# Evaluation of Methods for Sampling Rectal/Colonal Feces, Hides and Carcasses to Test for Presence of *Escherichia coli* O157:H7 and *Salmonella* spp.

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**SUMMARY**

The study was designed to compare sampling methods to test for the presence of *Escherichia coli* O157:H7 and *Salmonella* spp. in feces, on cattle hides and on the surface of carcasses. Of 30 lots tested, 36.6%, 13.3% and 0.0% were positive for presence of *E. coli* O157:H7 on hide, fecal and carcass samples, respectively, while corresponding percentages for *Salmonella* spp. positive samples were, 70.0%, 16.6% and 6.6% respectively. No differences (P>0.05) were observed between sampling methods when testing for *E. coli* O157:H7. However, when sampling for *Salmonella* spp., the hide rinsing method was most effective (P<0.05), as it recovered positive samples in 63.3% of hides.

**Key Words:** *Escherichia coli* O157:H7, *Salmonella* spp., sampling methods, fecal, hide, carcasses

## INTRODUCTION

The pattern of disease for foodborne illness in human populations is in continual evolution. As a result, emerging foodborne pathogens are posing new risk management problems, requiring the development of appropriate control strategies. The U.S Centers for Disease Control and Prevention (CDC) has estimated that foodborne pathogens are responsible for 76 million illnesses in the United States each year, resulting in 5,000 deaths (Mead et *al*., 1999). They are sustained by complex epidemiological factors, including the shift to highly intensive livestock production systems in combination with high-volume and high-speed processing facilities (Morales, 1998). Beef and dairy cattle are perceived to be the major reservoirs of *Escherichia coli* O157:H7. Animals harboring foodborne pathogens in their gastrointestinal tracts frequently appear non-symptomatic, but shed the pathogen in the environment (Gansheroff and O'Brien, 2000). The time required to test for these pathogens is not available during regular operation in high-volume facilities. Conventional inspection methods do not detect these pathogens. Prior to 2000, the prevalence of *E. coli* O157:H7 in the feces of dairy and beef cattle was believed to be less than 10%. Many estimates were lower than 2% (Gansheroff and O'Brien, 2000). Carcass contamination likely occurs during skinning when the hide is permitted to roll over so the contaminated hide surface contacts the exterior carcass surface (Gill et *al*., 1999).

With the development of more sensitive culture techniques to detect these *E. coli* O157:H7, the incidence has been found to be much higher than what was previously reported. Elder et *al*. (2000) sampled animals of 29 lots and found that 72% of lots sampled contained at least one EHEC O157-positive fecal sample and 38% of lots had positive hide samples.

For this study, we have used the most sensitive and economically feasible culture techniques available to identify the most practical methods for sampling feces, hides and carcasses.

## MATERIALS AND METHODS

***Experimental design.*** Sampling was conducted on at least six lots of cattle in each plant. Following sample collection at each plant, all samples for *E. coli* O157:H7 testing were placed in coolers without refrigeration and samples for the detection of *Salmonella* were refrigerated; all samples collected at each plant were shipped overnight via air express to a private laboratory (Food Safety Net Services, San Antonio, TX) for analysis.

***Fecal Sampling.*** In this study, a total of 120 samples were collected from animals post-exsanguination, but before hide opening. Of the 120 fecal samples collected, half were obtained by palpating the rectum and collecting 10g of feces using a clean, plastic palpation glove. The samples (n = 30) that were to be evaluated for presence of *E. coli* O157:H7, were suspended in 90 ml of GN broth according to the procedures of Elder et *al*. (2000). An additional 10 g sample (n = 30) from an animal in the same lot was collected for analysis of presence/absence of *Salmonella* spp. The sample was placed in a Whirl Pak® (Int'l Bioproducts, Bothell, WA) bag and stored under refrigeration before shipment.

The same individual animals that were rectally palpated were followed through the slaughter process. When each animal was eviscerated, its colon (approximately 750 cm proximal to the rectum) was removed and a fecal sample was obtained for microbial analysis. Each colon was laterally excised to expose the interior surface. Using a template, a 100 $cm^2$ area was swabbed using a pre-hydrated sponge kit.

