Determination of the incidence of *Escherichia coli* O157:H7 and *Salmonella* contamination on hides and carcasses at cow/bull packing plants

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**U.S. Department of Agriculture, Agricultural Research Service**

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**Background**
Outbreaks of foodborne illness linked to consumption of hamburger contaminated with *E. coli* O157:H7 have had a devastating effect on profitability of the beef industry. We have established that the prevalence of *E. coli* O157:H7 on the hides of fed cattle is high, that often pre-evisceration carcasses become contaminated with *E. coli* O157:H7, and that microbial interventions result in most (97%) fed-beef carcasses being negative for *E. coli* O157:H7. Yet little is known about the prevalence of *E. coli* O157:H7 on the hides and carcasses of cull cows and bulls. Thus, the experiments described here establish the prevalence of *E. coli* O157:H7 and *Salmonella* on the hides and carcasses of cull cows and bulls and evaluate the relationship between the prevalence of *E. coli* O157:H7 and other measures of microbial contamination including the prevalence of *Salmonella* and levels of other generic bacteria.

The stated objectives for this work were:

1) Determine the prevalence of *E. coli* O157:H7 and *Salmonella* and the level of generic microbial contamination on the hides of slaughter cows and bulls.

2) Determine the prevalence of *E. coli* O157:H7 and *Salmonella* and the level of generic microbial contamination on cow/bull carcasses before pre-evisceration microbial interventions.

3) Determine the prevalence of *E. coli* O157:H7 and *Salmonella* and the level of generic microbial contamination on cow/bull carcasses after all microbial interventions.

4) Determine the relationship between the prevalence of *E. coli* O157:H7 and the prevalence of *Salmonella* and measures of the level of generic microorganisms for cow/bull hides and carcasses.

**Methodology**
Hide, pre-evisceration carcass, and post-wash carcass sponge samples were obtained from 5 cow/bull slaughter plants. For each plant, sampling occurred on three days with 65 samples of each type obtained on each day for a total of 195 samples of each type per plant and a grand total of 975 samples of each type (Table 1). Samples were transported (2°C) to MARC and analyzed for *Enterobacteriaceae* counts, aerobic plate counts, and prevalence of *E. coli* O157:H7 and *Salmonella*. Most probable number of *E. coli* O157:H7 were determined for all positive pre-evisceration carcass and post-intervention carcass samples.

**In-plant sampling locations.** Sampling with wetted sponges was done at three locations on the processing line: (1) hide, sampled after hide opening before hide removal; (2) pre-evisceration, immediately following dehiding before any antimicrobial applications; (3) post-intervention, in the chill cooler after all antimicrobial interventions. Individual animals/carcasses were tagged and tracked throughout the process. The same carcass was sampled at (1) hide, (2) pre-evisceration, and (3) post-intervention processing points.

**Sample collection.** All samples were obtained following the previously described method (Arthur et al. 2004).

**Sample processing.** Sponge bags were massaged thoroughly and aliquots of 2.5 ml and 5 ml (12.5% of the diluent volume) were removed from the hide, post-intervention, and chilled samples and the pre-evisceration and post-evisceration samples, respectively, prior to the addition of
enrichment media. The sample aliquots were used for enumeration of total aerobic bacteria, *Enterobacteriaceae*, and *E. coli* O157.

**E. coli O157 detection.** Eighty milliliters of tryptic soy broth (TSB) were added to the hide, post-intervention, and chilled sample bags and 160 ml of TSB were added to the pre-evisceration and post-evisceration sample bags, each of which contained two sponges. All sample bags were incubated, subjected to immuno-magnetic separation and plated as previously described (Arthur et al. 2004). After the plates were incubated, up to three suspect colonies were picked and tested by latex agglutination (DrySpot *E. coli* O157; Oxoid, Basingstoke, England). Data from Barkocy-Gallagher et al. (2003) showed that > 90% of samples that were presumptively positive for *E. coli* O157 based on the above methods were confirmed positive for *E. coli* O157:H7. Therefore, for the purposes of this study, any sample that produced *E. coli* O157-characteristic colonies that gave positive reactions for the O157-latex agglutination assay was considered positive for *E. coli* O157:H7.

**Enumeration.** Total aerobic counts (APC) and *Enterobacteriaceae* counts (EBC) were processed on a Bactometer (BioMerieux, Hazelwood, Mo.) or, for those samples with too few organisms to count on the Bactometer, Petrifilm Aerobic Count Plates or *Enterobacteriaceae* Count Plates (3M Microbiology, St. Paul, Minn.) were used. Bacterial counts from Petrifilm were used to generate standard curve data for the Bactometer during calibration to facilitate agreement between the two systems.

