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Use of model super-shedders to define the role of pen floor and hide contamination in the transmission of *Escherichia coli* O157:H7[1]

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ABSTRACT: Super-shedders, cattle shedding at least 10^4 cfu of *Escherichia coli* O157:H7 per gram of feces, increase the risks of contaminating the food chain and disseminating the organism through cattle populations. Because detecting super-shedders in cattle populations is laborious and time-consuming, a study was conducted to evaluate the role of hide and pen-floor contamination by model super shedders (MSS) in transmission of *E. coli* O157:H7. Steers (n = 48) negative for *E. coli* O157:H7 were allocated to 6 pens, with 2 replicate pens per treatment. Treatment A consisted of 3,000 g of feces inoculated with 10^6 cfu/g of a 5-strain mixture of nalidixic acid-resistant *E. coli* O157:H7 and spread in simulated fecal pats on the pen floor for d 0 through 4 and d 14 through 18. For treatment B, 100 g of the feces per day was spread on the perineum of 1 MSS per pen, and the remaining feces was placed on the pen floor as fecal pats similar to treatment A. Treatment C differed from B in that 50 g of feces was spread on the perineum and 50 g on the brisket of the MSS steer. Fecal samples, perineal swabs (500-cm^2 area around the anus), freshly voided fecal pats and manila rope samples were collected during a 56-d experimental period. More positive rope samples were found in treatments B and C compared with A ($P = 0.05$), and steers within treatments B and C were 1.3 times more likely ($P = 0.05$) to shed *E. coli* O157:H7 in their feces than steers in treatment A. Even though the number of *E. coli* O157:H7 introduced into pens was similar, results indicate an increased importance of hide compared with pen-floor contamination for transmission of this organism to cattle. Because cattle within treatment B were persistently colonized with *E. coli* O157:H7, this design should prove suitable for future studies investigating the role of super-shedders in the transmission of *E. coli* O157:H7.

Key words: *Escherichia coli* O157:H7, feedlot cattle, hide, super-shedder

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INTRODUCTION

Super-shedders of *Escherichia coli* O157:H7 shed at least 10^4 cfu/g of feces (Chase-Topping et al., 2008) and are key contributors to environmental spread of this zoonotic pathogen (Matthews et al., 2006b; Cobbold et al., 2007), and contamination of hides (Stephens et al., 2009) and carcasses at slaughter (Fox et al., 2008). Because cattle typically shed less *E. coli* O157 than super-shedders, eliminating 5% of cattle with greatest infectivity may limit colonization of naïve cattle (Matthews et al., 2006a).

Hides are primary sources of carcass contamination with *E. coli* O157 (Loneragan and Brashears, 2005) and hygiene procedures have been developed to alleviate contamination of carcasses with this organism (Elder et al., 2000). However, prevalence of *E. coli* O157 before slaughter has been linked to contamination of carcasses after abattoirs adopted microbial interventions (Woerner et al., 2006). Because super-shedding cattle may excrete 10^9 cfu of *E. coli* O157/g of feces (Stephens et al., 2009), these animals are recognized as the greatest risks for food-chain contamination and maintenance of *E. coli* O157 in cattle populations (Cobbold et al., 2007).

Although a role of super-shedders in colonizing pen mates with *E. coli* O157:H7 has been established, dynamics of *E. coli* O157 transmission from super-shedders to pen mates is less clear. Transmission of *E. coli* O157 from cattle inoculated with $\geq 10^8$ cfu to pen mates has

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been evaluated (Besser et al., 1997). However, inoculated cattle typically show rapidly declining levels of *E. coli* O157 (Stanford et al., 2010), a pattern inconsistent with super-shedders. Stephens et al. (2008) simulated super-shedders by distributing feces inoculated with *E. coli* O157 to the floors of pens of naïve cattle, but this work did not assess animal-to-animal interaction in dissemination of *E. coli* O157. Consequently, the goal of the present study was to evaluate roles of hide and pen-floor contamination in transmission of *E. coli* O157:H7 to pen mates.

MATERIALS AND METHODS

Cattle were cared for according to guidelines established by the Canadian Council on Animal Care (1993).