***Hide Sampling.*** Samples (n = 300) were obtained from cattle hides using five sampling methods. The first method was the three-site sponging method. Using a template and a sterile sponge kit, researchers swabbed a 100 $cm^2$ area at each of the three sites (brisket, flank and rump) that are approved for carcass sampling by FSIS-USDA. The second sampling method involved the excision of pieces of hide. Using a sterile scalpel, approximately 100 $cm^2$ of hide surface area was removed from the left side of the animal along the midline and anterior to the navel. The excised sample was placed in a Whirl Pak® bag. The third hide sampling method was the gauze sampling method. A sterilized 4 X 4 inch piece of cotton gauze was hydrated in 25 ml of Butterfield's phosphate diluent. The excess diluent was pressed out of the gauze before swabbing the right side of the midline, anterior to the navel. The fourth hide sampling method was hair clipping. A 100 $cm^2$ area of the exterior of the hide was shaved with cordless clippers, directly anterior to the cotton gauze sampling site. The clipped hair was collected using a plastic Whirl Pak® bag as it fell from the hide. It should be noted that each animal (n = 60) was sampled at all of the four previously mentioned sampling areas. The final hide sampling procedure evaluated was hide washing. To prevent sampling of the same area twice, an additional animal was selected out of the same lot as the animals tested above. After stunning and prior to exsanguination, the brisket area was sprayed with double deionized water, using a livestock sprayer. A plastic bag was held under the brisket area to catch resulting effluent as it dripped from the carcass. After effluent collection, the sample was divided (half for *E. coli* O157:H7 analysis and half for the analysis of *Salmonella* spp.). After samples were collected from hides using all five sampling techniques, 20 ml of Brilliant Green Bile 2% (60g/liter, Difco Laboratories) was added to each sample bag before shipment to the laboratory for *E. coli* O157:H7 analysis.

The 4-point "mud score" system developed by Sofos et *al*. (1999) was applied to all of the cattle that were sampled during conduction of the hide sampling portion of this study. Due to the environmental conditions during the time that this study was conducted, all mud scores were "1" (no mud on the animals).

***Carcass Sampling.*** Carcasses (n = 240) were sampled by randomly selecting 6 adjacent sides (3 carcasses) for microbial analysis by four sampling methods. Collection of the four microbiological samples collected using the different methods from the six adjacent sides occurred before the final plant microbiological intervention system. Each plant used different intervention strategies throughout the slaughter process, which simulated current diversity within the industry. The first carcass surface sampling method evaluated was sponge sampling. Using a carcass sponge kit, 100 $cm^2$ areas were swabbed at the three carcass sites (brisket, flank and rump) as mandated by USDA for conducting generic *E. coli* testing as part of the 1996 Pathogen Reduction; HACCP Systems Final Rule. Sampling was performed aseptically using sterile, latex gloves, which, in addition to the template, were changed between samples. The same side of the sponge was used to first swab both the brisket and flank, and the opposite side of the sponge was used to swab the rump (last to be swabbed). The second carcass surface sampling method valuated was tissue excision. Using a sterile scalpel, a 100 $cm^2$ area of tissue was excised at each of the same three carcass sites as were tested using the sponge technique (brisket, flank and rump), and then placed in a Whirl Pak bag. The third carcass surface sampling technique involved swabbing the pattern-mark (hock, hindshank, inside round, cod/udder, flank midline, navel midline and foreshank) of the carcass. A sterile carcass sponge kit was used to swab all of the circumscribed area. The final carcass surface sampling technique was the thorax sponge. Using a sterile carcass sponge kit, the indeterminate area of the thorax (chest cavity) and throat area were swabbed.

## RESULTS AND DISCUSSION

***Overall Prevalence of Pathogens.*** Of the total 150 hide samples collected for detection of *E. coli* O157:H7, 27 (18.0%) were positive (Tables 1 and 2). When characterized by lots, 11 of the 30 (36.8%) lots sampled contained at least one hide that was positive for *E. coli* O157:H7. Of the total fecal samples, 6.7% were positive for *E. coli* O157:H7. Of 30 lots sampled, 4 (13.3%) had at lease one fecal sample that was positive for *E. coli* O157:H7. No carcass samples were positive for *E. coli* O157:H7 using any sampling procedure.

The prevalence of *Salmonella* spp was much higher than *E. coli* O157:H7 in all sampling units (Tables 2 and 3). Seventy percent (21/30) of lots had at least one hide that was positive for *Salmonella* spp. Of all hide samples collected, 68 of 150 samples (45.3%) tested positive for *Salmonella* spp. In addition, 8.3% (5/60) of all fecal samples were positive for *Salmonella* spp. When characterized by lots, 16.7% (5/30) of all lots had at least one fecal sample and 6.7% of lots had at least one carcass sample that was positive for *Salmonella* spp.

