Perineal swabs reveal effect of super shedders on the transmission of \textit{Escherichia coli} O157:H7 in commercial feedlots\textsuperscript{1}

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\textbf{ABSTRACT:} Cattle that shed more than $10^4$ cfu/g of \textit{Escherichia coli} O157 in feces have been described as super shedders (SS) and are thought to have major impacts on prevalence and transmission of this organism. Two Southern Alberta commercial feedlots (feedlot X, 7 pens averaging 183 steers; feedlot Y, 5 pens averaging 153 steers) were sampled from May 2007 to January 2008. Background samples [fecal pat (FP); water, ropes] were taken weekly from each pen for 2 wk before collection of samples from individuals [fecal grab (FG); perineal swab] at 2 different times [during spring and summer (S1); immediately before slaughter during fall and winter (S2)]. Immunomagnetic separation and selective media were used for detecting \textit{E. coli} O157:H7. Positive FG and FP were enumerated by direct plating onto sorbitol MacConkey agar supplemented with 2.5 mg/L of potassium tellurite and 0.05 mg/L of cefixime. Five sorbitol-negative colonies were agglutinated using an anti-O157 latex kit, and the proportion of positive colonies was adjusted for non-\textit{E. coli} O157:H7. Overall, there were 153 (7.16%) and 10 (0.45%) SS at S1 and S2, respectively. In feedlot X, SS and penmates of SS during S1 were more likely ($P < 0.01$) to shed \textit{E. coli} O157:H7 in their feces and have this organism on their perineum than cattle in a pen where no SS were identified. In feedlot Y, SS and penmates of SS during S1 were more likely ($P < 0.01$) to have \textit{E. coli} O157:H7 on their perineum than those from a pen where only 1 SS was identified, but steers in only 1 pen with multiple SS were more likely ($P < 0.01$) to shed this organism in feces. Overall, \textit{E. coli} O157:H7 was 1.85 times more likely ($P < 0.01$) to be detected in perineal swabs compared with FG and \textit{E. coli} O157:H7 was more likely ($P < 0.01$) to be detected at S1 compared with S2 for all sample types. Super shedders were a larger proportion of shedding cattle in S1 than in S2, but the presence of SS increased ($P < 0.01$) prevalence of this organism on the perineum of cattle throughout the year. Even when SS did not increase fecal shedding of \textit{E. coli} O157:H7, their presence increased contamination of hides, an outcome that could have important implications for contamination of carcasses at the abattoir.

\textbf{Key words:} \textit{Escherichia coli} O157, fecal, feedlot cattle, hide, super shedder

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that interaction among naïve penmates played a critical role in transmission. Monitoring \( E. \ coli \ O157:H7 \) in individual animals has been generally performed by collection of fecal grab (FG) samples (Omisakin et al., 2003; Fox et al., 2008; Niu et al., 2008; Stephens et al., 2008) or swabs at the rectal-anal junction (Naylor et al., 2003; Davis et al., 2006; Niu et al., 2008). Because of the invasiveness of these procedures and because opportunities and time available for monitoring \( E. \ coli \ O157:H7 \) in commercial feedlots are limited, alternative monitoring procedures are warranted. Therefore, we conducted a large-scale study to observe 1) the distribution of commercial feedlot cattle that shed more than 10^4 cfu/g of \( E. \ coli \ O157:H7 \) (super shedders) in their feces; 2) the impact of super shedders on transmission of \( E. \ coli \ O157:H7 \) to penmates and the feedlot environment; and 3) the utility of a perineal swab (PS) compared with that of a FG sample for identification of feedlot cattle carrying \( E. \ coli \ O157:H7 \).

**MATERIALS AND METHODS**

All steers were cared for according to the standards of the Canadian Council on Animal Care (Olfert et al., 1993).

**Animals**

Feedlot cattle were sampled at implantation from May to August in the spring and summer of 2007 (S1) and just before shipment to slaughter from September 2007 to January 2008 in the fall and winter (S2). Cattle from feedlot X weighed 516.7 ± 157.8 kg at S1 (n = 1,357) and 642.5 ± 47.7 kg at S2 (n = 1,281). Cattle from feedlot Y weighed 541.5 ± 45.3 kg at S1 (n = 779) and 687.0 ± 52.9 kg at S2 (n = 768). For feedlot X, S2 required multiple samplings for more than 1 mo due to the rate at which cattle were shipped for slaughter. The number of steers in pens 1 to 7 in feedlot X averaged 194 with a range from 152 to 258. Feedlot Y had an average of 156 steers in pens 8 to 12 with a range from 106 to 235. Cattle were fed a barley-based finishing diet containing 85 to 90% barley grain, 5 to 10% barley silage, and 5% pelleted supplement on a DM basis. Steers had continuous access to fresh water.

**Background Samples**

For each of the 12 pens surveyed, background samples (FP, ropes, water from troughs) were collected on at least 2 consecutive weeks before the sampling of individuals at S1 and S2. Fecal pat samples (5 g per pat; 1 pat per 20 animals in the pen) were collected from freshly voided FP, pooled by pen into a Whirl-Pak bag, and transported to the laboratory on ice. Rope samples were obtained by hanging 2 manila ropes (1.22 m) per pen to the water trough rail in a readily accessible area near the feed bunk. Ropes were tied to the rails using 3 half-hitch knots and remained in the pens for at least 1 h. Both ropes from each pen were then placed in a 1-L bottle containing 500 mL of buffered peptone water. Bottles were transported back to the laboratory at ambient temperatures. Water samples were obtained by swabbing a 100-cm² area on the side of each water trough with a sterile sponge (25 cm²) in each pen. The sponge was then placed in a 100-mL disposable jar containing 45 mL of modified \( E. \ coli \) broth with 20 mg/L of novobiocin (mEC-nov, EMD, Gibbstown, NJ). Jars were shaken for 30 s and transported to the laboratory at ambient temperature.