Cattle

Forty-eight Charolais-cross steers (415.1 ± 12.4 kg of initial BW) were randomly allocated to 6 pens (8 steers per pen and 2 pens per treatment) with replicate pens sharing a water bowl. An empty pen was used to prevent direct contact between treatment groups, and a separate handling system was used for collection of samples from individual animals in treatment A (*E. coli* O157:H7 on pen-floor only). Steers were adapted to their environment and a typical feedlot finishing diet consisting of 10% barley silage, 85% barley grain, and 5% protein supplement for 3 wk before initiation of the study. Fecal grab samples were collected weekly during the adaptation period to verify the absence of *E. coli* O157:H7.

Collection and Inoculation of Feces

Ten additional steers (donor cattle) receiving the same finishing diets as experimental steers were housed separately within 2 concrete-floored pens. Rectally collected fecal samples were obtained from donor cattle weekly for 3 wk to confirm absence of *E. coli* O157:H7. Cattle were provided access to a bedded area at night, but during the day were housed within the concrete floor area of the pen. During d 0 through 4 and d 14 through 18 of the study, approximately 20 kg of freshly voided fecal pats were collected on a daily basis from these animals to simulate 2 periods of active shedding separated by a 1-wk break. During each day of fecal collection, cattle were moved to a clean pen and all feces was removed from the floor of the vacated pen. Bags of feces were sealed within plastic containers and transported to the laboratory by 0830 h.

Collected feces were mixed at the laboratory with a large wooden paddle and divided into 6 aliquots of 2.9 kg. Each fecal aliquot was blended with 5 strains of naladixic acid-resistant (nal^R) *E. coli* O157:H7 in 80 mL of PBS to reach a final dilution of 10^6 cfu/g of feces. Strains E318Nm E32511, Co281–31N, and R508N (R. Johnson, Public Health Agency of Canada, Guelph, Ontario, Canada); and H4420N (V. Gannon, Public Health Agency of Canada, Lethbridge, Alberta, Canada) were grown separately in 100 mL of tryptic soy broth containing 50 μg/mL of nalidixic acid. After 4 centrifuges at 1,000 × *g* each for 12 min at 20°C, bacteria were washed 3 times in PBS (pH 7.2) and resuspended in PBS. Bacteria were enumerated both before and after fecal inoculation by standard dilution plating in duplicate onto sorbitol MacConkey agar supplemented with 2.5 mg/L of potassium tellurite, 0.05 mg/L of cefixime, and 0.05 mg/L of nalidixic acid (**CT-SMACnal**; Dalynn, Calgary, Alberta, Canada). Blending of feces with inoculum was as described by Stephens et al. (2008).

Sampling of Cattle and Pens

At 0900 h, before spreading of inoculated feces, fecal grab samples (~5 g) were collected from all experimental animals by rectal palpation using a fresh glove for each steer. At the same time, hide swab samples were collected from a 500-cm^2 area of the perineum using a sterile Spongecicle (Med-Ox Diagnostics, Ottawa, Ontario, Canada) moistened with 25 mL of PBS, with a new Spongecicle used for each steer. Three freshly voided, widely separated fecal pats per pen were also sampled (~5 g per pat), with care taken to avoid sampling previously placed artificial fecal pats. For each pen, a 1.2-m-long manila rope was tied to the feed bunk at 0930 h to allow oral access by steers, recovered after 4 h, and placed in a 500-mL bottle of buffered peptone water. All samples were collected daily on d 0 through 4, 5, 7, 10, 14 through 18, 21, and then weekly for the next 5 wk. After collection, samples were immediately transported to the laboratory for analysis according to transport of dangerous goods guidelines for class 6.2 (toxic/infectious substances; Transport Canada, 2010).

Distribution of Feces to Treatment Groups

Inoculated feces were bagged by treatment group before transport to the beef containment facility. Application of feces to pen floors occurred twice daily (1000 and 1430 h) with equal amounts of feces applied at each time and feces used in the afternoon stored at ambient temperature at the beef containment facility. For treatment A, a total of 3,000 g of inoculated feces were spread on the pen floor on each of the inoculation days. The corner of the plastic bag was cut, and 5 simulated fecal pats (~300 g each) were placed in the morning and afternoon. In the morning, 3 were placed on the bedding and 2 around the feed bunk area. In the afternoon, 1 fecal pat was placed on the bedding, 3 around the feed bunk area, and 1 by the water bowl. For treatment B, a steer from each pen was selected as a model super shedder (**MSS**) for the duration of the study.

