Evaluation of a model for Escherichia coli O157:H7 colonization in streptomycin-treated adult cattle

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Objective—To develop a repeatable model for studying colonization with streptomycin-resistant Escherichia coli O157:H7 in adult cattle.

Animals—5 adult mixed-breed beef cattle.

Procedures—Cattle were surgically cannulated in the duodenum, treated daily with streptomycin (33 mg/kg) via the duodenal cannula prior to and during experimental colonizations, and colonized with 10^5 CFUs of streptomycin-resistant E coli O157:H7 via the duodenal cannula. Colonization of rectal mucus and shedding in feces were monitored. Antimicrobials were administered to eliminate the colonizing strain so that 5 repeated colonization experiments could be performed. A comprehensive analysis of colonization was performed at necropsy.

Results—Streptomycin treatment resulted in improved experimental colonization variables, compared with untreated controls, during initiation (days 2 to 6) and early maintenance (days 7 to 12) of colonization. Elimination of the colonizing strain followed by 5 repeated colonizations in the same animals indicated the repeatability of the protocol. Positive results of bacteriologic culture of feces 7 and 12 days after colonization were obtained in 100% and 84% of samples, respectively, across all animals and trials. At necropsy, highest magnitude recovery was in terminal rectal mucus.

Conclusions and Clinical Relevance—The model was highly repeatable and novel with respect to streptomycin treatment, use of duodenal cannulas, and repeated colonizations of the same animals. Its use in adult cattle, from which most bovine-derived food originates, is critical to the study of preharvest food safety. The findings have implications for understanding intermittency of shedding in the field and for proposed vaccine-based interventions. (Am J Vet Res 2006;67:1914–1920)

Escherichia coli O157:H7 is an intestinal pathogen of humans that causes hemorrhagic colitis and the occasionally fatal sequela known as hemolytic-uremic syndrome, which is mediated by Shiga toxin. Since 1982, when E coli O157:H7 was first associated with human disease following consumption of undercooked hamburger, the epidemiologic link to food contamination has been established. Contamination of ground beef and other food products, including leafy vegetables, processed meats, and milk, usually occurs via exposure to bovine feces. Bovine rectal mucosa is the primary reservoir of this pathogen, but cattle are not usually affected by the infection, a trait possibly conferred by their lack of intestinal expression of the Shiga toxin receptor. Intimin and long polar fimbriae, virulence factors of E coli O157:H7, are known to be important factors in colonization. Although progress has occurred, a comprehensive understanding of the interaction between E coli O157:H7 and cattle is still lacking. The occurrence, magnitude, and duration of shedding of E coli O157:H7 by cattle are highly variable in field and experimental situations. Because such variability suggests that unknown host and environmental factors are at play, adult cattle models for E coli O157:H7 are needed to study colonization.

In the natural setting, factors affecting initial colonization of naïve animals are unknown, but horizontal transmission is presumed and is observed experimentally. With experimental infection, age-related differences in pathogenicity are observed. Neonatal calves (≤ 3 weeks) have diarrhea and attaching and effacing histopathologic changes that are most severe in the youngest calves. Conflicting results regarding the occurrence or the absence of pathogenicity of E coli O157:H7 following experimental colonization in young calves have been reported. Adherence of E coli O157:H7 was reported in 1 yearling steer, but pathogenicity is usually not observed in adults. Despite the lack of detectable pathogenicity, persistent colonization and shedding of E coli O157:H7 by adult cattle is the pri-
mmary reason for contamination of ground beef, an important vehicle for E. coli O157:H7 infection in humans. Because adult cattle are the primary source of contaminated ground beef, modeling adult colonization by E. coli O157:H7 should be a priority.

Fecal shedding by orally colonized adults is short-lived and of lower magnitude, compared with that in young calves. Low-level, short-duration shedding by adult cattle has also been observed in other experiments, which is consistent with what is seen in the field. The reasons for differing shedding patterns between adults and young calves are thought to be attributable to diet, age-related differences in rumen function, or immune response. To more fully explore the variables affecting colonization, robust adult models are needed.