**Individual Animal Samples**

Fecal grab and PS (100-cm² area around the anus in the center of the perineum) samples were simultaneously obtained from each individual animal enrolled in the study during S1 and S2. Fecal grab samples were obtained by rectal palpation using a clean glove for each animal. Feces were placed in Whirl-Pak bags and transported back to the laboratory on ice. Perineal swab samples were obtained using a sterile SpongeSicle hydrated with 25 mL of PBS (Med-Ox Diagnostics Inc., Ottawa, Ontario, Canada), and a new SpongeSicle was used for each animal. Each SpongeSicle was placed in a separate Whirl-Pak bag along with 45 mL of mEC-nov and transported to the laboratory at ambient temperature. All samples were delivered to the laboratory for analysis within a period of 12 h and refrigerated at 5°C until completion of \( E. \ coli \ O157:H7 \) detection and enumeration.

**\( E. \ coli \ O157 \) Detection**

Bags of pooled FP were manually blended before subsampling, and 1 g of feces from FG or FP was added to 9 mL of mEC-nov and incubated at 37°C for 6 h. Water samples were incubated at 37°C for 18 to 24 h. Rope samples were shaken (~450 rpm) for 3 × 5-min periods with 5 min of no agitation and then incubated for 16 h at 37°C. Perineal swab samples were incubated in the original transport media for 18 h at 37°C.

After enrichment, a 1-mL aliquot of each sample type (FP, water, rope, FG, and PS) was subjected to immunomagnetic separation using Dynabeads anti-O157 (Dynal, Lake Success, NY) and a PickPen (BioControl Systems Inc., Bellevue, WA) magnetic particle separation device as per manufacturers’ instructions. Fifty microliters of the bead-bacteria mixture was plated onto sorbitol MacConkey agar supplemented with 2.5 mg/L of potassium tellurite and 0.05 mg/L of cefixime (CT-SMAC), and the plates were incubated for 16 h at 37°C. Up to 3 sorbitol-negative (clear) colonies per plate were subjected to agglutination using an \( E. \ coli \) O157 latex kit (Oxoid, Nepean, Ontario, Canada). One isolate per sample was further subjected to multiplex PCR assays for the detection of the \( vt, \) eaeA, and \( flic \) genes.
genes as a final confirmation of \textit{E. coli} O157:H7 (Gannon et al., 1997).

\textbf{\textit{E. coli} O157 Enumeration}

The FG or FP that were positive for \textit{E. coli} O157 were serially diluted (1 g of feces in 9 mL of mEC-nov), and 100 µL of the $10^{-2}$ and $10^{-3}$ dilutions were plated in duplicate onto CT-SMAC. The CT-SMAC plates were incubated for 16 h at 37°C. Up to 5 sorbitol-negative (clear) colonies per plate were subjected to agglutination using an \textit{E. coli} O157 latex kit (Oxoid). Sorbitol-negative (clear) colonies were counted on each duplicate plate; dilution calculations were performed; adjustments for the proportion of positive agglutinations out of 5 were made; and counts were recorded in cfu per gram. Below the level of sensitivity of this method, counts <2 log cfu/g of feces were randomly assigned a number between 1 and 100 by utilizing the RANDBETWEEN worksheet function in Microsoft Excel (Microsoft Corp., Redmond, WA).

\textbf{Statistical Analysis}

Data were analyzed (SAS Institute Inc., Cary, NC), and descriptive statistics were generated. Fisher’s exact test was performed to compare background samples between pens for each feedlot with $H_0: \mu = \mu_0$. An analysis of \textit{E. coli} O157:H7 prevalence was performed using logistic regression methodology within the GLIMMIX procedure of SAS, with odds ratios generated using orthogonal contrasts. For these series of analyses, $H_0$: all pens = to referent pen, which for feedlot X was pen 7 (0 super shedders and 233 steers) and for feedlot Y was pen 12 (1 super shedder and 106 steers). Shedding levels of \textit{E. coli} O157:H7 were analyzed using linear mixed-model methodology within the MIXED procedure of SAS. For these series of analyses, $H_0: \mu = \mu_0$.

\textbf{Cattle shedding} \textit{E. coli} O157:H7 were categorized according to levels shed (category A, <2; category B, ≥2 to <4; category C, ≥4 to <6; category D, ≥6 log cfu/g of feces). Percentage (total cfu per group/total cfu per pen) contributed by each group (super shedders, category C + D; non-super shedders, other shedders) of \textit{E. coli} O157 cfu/g of feces based on results from enumeration of FG samples was also calculated.