These cattle had 100 g of inoculated feces spread across the perineum once per day in the morning. The remaining 2,900 g of inoculated feces was spread as fecal pats twice daily using the same pattern as for treatment A. For treatment C, 1 MSS per pen was also selected, with these cattle having 50 g of inoculated feces spread on the perineum and 50 g of inoculated feces spread across the brisket area. The remaining 2,900 g of inoculated feces was spread similarly to treatments A and B.

Detection and Enumeration of nal^R E. coli O157:H7

One gram of feces (fecal grab and fecal pat samples) was added to 9 mL of modified *E. coli* broth with 20 mg/L of novobiocin (**mEC-nov**) and incubated for 6 h at 37°C. Hide swabs were incubated for 16 h at 37°C with an additional 90 mL of mEC-nov. Ropes were shaken at 450 rpm for three 5-min periods with 5 min of rest (no agitation) and then incubated for 16 h at 37°C.

From these samples, a 1-mL aliquot was subjected to immunomagnetic separation using Dynabeads anti-O157 (Dynal, Lake Success, NY) and a Pickpen magnetic particle separation device (BioControl Systems Inc., Bellevue, WA) according to the manufacturer's instructions. Up to 3 sorbitol-negative (clear) colonies per plate were subjected to agglutination using an *E. coli* O157 latex kit (Oxoid, Nepean, Ontario, Canada).

Fecal grab samples positive for nal^R *E. coli* O157:H7 were serially diluted (1:10) in mEC-nov, and 100 μL of a range of dilutions was plated in duplicate onto CT-SMACnal, which was incubated for 16 h at 37°C. Only those plates having 30 to 300 colonies were used in determination of bacterial populations, and counts were recorded as colony-forming units per gram.

Statistical Analyses

In all statistical analyses, pen was the experimental unit. The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to compare number of *E. coli* O157:H7 shed over time in treatment groups (A, B_naive, B_super-shedder, C_naive, C_super-shedder), with week as the repeated variable and interactions (pen × treatment and pen × treatment group × week) as random variables. Orthogonal contrasts and odds ratios within the GENMOD procedure of SAS were used to compare impact of the 5 treatment groups (A, B_naive, B_super-shedder, C_naive, C_super-shedder) on the incidence of *E. coli* O157:H7 in samples collected from individual animals (fecal grab, hide swab) and from the feedlot environment (fecal pat, rope), with treatment group, week of study, and pen included in the model. Because all MSS had inoculated hides, these cattle were excluded from hide swab analyses.

The NLIN procedure of SAS was used to compare *E. coli* O157:H7 disappearance curves among the 3 treatments (A, B, and C) and evaluate inflection points as fecal counts of *E. coli* O157:H7 were not normally distributed. A 3-parameter single exponential decay model including a lag time was used:

$$\text{cfu} = \text{AS} + \text{b} \times \{1-[^{-\text{c}\times(\text{t}-\text{t0})}]\},$$

with colony-forming units of *E. coli* O157:H7 (log 10), AS as the curve asymptote, b as the slope of the curve, c as the fractional rate of accumulation of colony-forming units, t as the time in days after first inoculation with the organism, and t0 as the time in days before fecal shedding of the organism occurred.

RESULTS AND DISCUSSION

Spreading inoculated feces on the hides of MSS in treatments B and C increased ($P = 0.05$) the number of positive rope samples compared with treatment A (Table 1) even though similar numbers of *E. coli* O157:H7 were introduced into all pens. The presence of *E. coli* O157:H7 on ropes is correlated to the presence of this bacterium in the oral cavity of cattle (Smith et al., 2008). Consequently, grooming of hide-inoculated MSS by pen mates was likely responsible for increased detection of the organism in ropes from these treatments. Similarly, Kohari et al. (2009) demonstrated that grooming of calves by their dams reduced counts of coliform bacteria on calves by at least 1 log cfu/cm^2 of hide, although these authors did not determine the impact of this behavior on populations of oral microbial flora of the dams.