We hypothesized that problems associated with poor persistence and inconsistent colonization may be explained by colonization resistance, which refers to the ability of the intestinal microflora to resist colonization by an invading bacterium. The large intestine microflora includes hundreds of bacterial species, characterized by stable populations controlled by competition for limited nutrients and resistant to invading species unless perturbed by an exogenous factor or unless the invading bacterium can compete for a certain nutrient. This, the essence of Freter’s nutrient-niche hypothesis, has not been addressed in prior bovine E. coli O157:H7 colonization models, but has been addressed in a murine model. In that model, mice treated continuously with orally administered streptomycin are stably colonized with streptomycin-resistant E. coli O157:H7. Streptomycin treatment decreases the facultative anaerobic bacteria from 10^6 CFUs/g of feces to 10^2 CFUs/g of feces, allowing the invading E. coli O157:H7 strain to colonize the open niche. Results of another study suggest that E. coli O157:H7 colonizes the large intestinal mucosa in this niche, which influenced our decision to sample rectal mucus, as well as fecal samples.

With this background of murine models, we proposed to similarly develop a streptomycin-treated adult cattle model. We were, however, concerned that streptomycin could affect the rumen microflora, which controls normal digestive and fermentation processes, resulting in indigestion and anorexia. To avoid adverse effects of streptomycin on the rumen microflora, the drug was administered through a duodenal cannula, a device that can be easily maintained for up to 2 years in cattle, providing ready access to the lower portion of the gastrointestinal tract for sampling or dispensing. Thus, the cannula provided for postgastric administration of streptomycin as well as instillation of the initial experimental bacterial bolus.

The purpose of the study reported here was to develop a repeatable model for studying colonization with streptomycin-resistant E. coli O157:H7 in adult cattle. We intended to determine the success of colonization via a duodenal cannula, the effect of streptomycin treatment on colonization consistency, the difference between fecal shedding and rectal mucus recovery, and the possibility of using the same animals for repeated colonization trials, which lowers costs associated with use of the adult animal model.

Materials and Methods

Research cattle and environment—Five 8-month-old mixed-breed beef cattle (4 steers, 1 heifer; each approx 227 kg of body weight) were purchased and allowed to acclimatize to their initial environment, the Oklahoma State University Veterinary Teaching Hospital. Animal experiments and usage were approved by the Oklahoma State University Institutional Animal Care and Use Committee. Duodenal cannulas were placed 15 cm caudal to the pyloric junction and anchored and exteriorized between the last 2 ribs by use of a published technique. Cattle were treated alter surgery with penicillin G procaine (20,000 U/kg, IM, q 24 h) and ceftiofur sodium (0.5 mg/kg, SC, q 24 h) for 1 week; providone-iodine rinses were used daily for the cannula site for 2 weeks. During experimental colonizations, cattle were transferred to and maintained in an ABSL-2 large animal facility; each animal was placed in a pen alone. Research and animal care personnel followed strict ABSL-2 safeguards in the animal facility and laboratories in accordance with a protocol approved by the Oklahoma State University Institutional Biosafety Committee. Cattle were fed a pelleted total mixed ration composed of corn, ground alfalfa hay, and mineral supplements twice daily. Water was provided free choice. At termination of experiments, cattle with negative results of enrichment culture for E. coli O157:H7 were temporarily transferred to an outdoor facility and maintained similarly while the ABSL-2 facility was cleaned.

Bacterial strain and inoculation preparation—The E. coli O157:H7 (EDL 933) strain used was streptomycin and nalidixic acid resistant. Bacteria were grown overnight in 250 mL of Luria broth to an optical densityabs of 1.5 to 1.6. For inoculation, 100 mL of cell suspension was pelleted and washed 3 times with PBS solution and resuspended in 20 mL of PBS solution. Tenfold dilutions of the final inocula were prepared and plated with spreading on selective SMAC agar media to accurately assess the inoculation concentration.