\textbf{RESULTS}

Overall, FP, water, and rope samples were 15.38, 4.67, and 13.56% positive for \textit{E. coli} O157:H7 in feedlot X and 31.03, 27.59, and 34.48% positive in feedlot Y, respectively. Comparing background samples and pens during S1 and S2, no significant differences in detection of \textit{E. coli} O157:H7 were found for feedlot Y (Table 1). In contrast, pens within feedlot X differed in detection of positive FP, rope, and water samples over S1 and S2.

\begin{table}
\centering
\begin{tabular}{llllllllll}
\hline
\textbf{Sampling} & \textbf{and type} & \textbf{n} & \textbf{1} & \textbf{2} & \textbf{3} & \textbf{4} & \textbf{5} & \textbf{6} & \textbf{7} & \textbf{P-value} \\
\hline
\textbf{Feedlot X, pen} & & & & & & & & & & \\
\hline
\textbf{1} & FP & 19 & 0 (0.00) & 1 (50.00) & 0 (0.00) & 0 (0.00) & 1 (50.00) & 0 (0.00) & 0 (0.00) & 0.14 \\
R & 17 & 2 (100.00) & 0 (0.00) & 2 (66.67) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 0.02 \\
W & 19 & 0 (0.00) & 0 (0.00) & 1 (33.33) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 0.79 \\
\textbf{2} & FP & 44 & 1 (16.67) & 1 (12.50) & 1 (11.11) & 5 (71.43) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 0.02 \\
R & 41 & 0 (0.00) & 1 (14.29) & 0 (0.00) & 1 (14.29) & 1 (25.00) & 1 (20.00) & 0 (0.00) & 0.68 \\
W & 44 & 0 (0.00) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 2 (40.00) & 0 (0.00) & 0.02 \\
\hline
\textbf{Feedlot Y, pen} & & & & & & & & & & \\
\hline
\textbf{1} & FP & 12 & 0 (0.00) & 2 (66.67) & 2 (100.00) & 0 (0.00) & 1 (50.00) & 0.21 \\
R & 13 & 2 (66.67) & 3 (75.00) & 1 (50.00) & 1 (50.00) & 1 (50.00) & 1.00 \\
W & 13 & 2 (66.67) & 3 (75.00) & 0 (0.00) & 1 (50.00) & 1 (50.00) & 0.73 \\
\textbf{2} & FP & 17 & 3 (75.00) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 1 (25.00) & 0.11 \\
R & 16 & 1 (25.00) & 0 (0.00) & 0 (0.00) & 1 (50.00) & 0 (0.00) & 0.60 \\
W & 16 & 0 (0.00) & 1 (25.00) & 0 (0.00) & 0 (0.00) & 0 (0.00) & 1.00 \\
\hline
\end{tabular}
\caption{Number (percentage) of background samples: pooled fecal pats (FP), manila rope orally accessed by cattle (R), and drinking water and sediment from trough (W) that were positive for \textit{Escherichia coli} O157:H7 in each pen of cattle from feedlots X and Y.}
\end{table}
In feedlot Y, rope and water samples were 11 and 17 times, respectively, more likely (P = 0.02) to be positive for *E. coli* O157:H7 during S1 than S2.

Similar to the rope and water samples of feedlot Y, FG and PS from both feedlots were more likely to be positive for *E. coli* O157:H7 in S1 than in S2. During S1, there were a total of 612 (28.68%) and 1,012 (47.67%) positive FG and PS for *E. coli* O157:H7 from both feedlots. In contrast, during S2 there was a total of 105 (5.11%) and 148 (7.20%) positive for FG and PS from both feedlots. In feedlot Y, rope and water samples were 11 and 17 times, respectively, more likely (P < 0.01) to be detected in the spring and summer (S1) compared with the fall and winter (S2) in FG from cattle in feedlots X and Y, respectively. Accordingly, *E. coli* O157 was 10 and 111 times more likely (P < 0.01) to be detected in the spring and summer (S1) compared with the fall and winter (S2) in PS from cattle in feedlots X and Y, respectively.

Comparing S1 and S2, FG and PS showed similar trends across feedlots. *Escherichia coli* O157:H7 was 12.0 and 8.4 times more likely (P < 0.01) to be detected in the spring and summer (S1) compared with the fall and winter (S2) in FG from cattle in feedlots X and Y, respectively. Accordingly, *E. coli* O157 was 10 and 111 times more likely (P < 0.01) to be detected in the spring and summer (S1) compared with the fall and winter (S2) in PS from cattle in feedlots X and Y, respectively.

Comparing FG and PS, detection of *E. coli* O157:H7 was 1.9 times more likely (P < 0.01) in PS as compared with FG. This difference was evident in S1 and S2, where *E. coli* O157:H7 was 55% (P < 0.01) and 31% (P = 0.01) more likely to be detected in PS compared with FG, respectively. As well, the superiority of PS was evident in both feedlots, with *E. coli* O157:H7 34 (P < 0.01) and 48% (P < 0.01) more likely to be detected in PS compared with FG from feedlots X and Y, respectively (Table 2).

On a pen-level basis, FG or PS from feedlot X were 1.6 (P < 0.01) and 3.2 (P < 0.01) times more likely to have *E. coli* O157:H7 as compared with these same samples from feedlot X. During S1, *E. coli* O157:H7 from feedlot Y was 1.9 (P < 0.01) and 5.6 (P < 0.01) times more likely to be found in FG or PS, respectively, as compared with feedlot X on a pen-level basis. Similar results were found during S2, and *E. coli* O157:H7 from feedlot Y was 1.8 (P = 0.02) and 1.3 (P = 0.13) times more likely to be found in FG or PS, respectively, as compared with feedlot X on a pen-level basis (data not shown).