The likelihood of ropes being positive for *E. coli* O157:H7 was 4.48 times greater ($P < 0.001$) in the first 3 wk compared with subsequent weeks of the study (Table 1). Week 3 of the study had the greatest proportion of positive rope samples ($P = 0.05$, data not shown) and hide swabs ($P < 0.001$; Figure 1A). A simultaneous peak in positive rope and hide swab samples in all treatment groups at wk 3, 3 d after final spreading of inoculated feces, implies maximum contamination of the pen environment at this time, although fecal pats showed no similar temporal relationship (Table 1). The rapid decline in positive rope and hide swab samples after wk 3 was likely related to removal of *E. coli* O157:H7 from hides and pen surfaces by the cattle grooming and licking objects. Although recording such behaviors was beyond the scope of the present study, Ishiwata et al. (2008) determined that penned cattle fed ad libitum spent approximately 8% of daylight hours in oral-related behaviors such as grooming and licking.

Fecal pats positive for *E. coli* O157:H7 did not follow the temporal pattern of hide swab and rope samples because peak occurrence of positive fecal grab samples occurred across all treatment groups on wk 5 ($P < 0.001$; Figure 1B). A time lag from maximum hide and oral contamination until peak incidence of positive fecal samples would be expected because Shere et al. (2002)

Table 1. Odds ratios (OR) and confidence intervals (CI) for likelihood of samples positive (pos) for *Escherichia coli* O157:H7 collected over 8 wk from the environment of pens receiving feces inoculated with 10^6 cfu of *E. coli* O157:H7

	Fecal pat[2]				Rope[3]			
Treatment[1]	No. of pos	OR	CI	Signif.[4]	No. of pos	OR	CI	Signif.
A	10/28	1.74[5]	1.40 to 2.24	<0.001	8/32	0.73[5]	0.53 to 1.00	0.05
B	5/28	0.95[6]	0.83 to 1.09	0.46	13/32	1.09[6]	0.91 to 1.30	0.37
C	3/28	0.97[7]	0.63 to 1.51	0.90	14/32	4.48[7]	2.48 to 8.11	<0.001

[1]Treatment: feces (3,000 g) were spread on the pen floor in simulated fecal pats (treatment A); spread on pen floor (2,900 g) with 100 g spread on the perineum of 1 model super-shedder (MSS) steer per pen (treatment B); or spread on the pen floor (2,900 g) and spread on the perineum (50 g) and brisket (50 g) of 1 MSS per pen (treatment C).
[2]Freshly voided fecal pats were sampled and inoculated feces avoided.
[3]Rope, 120-cm manila rope available for oral access by cattle for 4 h.
[4]Significance, *P*-values for OR.
[5]Treatments B and C as referent compared with treatment A.
[6]Treatment B as referent compared with treatment C.
[7]Wk 4 through 8 of study as referent compared with wk 1 to 3 (during or within 1 wk of active distribution of inoculated feces).

noted an 8-d to 2-wk lag from introduction of a calf shedding *E. coli* O157:H7 until fecal shedding commenced in naïve calves. Concentration of the organism ingested during natural colonization would influence the lag time; McGee et al. (2004) proposed that multiple oral inoculations of 2 to 4 log cfu *E. coli* O157:H7 may be required before initiation of fecal shedding. The maximum length of the lag time before fecal shedding would depend on survival of *E. coli* O157:H7 in the environment, which would decline over time as influenced by climatic factors such as UV radiation and variable temperature (Oliver et al., 2010). Matthews et al. (2006b) estimated an average infectious period of 3 wk during which *E. coli* O157:H7 shed in the environment would readily colonize additional cattle. Accordingly, fecal shedding of the organism in the present study markedly declined by wk 6 (Figure 1B), 3 wk after the final environmental inoculation with *E. coli* O157:H7.