Enumeration of *E. coli* O157 was done using a 3-tube most probable number (MPN) method. Triplicate 10-fold dilution series were made by transferring 100 µl of the sample aliquot into 900 µl of TSB in deep well microtrays. The microtrays were incubated at 25°C for 2 h, then at 42°C for 10 h. After incubation the trays were kept at 4°C until the results from the *E. coli* O157 prevalence analysis were completed. For any sample that was positive for *E. coli* O157 in the prevalence analysis, samples from the corresponding BPW MPN dilutions were processed using the Bax lysis reagents (Qualicon). The culture lysate was used for PCR detection of sil DNA, representative of *E. coli* O157:H7. The combination of positive dilution tubes was used to obtain the MPN/ml by using the 3-tube MPN table from the Bacteriological Analytical Manual (http://vm.cfsan.fda.gov/~ebam/bam-a2.html).

**Findings**

**E. coli O157:H7.** The data presented here show that the hides of animals slaughtered at cow/bull processing plants carry *E. coli* O157:H7 at prevalences similar to that of fed cattle. In this study 57% of all cattle hides sampled carried O157:H7 and plant prevalence ranged from 39% at Plant 4 to 80% at Plant 2 (Table 1). From previous studies using similar detection and isolation procedures, *E. coli* O157:H7 prevalence on fed cattle hides ranged from 46.5% to 81.8% and 67.2% to 73.8% during non-winter months (Barkocy-Gallagher et al. 2003, Rivera-Betancourt et al. 2004). Defects in dressing practices can lead to transfer of bacterial pathogen from the hide to the carcass. Sampling of the carcass immediately after hide opening is done to determine the frequency of bacterial transfer from hide to carcass. Again prevalence rates for *E. coli* O157:H7 on pre-evisceration carcasses at cow/bull plants (31.5%; range 15.9% to 48.2%) were similar to those from plants that processed fed beef cattle (range 3.1% to 40.8%; Barkocy-Gallagher et al. 2003, Rivera-Betancourt et al. 2004). *E. coli* O157:H7 prevalence for fed cattle carcasses post-intervention has been reported to be very low (range 0% to 3.1%). There were no post-intervention carcasses found to harbor *E. coli* O157:H7 in this study.

**Salmonella.** Similarly, the prevalence of *Salmonella* on the hides of animals presented for slaughter at cow/bull processing plants (64.8%; range 38.5% to 90.3%; Table 2) was comparable to that reported for fed cattle at slaughter (range 26.7% to 99%; Rivera-Betancourt et al. 2004,
Barkocy-Gallagher et al. 2003). This study found pre-evisceration carcasses at cow/bull plants to carry *Salmonella* at a higher rate (40.5% vs. 26.8%) than pre-evisceration carcasses at fed cattle plants (Barkocy-Gallagher et al. 2003, Rivera-Betancourt et al. 2004). These numbers were reduced by the in-plant antimicrobial interventions to less than one percent.

*Enumeration of E. coli O157:H7.* MPN analysis of the pre-evisceration carcass samples is on-going. Results from these experiments will be forwarded as soon as they have been completed.

**Implications**

When the APC and EBC levels were grouped into classes a positive relationship was identified between the APC or EBC class and the prevalence of *E. coli* O157 and *Salmonella* on hides and pre-evisceration carcasses (Table 4 and 5). The samples from higher classes of APC and EBC were more likely to be positive for *E. coli* O157 and *Salmonella*. A similar relationship between *E. coli* O157:H7 and both APC and EBC for fed cattle carcasses has been reported previously (Arthur et al., 2004). While indicator organism levels cannot be used for direct presence or absence analysis of pathogens, they may be useful as a guideline for the minimization of pathogen contamination. By modifying intervention schemes to maintain APC and EBC levels below maximum target values, processors are likely to reduce the prevalence of microbial pathogens on carcasses.