Although feedlot Y had more positive FG and PS than did feedlot X, levels of *E. coli* O157:H7 detected did not differ between feedlots (Table 3). During S1, feedlot X averaged 2.67 ± 0.10 log cfu/g of feces of *E. coli* O157 in FG, and feedlot Y averaged 2.84 ± 0.12 log cfu/g of feces. During S2, feedlot X averaged 1.83 ± 0.16 log cfu/g of feces of *E. coli* O157 in FG, and feedlot Y averaged 2.18 ± 0.23 log cfu/g of feces. As well, the mean shedding level of *E. coli* O157 among non-super shedders did not differ among pens during S1 or S2.

Using the categories for shedding levels of *E. coli* O157 described in Table 4, in feedlot X during S1 super shedders (categories C + D) accounted for 25% of cattle shedding the organism, whereas 22% of animals sampled shed *E. coli* O157:H7 (A + B + C + D). Similar results for S1 were found for feedlot Y, for proportion of super shedders (27% of cattle shedding), although total shedders in feedlot Y (36%) were greater than for feedlot X as previously reported. During S2, the proportion of super shedders fell dramatically in both feedlots, accounting for 4 and 13% of cattle shedding *E. coli* O157:H7 in feedlots X and Y, respectively.

Fecal and hide contamination at the pen and feedlot level including the impact of super shedders on the prevalence of *E. coli* O157:H7 at S1 is shown in Table 5. In feedlot X, cattle from pens with super shedders were between 9 and 702 times more likely (P < 0.05) to have *E. coli* O157:H7 in their feces than animals in the pen where no super shedders were identified. In feedlot Y, the impact of super shedders on fecal shedding was less uniform. Cattle from pens in feedlot Y with 19 and 44 super shedders were 2.1 and 6.5 times more likely (P = 0.01) to have *E. coli* O157:H7 in their feces than those

### Table 2. Numbers (percentage individual animal samples) positive for *Escherichia coli* O157:H7 from fecal grab and perineum hide swab samples among cattle from 2 feedlots (X and Y) during sampling points 1 and 2

<table>
<thead>
<tr>
<th>Analysis type</th>
<th>FG</th>
<th>PS</th>
<th>OR</th>
<th>CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>717 (17.12)</td>
<td>1,160 (27.78)</td>
<td>0.54</td>
<td>0.48 to 0.60</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sampling point 1&lt;sup&gt;6&lt;/sup&gt;</td>
<td>612 (28.68)</td>
<td>1,012 (47.67)</td>
<td>0.45</td>
<td>0.39 to 0.51</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sampling point 2&lt;sup&gt;7&lt;/sup&gt;</td>
<td>105 (5.11)</td>
<td>148 (7.20)</td>
<td>0.69</td>
<td>0.53 to 0.91</td>
<td>0.01</td>
</tr>
<tr>
<td>Feedlot X&lt;sup&gt;8&lt;/sup&gt;</td>
<td>369 (13.96)</td>
<td>534 (20.30)</td>
<td>0.66</td>
<td>0.57 to 0.77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Feedlot Y&lt;sup&gt;9&lt;/sup&gt;</td>
<td>348 (16.38)</td>
<td>626 (40.52)</td>
<td>0.52</td>
<td>0.45 to 0.60</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1<sup>FG = fecal grab.</sup>  
2<sup>PS = perineum hide swab.</sup>  
3<sup>OR = pen level odds ratios.</sup>  
4<sup>CI = 95% confidence interval of the odds ratio.</sup>  
5<sup>P-values of *E. coli* O157:H7 detection in fecal grab samples in feedlot cattle using perineum hide swab samples as the referent.</sup>  
6<sup>Sampling 1, collected during spring and summer of 2007.</sup>  
7<sup>Sampling 2, collected just before shipment to slaughter during fall and winter of 2007.</sup>  
8<sup>Feedlot X included pens 1, 2, 3, 4, 5, 6, and 7.</sup>  
9<sup>Feedlot Y included pens 8, 9, 10, 11, and 12.</sup>
in the pen where only 1 super shedder was identified. In contrast, pens where 5 and 6 super shedders were identified in feedlot Y were not different ($P > 0.85$) in fecal shedding of *E. coli* O157:H7 when compared with the pen that had only 1 identified super shedder. For S2, because very few fecal samples positive for *E. coli* O157:H7 were identified, presence of super shedders did not affect fecal prevalence of *E. coli* O157:H7.

Although the presence of super shedders in a pen did not always affect fecal shedding of *E. coli* O157:H7, super shedders consistently increased detection of *E. coli* O157:H7 from PS. In feedlot X during S1, cattle from pens with super shedders were from 13 to 326 times more likely ($P < 0.01$) to have *E. coli* O157:H7 on their perineum than those from the pen where no super shedders were identified. Similar results were found for feedlot Y during S1 because cattle from pens with super shedders were between 3 and 46 times more likely ($P < 0.01$) to have *E. coli* O157:H7 on their perineum than those from the pen where only 1 super shedder was identified. Even in S2, when numbers of PS positive for *E. coli* O157:H7 dramatically declined, presence of super shedders in a pen increased the likelihood of positive PS ($P < 0.01$) in feedlots X and Y.

The percentage of *E. coli* O157 cfu/g in feces attributed to super shedders and other shedders is shown in Table 6. In feedlot X, the percentage of shedding attributed to super shedders ranged from 0.0 to 78.8% across pens. In feedlot Y, the percentage of shedding attributed to super shedders ranged from 9.9 to 61.3% across pens. There were no differences between groups (super shedder and other shedder) or pens for feedlot X and Y with regard to the percentage of shedding attributable to super shedders and other shedder penmates.