Inoculation and streptomycin treatment—To test the effect of streptomycin on experimental colonization, 5 study cattle were randomly assigned by use of a coin toss to a streptomycin-treated group (n = 3) and a nontreated control group (2) and colonized per cannula identically. After 18 days of sampling, the colonization was terminated by administration of antibiotics, a washout period of 21 days ensued, and the group assignments were reversed. All procedures were repeated so that 3 complete trials (repetitions) were performed. Following those 2 trials, 3 additional trials, each 15 days in length, were performed with streptomycin in continuous use on all 5 cattle; washout periods ranged from 6 days to 4 weeks. For inoculation, an endotracheal tube was placed in the opened cannula, followed by cuff inflation for sealing. Each animal received 10 mL of the prepared inoculum via the medicinal access port of the tube followed by a 5-mL flush with PBS solution via the same port, followed by a 40- to 120-mL flush with PBS solution via the main lumen of the tube. No loss of the inoculating bolus was ever observed. For cattle that received streptomycin treatment daily, a 1-g/mL solution of streptomycin sulfate in water was prepared and administered via the cannula at 33 mg/kg of body weight. On the morning of colonization initiation, streptomycin treatment was delayed approximately 12 hours until the evening sampling. Cattle receiving streptomycin daily began receiving the treatment regimen 3 days prior to...
colonization and continued to receive treatment until the colonization was terminated.

Sampling and media—Samples of feces and rectal mucus were collected at 0, 0.5, 1, 2, 3, 5, 7, 9, 12, 15, and 18 days after inoculation. Fecal samples ranged from 5 to 20 g and were collected directly from the terminal portion of the rectum. Feces were weighed and suspended at a 1:10 ratio in 1% tryptone with mixing and placed on ice for 1 hour prior to plating. Mucus samples were acquired from the rectum via palpation; briefly, following manual evacuation of the feces, mucus was collected from the ventral half of the most caudal 30 cm of the rectum by scraping with the lid from a sterile syringe casing. Fecal contamination of mucus was minimized by physical removal of feces, where possible, or reacquiring the sample if fecal contamination was grossly apparent. Mucus was scraped until 200 to 500 mg was acquired; mucus was suspended at a 1:2 ratio in 1% tryptone, vortexed vigorously, and placed on ice for 1 hour prior to plating. Feces and mucus samples were processed into 10-fold dilution series by diluting 100 µL of initial sample into 900 µL of sterile PBS solution, vortexing, and plating 100 µL of each dilution onto agar media by even spreading with an alcohol-flamed spreading tool. Bacterial plates were incubated 12 to 18 hours at 37°C prior to assessment and counting. Diluted feces and mucus samples were plated onto SMAC agar and SMAC agar with the antimicrobials streptomycin (40 µg/mL) and nalidixic acid (50 µg/mL). On the antimicrobial-selective media, sorbitol-negative (white) colonies were enumerated daily and, where colony morphology was unusual, subjected to confirmatory testing with a commercially available immunoassay. All colonies were also enumerated daily on the SMAC medium without antimicrobials, which gave an estimate of the total concentration of streptomycin-resistant, facultative anaerobic bacteria in samples. Enrichment cultures were used for all daily samplings. Briefly, 100 to 150 µL of primary sample was placed in tryptic soy broth with streptomycin (40 µg/mL) and grown at 37°C for 16 hours with shaking (210 oscillations/min). When the inoculated strain was not recovered from primary samples, the enrichment samples were subjected to dilution series and plated similarly. Enrichment samples yielded qualitative data of presence (enrichment positive) or absence (enrichment negative) of isolates. For purposes of computation of means, enrichment data were transformed to a semiquantitative estimate of 50 CFUs/g of feces or 10 CFUs/g of mucus, which was 50% of the lowest limit of detection for each of the primary sampling regimens. Rectal biopsy specimens, obtained at days 0, 3, 9, and 15 of each colonization trial, were procured from the dorsal half of the terminal 3 cm of rectum via an equine uterine biopsy instrument and fixed in neutral-buffered 10% formalin, processed through graded concentrations of alcohol, sectioned at 5 µm, stained with H&E, and cover slipped. The final trial, primarily descriptive and qualitative, was conducted with the knowledge that a valid statistical analysis of these groups was not possible.

