Values within a row with different letters are statistically ($P < 0.05$) different. EHEC, enterohemorrhagic *E. coli.*

Samples yielding presumptive positive PCR results for *Salmonella* were streaked onto xylose lysine tergitol 4 agar (Becton, Dickinson and Company) and incubated for 24 h at 37°C. Following incubation, suspect black colonies were picked and streaked onto TSA agar with 5% sheep blood (Hardy Diagnostics) and incubated for 18 to 24 h at 37°C. Colonies were then picked and tested with the S3 PCR assay for *Salmonella* (Molecular Epidemiology, Inc.) according to the same PCR protocols outlined above. Samples producing positive banding patterns following secondary screening with the S3 PCR assay for *Salmonella* were considered positive for *Salmonella*.

Statistical analysis. Two sample *t* tests were performed to determine differences in pathogen incidence between bung bagging procedures (pre- versus postwash bagging) with Minitab Statistical Software (Minitab Inc., State College, Pa). All differences were reported at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The incidence rates of EHEC, *E. coli* O157:H7, and *Salmonella* on carcass surfaces and in carcass surface wash water in response to common postwash and the alternative prewash bagging procedures are provided in Table 1.

Incidence rates for EHEC (35%) on carcass surfaces when the bung was bagged before preevisceration washing (prewash bagging) were lower than those on carcass surfaces when the bung was bagged after preevisceration washing (postwash bagging) (58.3%). Incidence rates for *E. coli* O157:H7 (1.7%) on carcass surfaces when the bung was bagged before preevisceration washing (prewash bagging) were significantly ($P < 0.05$) lower than those on carcass surfaces when the bung was bagged after preevisceration washing (postwash bagging) (5%). Incidence rates for *Salmonella* (0%) on carcass surfaces when the bung was bagged before preevisceration washing (prewash bagging) were significantly ($P < 0.05$) lower than those on carcass surfaces when the bung was bagged after preevisceration washing (postwash bagging) (8.3%).

Incidence rates for EHEC (18.3%) in carcass surface wash water when the bung was bagged before preevisceration washing (prewash bagging) were significantly ($P < 0.05$) lower than those in carcass surface wash water when the bung was bagged after preevisceration washing (postwash bagging) (28.3%). Incidence rates for *E. coli* O157:H7 (0%) on carcass surface wash water when the bung was bagged before preevisceration washing (prewash bagging) were lower than those in carcass surface wash water when the bung was bagged after preevisceration washing (postwash bagging) (1.7%). Incidence rates for *Salmonella* (8.3%) on carcass surfaces when the bung was bagged before preevisceration washing (prewash bagging) were slightly higher than those on carcass surfaces when the bung was bagged after preevisceration washing (postwash bagging) (5%).

Incidence rates of *E. coli* O157:H7 and *Salmonella* in carcass surface wash water from prewash bagging were not significantly ($P \geq 0.05$) different from postwash bagging. Overall, mean incident rates of EHEC, *E. coli* O157:H7, and *Salmonella* on carcass surfaces (mean of incidence rates from both pre- and postwash bagging) were 46.7, 3.3, and 4.2%, respectively (data not shown).

The rationale for investigating an alternative location for bung bagging was that contamination of wash water through contact with the rectal area and associated fecal material during the preevisceration wash would be reduced and that, in turn, the possibility of retained wash water spreading contamination to the exposed carcass would be reduced. The alternative procedure of bagging prior to the preevisceration wash (prewash bagging) acts to form a plug that when receded to its natural location covers the rectal opening and prevents spread of fecal material. Data from sponge sampling of carcass surfaces indicated that the alternative procedure of prewash bagging results in lower EHEC, *E. coli* O157:H7, and *Salmonella* incidence on carcass surfaces. Although the incidence of *E. coli* O157:H7 and *Salmonella* in carcass surface wash water from carcasses where bagging was performed prior to the preevisceration wash (prewash bagging) as compared to that where bagging occurred after preevisceration washing (postwash bagging) was not significantly different, the trend of lower incidence, especially for EHEC and *E. coli* O157:H7, was evident. The inconsistency regarding a slightly higher incidence rate of *Salmonella* in wash water of carcasses from prewash bagging was likely a result of chance detection for this pathogen in a natural substrate with nonhomogenous distribution. Pathogen incidence levels are highly variable in a natural system such as beef carcasses, and reduction in incidence due to perceived improvements in practice may not always be observed. Considering that this is true for observations of both decreases and increases in incidence,

it is more important to consider trends in changes of incidence. The trends in this study indicate that overall in wash water and on carcass surfaces, the practice of bung bagging before preevisceration washing leads to reduced incidence of pathogens as compared with postevisceration bung bagging.

Results highlight the relatively high mean incidence of EHEC (46.7%) contamination on carcass surfaces compared with that of *E. coli* O157:H7 (3.3%) and *Salmonella* (4.2%) on carcasses at the beginning of processing, which, during transit through the process, may influence EHEC incidence on and in retail meat. These data are important, for they provide evidence of the relative high frequency of EHEC contamination on beef carcasses and the need to assess the incidence and risk of this pathogenic group, the importance of which is often overshadowed by surveillance for *E. coli* O157:H7 in raw nonintact beef.

Current regulations do not permit bung bagging and dropping inside the body cavity before preevisceration washing unless the potential for contamination from the intestinal tract can be controlled. Results of this research indicate that the rectal area is a significant source of pathogen contamination on carcasses and that wash water is an important mechanism for potential transfer of pathogen contamination from the rectal area. There is merit for further research investigating the effect of prewash bagging versus postwash bagging on pathogen incidence in the carcass cavity. It should, however, be noted that by bagging the bung prior to the wash, the pooled water in the cavity will more likely have less incidence of pathogen contamination (as hypothesized and evidenced in this research) because bagging at this point eliminates the removal of bacteria from the open and exposed rectum by the physical effect of washing. In either case (prewash bagging versus postwash bagging), bung-bagging procedures will allow pooling of water and transfer of this water to the carcass cavity with the process of dropping the bung into the cavity. In the case of prewash bagging, the incidence in the pooled water is reduced and, thus, the likelihood of reducing contamination carriage consequently to the body cavity is also reduced. Results from this study provide evidence that bung bagging of the rectal opening by forming a plug prior to preevisceration washing is an effective means to control pathogen contamination and potential to spread from the rectal area of the carcass.

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