**DISCUSSION**

**Sampling Methodology**

Pearce et al. (2004) recommended testing two 1-g samples per FP due to variation in *E. coli* O157 distribution within individual FP. Because we collected 5-g samples from multiple FP and mixed the pooled sample before subsampling, it is likely that our methodology was equivalent to that of Pearce et al. (2004) for detection of *E. coli* O157. The limit of detection of the direct plating method used in this study to enumerate *E. coli* O157 in feces was $\geq 2$ log cfu/g of feces. Other more sensitive methods have been developed (Stephens et al., 2007a) but would not be practical for enumerating such a large number of samples. As well, decreased levels of *E. coli* O157 (<2 log/g) were readily detected by using immunomagnetic separation.

**Effect of Super Shedders on Transmission of *E. coli* O157 to the Environment**

Fecal pat samples were similar for prevalence between pens and sampling points indicating that *E. coli* O157:H7 persisted throughout the feedlot environment during the 9 mo of the study, regardless of presence of super shedders in a pen. Similarly, Williams et al. (2005) demonstrated long-term persistence of *E. coli* O157 under a range of environmental conditions. There

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**Table 3.** Mean (log cfu/g; ±SEM) levels of *Escherichia coli* O157 from pooled fecal pat samples$^1$ and individual animal fecal grab samples (excluding super shedders, but all other animals included) among cattle from 2 feedlots (feedlots X and Y) during sampling points 1 and 2

<table>
<thead>
<tr>
<th>FL/pen$^2$</th>
<th>S1$^3$—fecal pats</th>
<th>S2$^3$—fecal pats</th>
<th>S1$^4$—fecal grab</th>
<th>S2$^4$—fecal grab</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>2.05 ± 0.64</td>
<td>1.67 ± 0.12</td>
<td>2.67 ± 0.10</td>
<td>1.83 ± 0.16</td>
</tr>
<tr>
<td>1</td>
<td>0.00 ± 0.00</td>
<td>1.76 ± 0.00</td>
<td>3.94 ± 0.16</td>
<td>1.21 ± 0.19</td>
</tr>
<tr>
<td>2</td>
<td>1.41 ± 0.00</td>
<td>1.85 ± 0.00</td>
<td>2.06 ± 0.13</td>
<td>1.74 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>0.00 ± 0.00</td>
<td>1.69 ± 0.00</td>
<td>2.45 ± 0.20</td>
<td>1.87 ± 0.20</td>
</tr>
<tr>
<td>4</td>
<td>0.00 ± 0.00</td>
<td>1.61 ± 0.19</td>
<td>2.24 ± 0.43</td>
<td>2.09 ± 0.45</td>
</tr>
<tr>
<td>5</td>
<td>2.69 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>2.53 ± 0.31</td>
<td>2.16 ± 0.32</td>
</tr>
<tr>
<td>6</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>3.61 ± 1.01</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>7</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.85 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Y</td>
<td>2.21 ± 0.61</td>
<td>1.29 ± 0.31</td>
<td>2.84 ± 0.12</td>
<td>2.18 ± 0.23</td>
</tr>
<tr>
<td>8</td>
<td>0.00 ± 0.00</td>
<td>1.29 ± 0.31</td>
<td>2.90 ± 0.25</td>
<td>2.56 ± 0.30</td>
</tr>
<tr>
<td>9</td>
<td>1.87 ± 1.26</td>
<td>0.00 ± 0.00</td>
<td>3.13 ± 0.18</td>
<td>1.29 ± 0.20</td>
</tr>
<tr>
<td>10</td>
<td>2.54 ± 0.65</td>
<td>0.00 ± 0.00</td>
<td>2.50 ± 0.29</td>
<td>1.00 ± 0.52</td>
</tr>
<tr>
<td>11</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>2.27 ± 0.32</td>
<td>0.98 ± 0.49</td>
</tr>
<tr>
<td>12</td>
<td>NA$^5$</td>
<td>NA</td>
<td>1.97 ± 0.20</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

$^1$Five grams per pat; 1 pat per 20 animals in the pen.
$^2$X and Y overall refers to an analysis at the feedlot (FL) level.
$^3$S1 = samples taken during spring and summer of 2007, with fecal pat samples collected 2 wk before fecal grabs.
$^4$S2 = samples taken just before shipment to slaughter during fall and winter of 2007, with fecal pat samples collected 2 wk before fecal grabs.
$^5$NA = not available; sample was not enumerated.
Table 4. Numbers of positive (% of positive samples per category) fecal grab samples for *Escherichia coli* O157:H7 among feedlot cattle that shed in 4 different categories from feedlots (FL) X and Y