Statistical analysis—The nonparametric Wilcoxon signed rank test was used in a 1-tailed fashion to test the hypothesis that streptomycin-treated cattle would have significantly (P < 0.05) better colonization variables, compared with untreated control cattle. Data are given as mean ± SD. In comparing treated with untreated cattle, accurate assessment of colonization consistency and persistency was performed by use of not only numeric data, but also the phases of colonization, which were divided into 3 functional stages: initiation (days 2 to 6; sampling on days 2, 3, and 5), early maintenance (days 7 to 12; sampling on days 7, 9, and 12), and late maintenance (days 13 to 18; sampling on days 15 and 18). Support for this concept was provided by studies by Sheng et al. and Rice et al. in which culture-positive results obtained for ≤ 1 week indicated lack of a stable association between the host and bacterium.

Results

Cattle health—All cattle remained healthy throughout the course of the 6 repeated colonizations. Some cattle had transient, moderate bloat during the termination of colonization trials. This was presumed to be secondary to the effects of the 2 antimicrobials that were administered. The cunella of 1 steer became broken and displaced during the course of 1 experiment and was replaced. Sporadic diarrhea was seen in some cattle but was not correlated with any colonization event. All cattle had moderate average daily gains of 0.71 ± 0.05 kg over the 221 days of these studies. No attaching and effacing histopathologic changes were recognized in any animal during the course of the colonizations or the necropsy study (4 rectal biopsy specimens examined per animal per trial).

Effects of streptomycin—Recovery of the input strain in a time-relative manner for feces and mucus was determined (Figure 1). Streptomycin treatment increased E.coli O157:H7 shedding in mucus during initiation (P = 0.01) and early maintenance (P = 0.02), but not late maintenance. Streptomycin treatment increased E.coli O157:H7 shedding in feces only during
initiation ($P = 0.01$), but not during early and late maintenance.

Persistence and magnitude of bacterial recovery in mucus and feces—Greater persistence of recovery was evident in mucus than feces during the 2-phased trial (trials 1 and 2) that tested the effect of streptomycin (Figure 1). In determination of persistence of colonization, days of persistence were defined as the last sample day on which positive results were obtained unless $≥ 2$ intervening samples yielded negative results between 2 final samples that yielded positive results. The latter situation was negated, as it possibly implied a reinfection from another animal. In cattle treated with streptomycin, duration of $E$ coli O157:H7 persistence (ie, detection in samples) was $16.8 ± 2.7$ days in mucus and $14.0 ± 4.6$ days in feces. In cattle that were not treated, duration of $E$ coli O157:H7 persistence was $15.6 ± 2.5$ days in mucus and $9.8 ± 5.4$ days in feces. In analyses of the other 3 trials in which streptomycin was in continuous use (Table 1; trials 3, 4, 5), the input strain was recovered in mucus for $13.13 ± 3.25$ days and in feces for $10.93 ± 3.97$ days ($P = 0.02$). Higher magnitude colonization was seen in mucus, compared with feces, on day 2 ($P = 0.025$), day 3 ($P = 0.005$), and day 5 ($P < 0.05$), but was not significantly different between feces and mucus on days 7, 9, 12, and 13.

Repeatability of colonization—Five full-length trials of 15 or 18 days were conducted on the same 5 cattle. For bacterial recovery from mucus across all 5 trials, $92\%$ of all samples yielded positive results at day 9, $84\%$ of all samples yielded positive results at day 12, and $72\%$ of all samples yielded positive results at day 15. No substantial diminished colonization magnitude or persistence was detected in any subsequent trial (Table 1).