Increased contamination of the pen floor environment in freshly voided fecal pats was found in treatment A as compared with treatments B and C ($P < 0.001$; Table 1) because in the latter treatments MSS received 3.4% of the pen load of *E. coli* O157:H7 on their hides. Cattle walking through inoculated fecal pats would have facilitated the transfer of *E. coli* O157:H7 to freshly voided fecal pats. However, contamination of the pen floor with *E. coli* O157:H7 was less effective for producing fecal shedding of the organism than was contamination of the hides of MSS. Steers within treatments B and C were 1.3 times more likely ($P = 0.05$) to shed *E. coli* O157:H7 in their feces than steers in treatment A (Table 2). As expected, MSS were 8.1 times more likely than naïve steers to have perineal swabs positive for *E. coli* O157:H7 ($P < 0.001$), but fecal shedding of *E. coli* O157:H7 by MSS did not differ from that of pen mates, likely due to the inability of MSS to lick their own brisket or perineal regions (Hasker et al., 1989). These results demonstrate a greater effect of hide as compared with pen floor contamination for transmission of *E. coli*

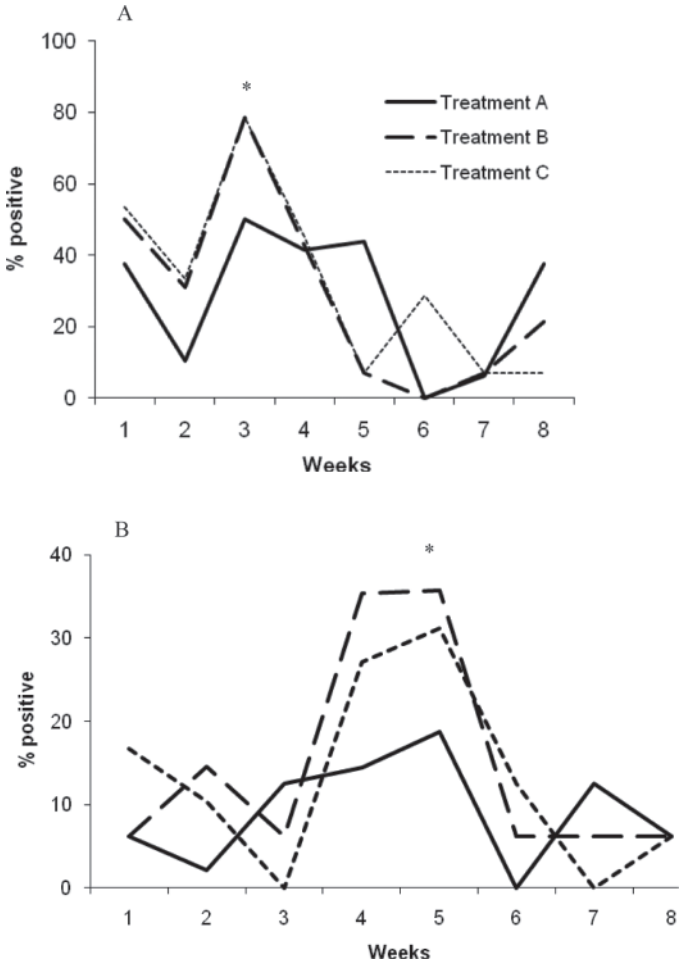

Figure 1. Proportion of perineal swabs (panel A) and fecal grab samples (panel B) that were positive for *Escherichia coli* O157:H7 from steers in treatments A (feces spread on pen floor from d 0 through 4 of wk 1 and d 14 through 18 of wk 3), B (feces spread on pen floor and perineum of 1 model super-shedder per pen), and C (feces spread on pen floor and on perineum and brisket of 1 model super-shedder per pen). The week with the greatest proportion of positive samples in all treatments ($P < 0.001$) is identified by an asterisk. Perineal swabs (panel A) collected from model super-shedders were excluded from treatments B and C.

Table 2. Odds ratios (OR) and confidence intervals (CI) for likelihood of perineal swabs and rectally collected fecal samples positive (pos) for *Escherichia coli* O157:H7 from steers in pens receiving feces inoculated with 10^6 cfu of *E. coli* O157:H7

			Perineal swabs				Fecal grabs			
Treatment[1]	Steer type	n[2]	No. of pos	OR	CI	Signif.[3]	No. of pos	OR	CI	Signif.
A	Naïve	16	59/208	8.10[4]	3.85 to 17.03	<0.001	19/240	1.43[4]	0.82 to 2.51	0.20
B	Naïve	14	61/182				30/210			
B	MSS	2	16/26	1.06[5]	0.91 to 1.23	0.46	6/30	1.30[5]	1.00 to 1.71	0.05
C	Naïve	14	66/182				31/210			
C	MSS	2	21/26	0.83[6]	0.64 to 1.07	0.15	3/30	0.83[6]	0.64 to 1.07	0.17