***Prevalence of Pathogens on the Hides.*** This study confirms previous reports that animals carry pathogens onto the slaughter floor via hide contamination (Tables 1-3). All hide sampling techniques recovered both *E. coli* O157:H7 and *Salmonella* spp. For *E. coli* O157:H7, the three-site sponge and hair clipping sampling methods appeared to be the most effective, both detecting the organism on 7 of 30 hides; but, results did not differ significantly ($P$>0.05) among all sampling techniques. When a large population of cattle is tested, the three-site sponge sampling technique would likely be the most proficient in detecting the prevalence of *E. coli* O157:H7 on the hide.

For detection of *Salmonella* spp., the hide rinse sampling method recovered the highest numbers of positive samples (19 of 30 or 63% of hides sampled). This technique was more effective ($P$<0.05) in the recovery of *Salmonella* spp. than hide excision of hair clippings, but there was no difference between the hide rinsing method and the sponge or gauze sampling techniques in detecting the pathogen. Unfortunately, random limb movement can create a human safety hazard when the unconscious cattle kick while the diluent is being collected from the hide. The second most effective hide sampling technique for detecting *Salmonella* spp. was the three-site sponge method. This method detected *Salmonella* spp. on 14 of 30 (47%) of the hides sampled; however, it was not statistically more effective than the other sampling methods used. Again, the trends suggested that with a greater number of observations, the three-site sponge sampling method would prove to be the safest and most effective way to detect *Salmonella* spp. on cattle hides.

***Prevalence of Pathogens in Feces.*** Using the same methods as were used by Elder et *al*. (2000), *E. coli* O157:H7 was isolated from at least one animal in 13% of the lots sampled in the present study, versus the 70% of lots that contained at least one positive sample in the Elder et *al*. (2000) study. Elder et *al*. (2000) collected samples during July and August, whereas this study was conducted in October and November. This seasonal difference could have affected the amount of fecal shedding of *E. coli* O157:H7 (Chapman et *al*. 1997, Sargeant et *al*. 2000). In addition, samples from the present study had to be strained using sterile gauze because the fecal slurry was too thick for immunomagnetic bead isolation. The pathogens may have been attached to the fibrous material that was not sampled. Neither sampling method was statistically more effective than the other (Tables 1 and 3) at detecting either *E. coli* O157:H7 or *Salmonella* spp. in feces samples.

***Prevalence of Pathogens on the Carcasses.*** Although the prevalence and incidence of *E. coli* O157:H7 were moderately high in the feces and on the hides of the cattle in the present study, *E. coli* O157:H7 was not detected on any of the carcasses. In addition, only 1.6% of all carcass samples tested positive for *Salmonella* spp.; this was a very low incidence considering that such a high percentage of lots and individual animals had this pathogen in their feces and/or on their hides (Tables 2 and 3).

## CONCLUSIONS

Incidence and prevalence of pathogens on the surface of cattle when they entered the slaughter floor was relatively high, and in some cases, dangerously high. Animal cleaning systems, to minimize contamination on the hide of cattle before slaughter, would help to minimize the microbial risk that live cattle present. Two of the plants from which samples were collected in this study "sprayed" water onto the cattle in the holding pens, before stunning, primarily to wash off any debris that potentially was present on the hide and, secondly, to prevent the hide from producing a "dust cloud" when mechanically removed from the carcass. It should be noted that Byrne et al. (2000) reported no reduction in *E. coli* O157:H7 levels when cattle were washed with a power hose for 1 min before stunning. One method of minimizing the presence of debris and the need for carcass trimming after hide removal is dehairing post-exsanguination as described by Castillo et *al*. (1998).

A second important conclusion from this study was that the three-site sponge sampling method appeared to be an adequate technique for sampling hides. Although sponge sampling did not result in incidence or prevalence levels statistically different ($P > 0.05$) from other sampling methods, results suggested that sponge sampling was, at a minimum, as effective as was any other sampling method. In addition, sponge sampling is presently practiced as the method of collecting microbiological samples from carcasses. Also, it is easily performed at line speed and offers no danger to the lab technician collecting samples.

Results from this study indicated that the laboratory techniques used for the detection and isolation of *E. coli* O157:H7 from feces require more research. Following the same basic procedures as described by Elder et *al*. (2000), researchers were unsuccessful in efforts to consistently recover (when present) *E. coli* O157:H7 from fecal samples. Finally, if detection of *E. coli* O157:H7 is to be mandatory at the carcass level, a more economically reasonable analytical protocol for testing feces and hides must be identified.