Sites of colonization—In a final colonization trial, cattle were euthanized and necropsied with a goal to describe the temporo-spatial distribution of the colonizing strain of $E$ coli O157:H7. The $E$ coli were not detected in the abomasum or rumen (Table 2), but were recovered from the gall bladder in 3 of the 5 cattle. Generally, the highest concentrations of bacteria were detected in the rectal mucus samples. However, this was not true for 1 steer that yielded positive results of enrichment cultures for many samples even though it was euthanized 24 hours after colonization. This animal inadvertently received a $1$-log order of magnitude lower colonization bolus (as determined by CFU determination on the inoculum), which could explain the reduced magnitude of recovery. The chief general observation was that, within 7 days, the terminal portion of the rectum became the primary site of localization, with ileal mucus being a secondary site of recovery. It was also evident that the colonizing bolus was cleared from the duodenum quickly and was only detected in the duodenum of the animal euthanized 1 day after colonization. Histologic specimens of the gastrointestinal tract (10 sites) and gall bladder (1 site) revealed no evidence of attaching and effacing histopathologic changes.

![Figure 1](image-url) —Results of bacteriologic culture of feces (A) and rectal mucus (B) for $Escherichia$ $coli$ O157:H7 in cattle ($n = 5$) that were or were not treated with streptomycin and were inoculated with $10^8$ CFUs via cannula into the duodenum. Bars represent SE of the log$_2$ values. Values at time 0 represent samples acquired 12 hours after inoculation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 7</th>
<th>Day 12</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feces</td>
<td>Mucus</td>
<td>Feces</td>
</tr>
<tr>
<td><strong>Cattle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial concentration</td>
<td>0–10$^3$</td>
<td>10–10$^2$</td>
<td>0–10$^3$</td>
</tr>
<tr>
<td><strong>Cattle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial concentration</td>
<td>10–10$^6$</td>
<td>10–10$^2$</td>
<td>0–10$^6$</td>
</tr>
<tr>
<td><strong>Cattle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial concentration</td>
<td>10–10$^6$</td>
<td>10–10$^2$</td>
<td>0–10$^6$</td>
</tr>
</tbody>
</table>

*No. of cattle with positive results of bacteriologic culture. †Range of values for all 5 cattle.

Table 1—Concentrations (CFUs/g of feces or mucus) of $Escherichia$ $coli$ O157:H7 in feces and rectal mucus of 5 streptomycin-treated adult cattle on various days after inoculation through a duodenal cannula in 3 trials.

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Discussion

The primary goal of developing an adult cattle model for the study of colonization by E. coli O157:H7 is a model that consistently yields reliable and repeatable establishment of colonization. The streptomycin-treated adult cattle model described here had several positive attributes that were consistent with this goal and were novel in 3 respects: initiation of colonization via the duodenal cannula, facilitation of occupation of that niche by use of the continuous streptomycin treatment, and use of the same cattle for repeated colonization experiments. In particular, we believe this model will be useful for studying the role of various factors in colonization of cattle by use of streptomycin-resistant mutant strains for these factors.

The most important benefit of the model is its reliability and repeatability in adult cattle. Adult cattle are more difficult to experimentally colonize than calves, and this difficulty is experienced in the areas of shedding concentrations and proportions of culture-positive study animals 1 week after colonization. The shedding concentrations of these streptomycin-treated cattle at 7 and 15 days after inoculation (10⁷ to 10⁸ CFUs/g and 10⁸ to 10⁹ CFUs/g, respectively) were similar to those found in other adult cattle colonization models. Furthermore, the model appears comparable to other adult models with respect to proportions of culture-positive study cattle 1 week after colonization. In this model, 100% of the cattle were actively shedding at day 12 in 4 trials; in the fifth trial, 100% were actively shedding through 7 days. Also, colonization persisted in all 5 animals for 15 days in at least 2 of the 5 trial repetitions. By comparison, Cray and Moon reported that 9 of 9 orally colonized adults were actively shedding at 14 days. Sheng et al. reported that 6 of 10 orally colonized cattle were shedding on day 12, and 8 of 8 rectally colonized cattle were shedding at day 14. Additionally, 12 of 18 adult cattle administered bacteria PO were shedding at day 12 to 14 in a study by Grauke et al. Further difficulty in adult colonization was reported for studies by Ohya et al. and Buchko et al., in which 4 of 8 calves and 9 of 18 yearling steers, respectively, yielded negative results of bacteriologic culture of feces by 7 and 9 days, respectively.