[1]Feces (3,000 g) were spread on the pen floor in simulated fecal pats (treatment A); spread on pen floor (2,900 g) with 100 g spread on the perineum of 1 model super-shedder (MSS) steer per pen (treatment B); or spread on the pen floor (2,900 g) and spread on the perineum (50 g) and brisket (50 g) of 1 MSS per pen (treatment C).
[2]n = numbers of steers sampled.
[3]Significance, *P*-values for OR.
[4]Naïve steers (all treatments) as referent compared with MSS (all treatments).
[5]Treatment A as referent (all steers) compared with naïve steers in treatments B and C.
[6]Treatment C as referent (all steers) compared with treatment B (all steers).

O157:H7, in accordance with McGee et al. (2004) who recognized grooming of hides as an important source of transmission of the organism among penned cattle.

Enumeration of *E. coli* O157:H7 in fecal grab samples did not differ across treatments, although maximum colony-forming units per gram were 1 log greater in naïve cattle within treatments B and C compared with cattle within treatment A (Table 3). Some cattle within treatments B and C may have obtained more *E. coli* O157:H7 from grooming hides than cattle within treatment A received from the pen floor. In contrast, the likelihood of cattle shedding quantifiable (>10 cfu/g) nal^R *E. coli* O157:H7 was 1.15 times greater in treatment B than A ($P = 0.02$), with treatment C intermediate of A and B. Shedding quantifiable *E. coli* O157:H7 may have been related to the lag time from study initiation until fecal shedding of the organism. Mean lag time before shedding was more than 15 d longer for steers in treatment A than B ($P = 0.04$; Table 4), with treatment C intermediate of A and B. The rapid colonization of steers in treatment B was also reflected in a greater proportion ($P < 0.001$) of steers fecal shedding quantifiable *E. coli* O157:H7 (>75%) compared with <35% of steers in the other 2 treatments.

Differences between treatments B and C may be related to limited grooming of the brisket because the anal and head/neck regions receive most attention during social licking in cattle (Simonsen, 1994). Treatment C was chosen because the brisket region of the hide is frequently contaminated with *E. coli* O157:H7 after cattle lay on contaminated fecal pats (Reid et al., 2002), but based on these results brisket contamination may have a limited impact on animal-to-animal transmission of this organism. In contrast, contamination of the perineum would be expected from fecal buildup in this region as commonly occurs in super-shedding cattle (Stephens et al., 2009), and the likelihood of hide-to-hide transmission (Collis et al., 2004) would also be

Table 3. Mean and maximum levels (cfu, $\log_{10}$) of *Escherichia coli* O157:H7, odds ratios (OR), and confidence intervals (CI) from steers shedding quantifiable (>10 cfu/g) naladixic-acid-resistant *E. coli* O157:H7 in feces

Treatment[1]	Steer type	Mean, cfu/g, $\log_{10}$	SEM	Maximum, cfu/g, $\log_{10}$	Feces >10 cfu/g	OR	CI	Signif.[2]
A	Naïve	2.11	0.3	3.66	10/240	1.42[3]	0.94 to 2.17	0.42
B	Naïve	2.62	0.2	4.69	20/210	1.17[4]	0.97 to 1.43	0.11
B	MSS	1.95	0.5	2.74	4/30	1.15[5]	1.02 to 1.30	0.02
C	Naïve	2.40	0.3	4.74	11/210	1.13[6]	0.98 to 1.30	0.09
C	MSS	1.92	0.7	1.98	2/30	0.83[7]	0.66 to 1.05	0.12

[1]Feces (3,000 g) were spread on the pen floor in simulated fecal pats (treatment A); spread on pen floor (2,900 g) with 100 g spread on the perineum of 1 model super-shedder (MSS) steer (treatment B); or spread on the pen floor (2,900 g) and spread on the perineum (50 g) and brisket (50 g) of 1 MSS steer per pen (treatment C).
[2]Significance, *P*-values for OR.
[3]The MSS from all treatments as referent compared with naïve steers from all treatments.
[4]Treatment A as referent (all steers) compared with treatments B and C (all steers).
[5]Treatment A as referent (all steers) compared with treatment B (all steers).
[6]Treatment B as referent (all steers) compared with treatment C (all steers).
[7]Wk 4 through 8 of study as referent compared with wk 1 to 3 (during or within 1 wk of active distribution of inoculated feces).