<table>
<thead>
<tr>
<th>FL/pen</th>
<th>Animal count</th>
<th>Category A (^1)</th>
<th>Category B (^2)</th>
<th>Category C (^3)</th>
<th>Category D (^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>1,357</td>
<td>195 (63.11)</td>
<td>36 (11.65)</td>
<td>61 (19.74)</td>
<td>17 (5.50)</td>
</tr>
<tr>
<td>1</td>
<td>152</td>
<td>7 (10.94)</td>
<td>22 (34.38)</td>
<td>31 (48.44)</td>
<td>4 (6.25)</td>
</tr>
<tr>
<td>2</td>
<td>153</td>
<td>94 (63.39)</td>
<td>5 (4.46)</td>
<td>10 (8.93)</td>
<td>3 (2.68)</td>
</tr>
<tr>
<td>3</td>
<td>253</td>
<td>60 (70.59)</td>
<td>6 (7.06)</td>
<td>12 (14.12)</td>
<td>7 (8.27)</td>
</tr>
<tr>
<td>4</td>
<td>153</td>
<td>9 (75.00)</td>
<td>1 (8.33)</td>
<td>2 (16.67)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>5</td>
<td>258</td>
<td>21 (72.41)</td>
<td>2 (6.90)</td>
<td>4 (13.79)</td>
<td>2 (6.90)</td>
</tr>
<tr>
<td>6</td>
<td>155</td>
<td>3 (50.00)</td>
<td>0 (0.00)</td>
<td>2 (33.33)</td>
<td>1 (16.67)</td>
</tr>
<tr>
<td>7</td>
<td>233</td>
<td>1 (100.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Y</td>
<td>779</td>
<td>175 (61.84)</td>
<td>33 (11.66)</td>
<td>46 (16.25)</td>
<td>29 (10.25)</td>
</tr>
<tr>
<td>8</td>
<td>151</td>
<td>32 (58.18)</td>
<td>4 (7.24)</td>
<td>14 (25.45)</td>
<td>5 (9.09)</td>
</tr>
<tr>
<td>9</td>
<td>235</td>
<td>83 (54.97)</td>
<td>24 (15.89)</td>
<td>23 (15.23)</td>
<td>21 (13.91)</td>
</tr>
<tr>
<td>10</td>
<td>121</td>
<td>17 (70.83)</td>
<td>2 (8.33)</td>
<td>4 (16.67)</td>
<td>1 (4.17)</td>
</tr>
<tr>
<td>11</td>
<td>166</td>
<td>26 (81.25)</td>
<td>0 (0.00)</td>
<td>4 (12.50)</td>
<td>2 (6.25)</td>
</tr>
<tr>
<td>12</td>
<td>106</td>
<td>17 (80.95)</td>
<td>3 (14.29)</td>
<td>1 (4.76)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sampling 1</th>
<th>Animal count</th>
<th>Category A</th>
<th>Category B</th>
<th>Category C</th>
<th>Category D</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>1,284</td>
<td>41 (89.13)</td>
<td>3 (6.52)</td>
<td>1 (2.17)</td>
<td>1 (2.17)</td>
</tr>
<tr>
<td>1</td>
<td>162</td>
<td>10 (100.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>4 (100.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>3</td>
<td>212</td>
<td>6 (100.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>7 (87.50)</td>
<td>0 (0.00)</td>
<td>1 (12.50)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>5</td>
<td>252</td>
<td>14 (77.78)</td>
<td>3 (16.67)</td>
<td>0 (0.00)</td>
<td>1 (5.50)</td>
</tr>
<tr>
<td>6</td>
<td>210</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>7</td>
<td>168</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Y</td>
<td>768</td>
<td>49 (83.05)</td>
<td>2 (3.39)</td>
<td>4 (6.78)</td>
<td>4 (6.78)</td>
</tr>
<tr>
<td>8</td>
<td>135</td>
<td>33 (76.74)</td>
<td>2 (4.65)</td>
<td>4 (9.30)</td>
<td>4 (9.30)</td>
</tr>
<tr>
<td>9</td>
<td>233</td>
<td>10 (100.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>10</td>
<td>135</td>
<td>3 (100.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>11</td>
<td>148</td>
<td>3 (100.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>12</td>
<td>106</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

\(^1\)Category A, <2 log cfu/g.
\(^2\)Category B, ≥2 log cfu/g to <4 log cfu/g.
\(^3\)Category C, ≥4 log cfu/g to <6 log cfu/g.
\(^4\)Category D, ≥6 log cfu/g.

*Feedlots X and Y refer to an analysis at the feedlot level.*
was no correlation between the proportion of positive background samples in pens with low compared with high super shedder numbers in the current study. An other study with more intensive background sampling after super shedders have been shipped from the pen, combined with intensive individual sampling of new arrivals, would be useful in assessing the impact of super shedders on the incidence and levels of *E. coli* O157 in the feedlot environment.

**Table 5.** Odds ratios (OR), 95% confidence intervals (CI) of the OR, and *P*-values for the likelihood of detecting *Escherichia coli* O157:H7 in fecal grab and perineum hide swab samples collected in the spring and summer from cattle in 2 feedlots using pen 7 (0 super shedders; *n* = 233 cattle) as a referent for all other pens from feedlot X and pen 12 (1 super shedder; *n* = 106 cattle) as a referent for all other pens from feedlot Y.