Furthermore, in the analysis of animal-specific shedding in studies by Grauke et al. and Sheng et al., culture-positive cattle at 25 days after infection and beyond were the same cattle that typically yielded positive results at days 12 to 14. Therefore, it appears that positive results of culture at days 12 to 14 are a strong predictor of long-term persistence of infection. Although longer colonizations were not a goal of the present study and were not attempted in these cattle, this trend indicates that long-term colonization is likely achievable with this protocol.

Reproducible colonizations of the same individual cattle were achieved in the study reported here. Successful colonization of the same 5 cattle 6 times without substantial variations of shedding concentrations and proportions colonized was achieved. Reproducibly colonizing the same cattle has been reported, but in those studies, cattle were reisolated once instead of 5 times as in the study reported here. In 1 study, there was a significant decrease in duration of colonization after reisolation, but in the other study, no differences in shedding patterns after reisolation were detected. Furthermore, reisolation in those studies was performed after a lengthy interval to allow for natural elimination of E. coli O157:H7. Attaching and effacing histopathologic changes were not detected in the experiments reported here, and this raises the possibility that, although multiple colonizations occurred, a host response was not induced. However, lack of such lesions or response could be caused by sample timing or the use of light microscopy instead of immunofluorescence or electron microscopy. Such absence is also consistent with a report of other experimentally infected adult cattle and the findings typically seen in naturally infected cattle. Furthermore, results of 1 study suggest that E. coli O157:H7 cannot be detected attached to epithelium in histologic sections when shedding concentration is < 10⁶ CFUs/g of feces, a threshold established previously. Finally, it seems reasonable to consider that the immune response of cattle to E. coli O157:H7 is manifested as intermittent shedding, but Johnson et al. determined that the serologic responses of cattle were not correlated with elimina-

Table 2—Sites of isolation of E. coli O157:H7 (CFUs/g of sample) on various days after inoculation through a duodenal cannula in 5 streptomycin-treated adult cattle that were euthanized on various days.

<table>
<thead>
<tr>
<th>Site</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steer</td>
<td>Heifer</td>
<td>Steer</td>
</tr>
<tr>
<td>Rumen, abomasum</td>
<td>0, 0</td>
<td>0, 0</td>
<td>0, 0</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>E+</td>
<td>E+</td>
<td>E+</td>
</tr>
<tr>
<td>Duodenum contents</td>
<td>1.0 x 10⁹</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duodenum mucus</td>
<td>E+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ileum contents</td>
<td>4.0 x 10⁹</td>
<td>3.0 x 10⁹</td>
<td>2.1 x 10⁹</td>
</tr>
<tr>
<td>Ileum mucus</td>
<td>E+</td>
<td>E+</td>
<td>2.0 x 10⁹</td>
</tr>
<tr>
<td>Cecum contents</td>
<td>E+</td>
<td>E+</td>
<td>4.0 x 10⁹</td>
</tr>
<tr>
<td>Cecum mucus</td>
<td>E+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Colon contents</td>
<td>E+</td>
<td>1.0 x 10⁹</td>
<td>0</td>
</tr>
<tr>
<td>Colon mucus</td>
<td>E+</td>
<td>E+</td>
<td>2.0 x 10⁹</td>
</tr>
<tr>
<td>Feces</td>
<td>E+</td>
<td>E+</td>
<td>1.4 x 10⁹</td>
</tr>
<tr>
<td>Rectal mucus</td>
<td>E+</td>
<td>E+</td>
<td>8.0 x 10⁹</td>
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</table>