Table 4. Lag times before fecal shedding of *Escherichia coli* O157:H7 and proportion of steers fecal colonized (quantifiable and detectable levels)[1]

Treatment[2]	Lag time before fecal shedding, d	SEM	Steers quantify *E. coli*[3]	OR	CI	Signif.[4]	Steers detect *E. coli*[5]	OR	CI	Signif.
A	44.2[b]	5.0	2/16	3.65	3.44 to 3.89	<0.001	11/16	1.37	1.21 to 1.55	<0.001
B	28.6[a]	5.1	12/16				14/16			
C	38.2[ab]	5.3	5/16				11/16			

[a,b]Means with different superscript differ, $P = 0.04$.

[1]Odds ratios (OR) and confidence intervals (CI) comparing likelihood of steers fecal shedding *E. coli* O157:H7 in treatments A and C as compared with treatment B.

[2]Feces (3,000 g) were spread on the pen floor in simulated fecal pats (treatment A); spread on pen floor (2,900 g) with 100 g spread on the perineum of 1 model super-shedder (MSS) steer (treatment B); or spread on the pen floor (2,900 g) and spread on the perineum (50 g) and brisket (50 g) of 1 MSS steer per pen (treatment C).

[3]Proportion of steers with fecal samples where *E. coli* O157:H7 was quantifiable by dilution plating (>10 cfu/g of feces).

[4]Significance, *P*-values for odds ratios.

[5]Proportion of steers with fecal samples where *E. coli* O157:H7 was detectable by immunomagnetic separation but was at levels insufficient for quantification by dilution plating (1 to 10 cfu per g of feces).

greater for contamination in the perineal compared with the brisket region.

Alternatively, some of the differences noted between treatments B and C may have been related to the social dominance of the cattle selected as MSS. Generally, dominant individuals spend less time grooming pen mates and are groomed more often (Val-Laillet et al., 2009). Others have documented that highly aggressive individuals are groomed less often by pen mates (Hasker et al., 1989). In our study, we randomly selected MSS, and it would be interesting to investigate if selection of individuals based on dominance behavior would alter transmission patterns of *E. coli* O157:H7. Chase-Topping et al. (2008) defined a super-spreader of *E. coli* O157H7 as an individual whose behavior leads to more opportunities to infect other hosts than the majority of cattle. The present study included aspects of the super-shedder through investigation of *E. coli* O157:H7 in feces on the pen floor and of the super-spreader as a result of *E. coli* O157:H7 associated with feces on the hide.

The greater likelihood of cattle shedding *E. coli* O157:H7 in feces in treatment B as compared with A was likely related to enhanced persistence of shedding in treatment B. Within treatment A, steers had a maximum of 2 positive fecal samples over the course of the study (Figure 2). In comparison, 3 steers per pen in treatment B had 3 or more positive fecal samples, and 2 steers (1 per pen) had 4 consecutively positive fecal samples. Treatment C was intermediate of A and B with 2 steers per pen having 3 or more positive fecal samples. The intermittent shedding of *E. coli* O157:H7 by cattle has been well-documented (Besser et al., 1997; Loneragan and Brashears, 2005; Matthews et al., 2006a), and even super-shedding cattle are thought to shed *E. coli* O157:H7 intermittently (Matthews et al., 2006b; Chase-Topping et al., 2008; Stephens et al., 2009). However, the development of multiple persistently colonized steers in treatment B demonstrates the increased transmission of the organism when it is hide-associated compared with being only associated with the pen floor (Stephens et al., 2008).