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**Table 1. Summary of Fecal, Carcass and Hide Sampling Data for *E. coli* O157:H7 by Plant**

| | Fecal[1] | | Carcass[2] | | | | Hide[3] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant | Palpation | Colon Swab | Sponge | Excision | Pattern Mark | Thorax | Sponge | Excision | Gauze | Hair Clip | Rinse |
| 1 | 1/6 (16.6%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 2/6 (33.3%) | 0/6 (0.0%) | 0/6 (0.0%) | 1/6 (16.6%) | 0/6 (0.0%) |
| 2 | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 1/6 (16.6%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) |
| 3 | 0/6 (0.0%) | 2/6 (33.3%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 5/6 (83.3%) | 3/6 (50.0%) | 3/6 (50.0%) | 5/6 (83.3%) | 5/6 (83.3%) |
| 4 | 0/6 (0.0%) | 1/6 (16.6%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 1/6 (16.6%) | 1/6 (16.6%) | 0/6 (0.0%) |
| 5 | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) |
| **Total** | **1/30 (3.3%)** [a] | **3/30 (10.0%)** [a] | **0/30 (0.0%)** [a] | **0/30 (0.0%)** [a] | **0/30 (0.0%)** [a] | **0/30 (0.0%)** [a] | **7/30 (23.3%)** [a] | **4/30 (13.3%)** [a] | **4/30 (13.3%)** [a] | **7/30 (23.3%)** [a] | **5/30 (16.6% )** [a] |

[1, 2, 3] Sampling units

[a, b] Means within each sampling unit bearing a common superscript letter are not different ($P > 0.05$). Differences among sampling units were not tested for statistical differences.

**Table 2. Summary of Percent Positive Samples and Percent Positive Lots**

| **Prevalence** | **Percent Positive Samples** | | |
|---|---|---|---|
| | **Fecal** | **Carcass** | **Hide** |
| Prevalence in all samples | | | |
| *E. coli* O157:H7 | 4/60 (6.6%) | 0/120 (0.0%) | 27/150 (18.0%) |
| *Salmonella* spp | 5/60 (8.3%) | 2/120 (1.6%) | 68/150 (45.3%) |
| Prevalence in lots | | | |
| *E. coli* O157:H7 | 4/30 (13.3%) | 0/30 (0.0%) | 11/30 (36.6%) |
| *Salmonella* spp | 5/30 (16.6%) | 2/30 (6.6%) | 21/30 (70.0%) |

**Table 3. Summary of Fecal, Carcass and Hide Sampling Data for *Salmonella* spp. by Plant**

| | Fecal[1] | | Carcass[2] | | | | Hide[3] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant | Palpation | Colon Swab | Sponge | Excision | Pattern Mark | Thorax | Sponge | Excision | Gauze | Hair Clip | Rinse |
| 1 | 1/6 (16.6%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 2/6 (33.3%) | 2/6 (33.3%) | 1/6 (16.6%) | 2/6 (33.3%) | 4/6 (66.6%) |
| 2 | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 1/6 (16.6%) |
| 3 | 1/6 (16.6%) | 1/6 (16.6%) | 0/6 (0.0%) | 1/6 (16.6%) | 0/6 (0.0%) | 0/6 (0.0%) | 5/6 (83.3%) | 3/6 (50.0%) | 5/6 (83.3%) | 3/6 (50.0%) | 6/6 (100.0%) |
| 4 | 1/6 (16.6%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 1/6 (16.6%) | 1/6 (16.6%) | 1/6 (16.6%) | 1/6 (16.6%) | 2/6 (33.3%) |
| 5 | 0/6 (0.0%) | 1/6 (16.6%) | 1/6 (16.6%) | 0/6 (0.0%) | 0/6 (0.0%) | 0/6 (0.0%) | 6/6 (100.0%) | 5/6 (83.3%) | 6/6 (100.0%) | 5/6 (83.3%) | 6/6 (100.0%) |
| **Total** | **3/30 (10.0%)**[a] | **2/30 (6.6%)**[a] | **1/30 (3.3%)**[a] | **1/30 (3.3%)**[a] | **0/30 (0.0%)**[a] | **0/30 (0.0%)**[a] | **14/30 (46.6%)**[ab] | **11/30 (36.6%)**[b] | **13/30 (43.3%)**[ab] | **11/30 (36.6%)**[b] | **19/30 (63.3%)**[a] |

[1, 2, 3] Sampling units

[a, b] Means within each sampling unit bearing a common superscript letter are not different ($P > 0.05$). Differences among sampling units were not tested for statistical differences.