**Table 1.**

<table>
<thead>
<tr>
<th>Average of Percent E. coli O157:H7</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plant 3</th>
<th>Plant 4</th>
<th>Plant 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hide</td>
<td>69.7</td>
<td>80.0</td>
<td>47.2</td>
<td>39.0</td>
<td>58.5</td>
<td>57.3</td>
</tr>
<tr>
<td>Pre-evis</td>
<td>15.9</td>
<td>44.6</td>
<td>25.1</td>
<td>48.2</td>
<td>27.0</td>
<td>31.5</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2.**

<table>
<thead>
<tr>
<th>Average of Percent Salmonella</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plant 3</th>
<th>Plant 4</th>
<th>Plant 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hide</td>
<td>53.3</td>
<td>38.5</td>
<td>90.3</td>
<td>56.9</td>
<td>78.6</td>
<td>64.8</td>
</tr>
<tr>
<td>Pre-evis</td>
<td>11.3</td>
<td>3.9</td>
<td>84.1</td>
<td>59.5</td>
<td>29.6</td>
<td>40.5</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>0</td>
<td>0.8</td>
<td>0.5</td>
<td>2.6</td>
<td>0.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**Table 3.**

<table>
<thead>
<tr>
<th>Average of APC (log CFU/100 cm²)</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plant 3</th>
<th>Plant 4</th>
<th>Plant 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hide</td>
<td>7.9</td>
<td>8.3</td>
<td>8.8</td>
<td>8.1</td>
<td>8.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Pre-evis</td>
<td>4.4</td>
<td>4.8</td>
<td>6.5</td>
<td>6.6</td>
<td>5.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>2.7</td>
<td>1.7</td>
<td>2.2</td>
<td>2.2</td>
<td>1.2</td>
<td>2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average of EBC (log CFU/100 cm²)</th>
<th>Plant 1</th>
<th>Plant 2</th>
<th>Plant 3</th>
<th>Plant 4</th>
<th>Plant 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hide</td>
<td>5.5</td>
<td>6.1</td>
<td>5.8</td>
<td>4.8</td>
<td>5.3</td>
<td>5.5</td>
</tr>
<tr>
<td>Pre-evis</td>
<td>2.1</td>
<td>2.2</td>
<td>3.7</td>
<td>3.3</td>
<td>2.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>1.1</td>
<td>0</td>
<td>0.4</td>
<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Table 4. Relationship between generic bacterial loads on the hides of cull cows and bulls and the frequency of samples positive for *E. coli* O157:H7 and *Salmonella*.

<table>
<thead>
<tr>
<th>APC range, CFU/100 cm²</th>
<th>N</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em> O157:H7</td>
</tr>
<tr>
<td>&lt; 10⁷</td>
<td>42</td>
<td>29</td>
</tr>
<tr>
<td>10⁷ to 10⁸</td>
<td>320</td>
<td>53</td>
</tr>
<tr>
<td>10⁸ to 10⁹</td>
<td>421</td>
<td>58</td>
</tr>
<tr>
<td>10⁹ to 10¹</td>
<td>117</td>
<td>68</td>
</tr>
<tr>
<td>≥ 10¹</td>
<td>39</td>
<td>28</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EBC range, CFU/100 cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10⁴</td>
</tr>
<tr>
<td>10⁴ to 10⁵</td>
</tr>
<tr>
<td>10⁵ to 10⁶</td>
</tr>
<tr>
<td>10⁶ to 10⁷</td>
</tr>
<tr>
<td>10⁷ to 10⁸</td>
</tr>
<tr>
<td>≥ 10⁸</td>
</tr>
</tbody>
</table>

Table 5. Relationship between generic bacterial loads on the pre-evisceration carcasses of cull cows and bulls and the frequency of samples positive for *E. coli* O157:H7 and *Salmonella*.

<table>
<thead>
<tr>
<th>APC range, CFU/100 cm²</th>
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<th>Percent positive</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em> O157:H7</td>
</tr>
<tr>
<td>&lt; 10⁴</td>
<td>52</td>
<td>10</td>
</tr>
<tr>
<td>10⁴ to 10⁵</td>
<td>274</td>
<td>24</td>
</tr>
<tr>
<td>10⁵ to 10⁶</td>
<td>270</td>
<td>30</td>
</tr>
<tr>
<td>10⁶ to 10⁷</td>
<td>244</td>
<td>38</td>
</tr>
<tr>
<td>10⁷ to 10⁸</td>
<td>75</td>
<td>41</td>
</tr>
<tr>
<td>≥ 10⁸</td>
<td>24</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EBC range, CFU/100 cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10⁴</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>10⁶ to 10⁷</td>
</tr>
<tr>
<td>10⁷ to 10⁸</td>
</tr>
<tr>
<td>≥ 10⁸</td>
</tr>
</tbody>
</table>
For more information contact:
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