The experimental design of Stephens et al. (2008) and the present study were similar, although more *E. coli* O157:H7 was used in the present study (10^6 cfu/g of feces) compared with 10^5 cfu/g in the high treatment of the previous study. The limited impact on fecal shedding of pen mates (total 12 positive samples) in the previous study led us to marginally increase the quantity of *E. coli* O157:H7 (from 10^5 to 10^6 cfu/g) used in the current study. Cray et al. (1998) did not produce fecal shedding in cattle after a single oral inoculation of 10^4 cfu, and fecal shedding was also not observed in the previous study after environmental inoculation with feces containing 10^2 cfu/g of *E. coli* O157:H7. Additionally, cattle shedding at levels of 10^6 to 10^9 cfu/g are present in commercial feedlot environments (Stephens et al., 2009), and use of feces inoculated at 10^5 cfu/g may have underestimated the impact of super-shedders in our previous study (Stephens et al., 2008).

Of interest, the current study was conducted during late September through December (mean temperature 3.1°C, mean day length 10.2 h; Environment Canada, 2010) when a seasonal decline in detection of *E. coli* O157:H7 would be expected, in contrast to the study of Stephens et al. (2008), which was conducted from May through July (mean temperature 15.1°C, mean day length 15.8 h; Environment Canada, 2010) during months of greater natural prevalence of the organism (Van Donkersgoed et al., 2001; Stanford et al., 2005) and when growth of the organism within the environment would have been likely (Oliver et al., 2010). Consequently, the combination of 10^6 cfu/g in the fecal inoculum and inoculation of both hides and pen floor used in the present study produced a robust design that would be useful for future studies investigating methods of mitigating transmission of *E. coli* O157:H7 from super-shedders to naïve pen mates.

Contamination of the pen floor influences dissemination of *E. coli* O157:H7 within pens of feedlot cattle,

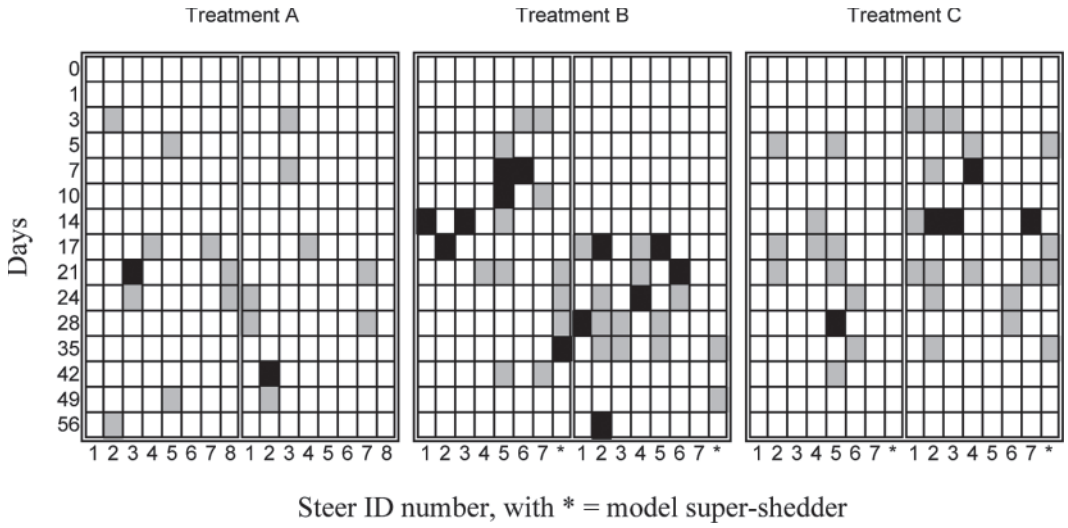

Figure 2. Fecal grab samples positive for *Escherichia coli* O157:H7 by pen, treatment, and steer over the course of the study. Treatment A is pen floor inoculation only. Clear squares = no detection, gray squares = positive detection, and black squares = positive by enumeration, asterisks = model super-shedder receiving inoculated feces on the perineum (treatment B) or perineum and brisket areas (treatment C) during d 0 through 4 and d 14 through 18. Pens within a treatment shared a water bowl, but an empty pen separated each treatment group.

but animal-to-animal contact may play a more important role in the transmission of this organism among cattle. Consequently, studies evaluating dissemination of *E. coli* O157:H7 should consider not only the quantity of the organism that are shed, but also the effects of animal behavior on transmission of this organism among pen mates.

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