<table>
<thead>
<tr>
<th>Feedlot and pen</th>
<th>SS1</th>
<th>Other shedders2</th>
<th>n3</th>
<th>OR (95% CI)</th>
<th>P-value</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fecal grab</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>29</td>
<td>152</td>
<td>214.38 (29.26 to &gt;999.99)</td>
<td>&lt;0.01</td>
<td>163.17 (39.04 to 681.89)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>99</td>
<td>153</td>
<td>702.1 (95.03 to &gt;999.00)</td>
<td>&lt;0.01</td>
<td>326.29 (77.37 to &gt;999.99)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>66</td>
<td>253</td>
<td>119.47 (16.44 to 868.00)</td>
<td>&lt;0.01</td>
<td>182.67 (44.33 to 752.72)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>10</td>
<td>153</td>
<td>21.54 (2.78 to 166.77)</td>
<td>&lt;0.01</td>
<td>13.64 (3.07 to 60.69)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>23</td>
<td>258</td>
<td>29.38 (3.96 to 217.87)</td>
<td>&lt;0.01</td>
<td>14.63 (3.44 to 62.10)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3</td>
<td>155</td>
<td>9.34 (1.11 to 78.52)</td>
<td>0.04</td>
<td>41.53 (9.85 to 175.01)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Perineum hide swabs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>163.17 (39.04 to 681.89)</td>
<td>&lt;0.01</td>
<td>326.29 (77.37 to &gt;999.99)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>182.67 (44.33 to 752.72)</td>
<td>&lt;0.01</td>
<td>326.29 (77.37 to &gt;999.99)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>119.47 (16.44 to 868.00)</td>
<td>&lt;0.01</td>
<td>182.67 (44.33 to 752.72)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>21.54 (2.78 to 166.77)</td>
<td>&lt;0.01</td>
<td>13.64 (3.07 to 60.69)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td>29.38 (3.96 to 217.87)</td>
<td>&lt;0.01</td>
<td>14.63 (3.44 to 62.10)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9.34 (1.11 to 78.52)</td>
<td>0.04</td>
<td>41.53 (9.85 to 175.01)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Number of super-shedder cattle within a pen from samples taken in spring and summer of 2007 (sampling 1).
2Number of animals within a pen also shedding *E. coli* O157:H7 but at <10⁴ cfu/g of feces from sampling 1.
3Total number of cattle in the pen for sampling 1.

Table 6. Per gram of feces, percentage (total cfu per group/total cfu per pen) attributed to each group (super shedders; other shedders of *Escherichia coli* O157) from fecal grab samples.

<table>
<thead>
<tr>
<th>Feedlot and pen</th>
<th>SS1</th>
<th>non-SS2</th>
<th>n3</th>
<th>SS% load</th>
<th>Non-SS% load</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>78</td>
<td>241</td>
<td>1,357</td>
<td>51.30⁴</td>
<td>56.03⁵</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>39</td>
<td>152</td>
<td>67.82</td>
<td>32.18</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>99</td>
<td>153</td>
<td>30.01</td>
<td>69.99</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>66</td>
<td>253</td>
<td>48.45</td>
<td>51.55</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>10</td>
<td>153</td>
<td>38.52</td>
<td>61.48</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>23</td>
<td>258</td>
<td>44.14</td>
<td>55.86</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3</td>
<td>155</td>
<td>78.84</td>
<td>21.16</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>1</td>
<td>233</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Y</td>
<td>75</td>
<td>208</td>
<td>779</td>
<td>43.03⁶</td>
<td>56.97⁷</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>36</td>
<td>151</td>
<td>61.28</td>
<td>38.72</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>107</td>
<td>235</td>
<td>56.73</td>
<td>43.27</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>19</td>
<td>121</td>
<td>40.65</td>
<td>59.35</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>26</td>
<td>166</td>
<td>46.56</td>
<td>53.44</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>20</td>
<td>106</td>
<td>9.91</td>
<td>90.09</td>
</tr>
</tbody>
</table>

1Number of super shedder (SS) animals within a pen from samples taken during spring and summer of 2007 (sampling 1).
2Number of other animals shedding (non-SS) *E. coli* O157:H7 at <10⁴ cfu/g within a pen from sampling 1.
3Total number of cattle in the pen for sampling 1.
4Least squares mean of the pen percentages of *E. coli* O157 cfu/g in fecal grab samples contributed by SS for feedlot X.
5Least squares mean of the pen percentages of *E. coli* O157 cfu/g in fecal grab samples contributed by non-SS for feedlot X.
6Least squares mean of the pen percentages of *E. coli* O157 cfu/g in fecal grab samples contributed by SS for feedlot Y.
7Least squares mean of the pen percentages of *E. coli* O157 cfu/g in fecal grab samples contributed by non-SS for feedlot Y.

Effect of Super Shedders on Transmission of *E. coli* O157 to Pen Mates

Our data did not support previous mathematical models (Omisakin et al., 2003; Matthews et al., 2006a) that suggested super shedders contribute the majority of the *E. coli* O157 load at the pen level. Matthews et al. (2006a) estimated that 80% of the transmission of *E. coli* O157 arises from the 20% of cattle that shed the...
most \textit{E. coli} O157 based on a cross-sectional FP study on 474 Scottish cattle farms. Omisakin et al. (2003) estimated that the super shedders, which were 9\% of the population sampled, represented >96\% of the total \textit{E. coli} O157 burden produced by the entire population of feedlot cattle sampled at slaughter. According to data collected in the current study, super shedders contributed 9.91 to 78.84\% of the \textit{E. coli} O157 in a feedlot pen, suggesting that mathematical approaches may overestimate the contribution of these individuals to the \textit{E. coli} O157 burden in feedlots. Omisakin et al. (2003) based their estimation on cattle sampled at slaughter after transport from the feedlot, and transportation stress may have caused these animals to shed at greater levels.

\textit{Distribution of Super Shedders in Feedlot Pens}

Although the contribution of super shedders to \textit{E. coli} O157 burden differs in the present study, the proportion of super shedders per pen shows agreement with previous studies. The study of Matthews et al. (2006a) estimated 20\% of positive animals shedding \(>4\) log cfu/g of feces, comparable with the present study where 25.8\% of shedders were super shedders during S1. However, proportion of super shedders was influenced by season of sampling, because only 9.5\% of positive cattle shed \(>10^4\) cfu/g feces during S2.

Shedding of \textit{E. coli} O157 by super shedders ranged from 4.00 to 9.29 log cfu/g of feces and did not differ between sampling periods. Although shedding by super shedders remained similar across seasons during the fall and winter along with a decreased proportion of shedding cattle as super shedders, numbers of super-shedding cattle were markedly reduced with 10 and 153 super shedders identified in the fall and winter months as compared with spring and summer months, respectively. Consequently, super shedders are obviously affected by the same forces responsible for seasonality of shedding as are other shedding animals (Barkocy-Gallagher et al., 2003).

Considering the intermittent nature of \textit{E. coli} O157:H7 shedding (Besser et al., 1997; McAllister et al., 2006), it is possible that more cattle than were identified may have shed at the level of super shedders. Due to the labor required for identification of super shedders, studies to document the long-term nature of shedding in super shedders have yet to be conducted. Collecting samples daily or weekly would have detected more super shedders among a pen of feedlot cattle, but would be logistically difficult considering the number of samples that would be obtained and the desire of commercial feedlots to minimize handling of cattle. Reducing the size of pen and animal numbers tested would make this type of experiment possible, but would reduce the chances of identifying supper shedders due to smaller experimental populations.

Likewise the dynamics of transmission of \textit{E. coli} O157:H7 may be quite different between experimental pens that typically contain 10 to 20 animals and commercial pens that usually house 100 or more. For example, Chase-Topping et al. (2007) examined 13,000 FP samples from 481 farms in Scotland and found 441 samples from 91 farms tested positive for \textit{E. coli} O157:H7. These authors then identified a point estimate for super shedders to be \(\geq 3\) or \(\geq 4\) log cfu/g of feces, which provided a useful estimate of farm-level prevalence but did not identify super shedders at the animal level. Cobbold et al. (2007) conducted a study of 160 feedlot cattle in which recto-anal mucosal swabs and FG were collected from each animal during a 14-wk sampling period (July to October 2003). These authors identified 5 super shedders from 3 pens at an incidence of 0.22\% of the total samples taken (\(n = 2,240\)). In the current study, 163 super shedders were identified, an incidence of 3.82\% of the total samples taken (\(n = 4,272\)). Consequently, the strategy used in the present study of enrolling more animals with less-intensive sampling appears more likely to identify super shedders than intensive sampling of fewer cattle.

\textit{Utility of Perineum Swabs Compared with Fecal Grab Samples for Detection of \textit{E. coli} O157}

The PS procedure used in this study may provide a more accurate picture of the prevalence and distribution of \textit{E. coli} O157:H7 among individual cattle within feedlots than FG; swabbing the anal region may sample feces from multiple defecations persisting in the folds of the anus. Although FG have been previously found to be more sensitive than PS for detection of \textit{E. coli} O157 (Stephens et al., 2007b), previous studies used different media (tryptic soy broth) and incubation times (2 h at 25°C and 6 h at 42°C) for preenrichment of PS samples, as described by Keen and Elder (2002). The present study used mEC-nov and incubation of swabs at 37°C for 18 h, which likely improved recovery of this organism. Acquiring a PS is less invasive and time-consuming than collecting a FG from individual cattle and may prevent underestimation of pathogen prevalence. Whether numbers of cattle shedding were elevated (S1) or reduced (S2), PS always detected more positive cattle than FG in the present study.

\textit{Implications for Control of \textit{E. coli} O157:H7}

Targeting super-shedder cattle for mitigation strategies has been proposed as a means of reducing the incidence and spread of \textit{E. coli} O157:H7 to pen-mates and the feedlot environment (Matthews et al., 2006a). The majority of cattle shed \textit{E. coli} O157:H7 at low levels (<10^2 cfu/g; Omisakin et al., 2003) and for short durations in their feces (Bach et al., 2002), which suggests that most cattle positive for \textit{E. coli} O157:H7 are
not super shedders. Several studies (Barkocy-Gallagher et al., 2001, 2003; Nou et al., 2003) have emphasized the importance of minimizing the presence of \( E.\ coli\) O157:H7 on the hide because fecal contamination of hides is correlated with carcass contamination (Elder et al., 2000). Due to the link discovered in the present study between presence of super shedders and increased contamination of the perineum with \( E.\ coli\) O157:H7, focusing pre-slaughter mitigation strategies on super shedders should reduce the risk of contamination at the abattoir.

Based on the results of this study, the presence of super shedders in a pen greatly increases the prevalence of \( E.\ coli\) O157:H7, especially on the hides of non-super shedder penmates. Fox et al. (2008) demonstrated that presence of a super shedder in a truckload of cattle traveling to the abattoir significantly increases the chance of isolating \( E.\ coli\) O157:H7 from a carcass within that truckload. Consequently, super-shedding cattle are important to consider when implementing food-safety strategies. In this study, super shedders were found to be more prevalent in the spring and summer than the fall and winter, but were shown to have a significant impact on the prevalence of this pathogen throughout the year. The impact of super shedders on the feedlot environment and other pen-mates makes super-shedder animals an important mitigation strategy target. Further studies to understand the mechanism(s) responsible for an animal becoming a super shedder of \( E.\ coli\) O157:H7 are needed.

**LITERATURE CITED**


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