0 = Not detected by use of primary or enrichment cultures. E+ = Positive results obtained by use of enrichment culture only.
tion or reinfection with *E. coli* O157:H7. As a result of their findings, they speculated that persistence and reinfection in the face of an immune response contributed to persistent infection in the herd and that vaccine-based interventions could be of questionable efficacy. The reproducible colonizations attained in the present study seem to add additional weight to that argument and may also have broader implications for epidemiologic surveillance and food safety. For surveillance of herds, this confirmed potential for reproducible colonization largely restricts such data to point-in-time relevance and renders such surveillance powerless to predict future trends. For food safety, similarly, our findings suggest that samples must be obtained from every animal at the point of slaughter. Obtaining samples from a fraction of a herd or from animals 1 day before slaughter would likely be poorly predictive of which cattle were actively shedding *E. coli* O157:H7 at the time of slaughter.

Administration of the colonizing bolus via the duodenal cannula conferred the principal advantage of avoiding ruminal dilution of the bolus. Whether or not dilution of the inocula in ruminal contents (estimated to be 100 to 120 L in these cattle) affects colonization success is not known and was not tested here. However, the data acquired from the streptomycin-treated adult cattle were more similar to that of the rectal administration model than the oral or ruminal colonization models. Thus, results of the present study and those obtained by use of the rectal colonization model indirectly indicate that success of experimental colonization is negatively affected by transit through the upper portion of the gastrointestinal tract. To further support this assertion, conditions simulating rumen fluid of well-fed cattle negatively affect growth of *E. coli* O157:H7.

Streptomycin caused a substantial reduction of susceptible facultative anaerobic bacteria by 6 log-orders of magnitude in a murine *E. coli* O157:H7 colonization model. However, although the results indicated a beneficial effect of streptomycin on success of colonization with *E. coli* O157:H7, such a magnitude of reduction was not observed in the streptomycin-treated cattle, in which reduction by 2 log-orders of magnitude (10^6 to 10^4 CFUs/g) was usually observed. The reasons for this difference could be a higher proportion of naturally streptomycin-resistant microflora in cattle than in mice, the cumulative acquisition of resistance by facultative anaerobes in the present study, or the diminishing effect on the efficacy of the drug caused by the acidity of the bovine rectum.

In sampling rectal mucus as well as feces, it was determined that rectal mucus samples yielded higher magnitudes of shedding during early colonization and that use of rectal mucus also resulted in more extended recovery of the organism. Mucus was processed differently than feces because initial vortexing was required to counteract the viscosity of the former but introduced problems with the latter. Although this could have affected recovery numbers, it should not have affected the analysis of colonization longevity or shedding occurrence in cattle and likely had a negligible effect on the quantitative data during early colonization, when the difference was large. Although our mucus sampling and resultant improved recovery of *E. coli* O157:H7 were broadly similar to the rectoanal mucosal swab culture method described by Rice et al., the basis for and implementation of mucus sampling in our study were 3-fold: generic *E. coli* reside in the mucus layer of the intestine and metabolize mucin-derived sugars; murine cecal and colonic mucus support growth of *E. coli* O157:H7 strain EDL933; the strain used in this study; and the mucus mass required and protection of the biopsy sites in the dorsal portion of the rectum necessitated sampling the ventral 30 cm of the distal portion of the rectum. It should be further stated that the mucus sampling technique necessarily included mucosal epithelium, and its potential as a confounder has been considered. Because *E. coli* O157:H7 cannot be detected attached to epithelium in histologic sections when shedding concentrations are < 10^6 CFUs/g of feces, it seems reasonable to suggest that the inclusion of epithelium in our mucus samples had a largely negligible effect on bacterial counts. It might also suggest that the most promising locale for interruption of the bovine-*E. coli* O157:H7 relationship is within the mucus, not at the mucosal surface.

In the terminal (euthanasia) colonization trial, site-specific localization and magnitude of *E. coli* O157:H7 colonization were assessed at necropsy. Low-level, widespread dilution of the bolus occurred at day 1 in intestinal contents. At days 3 and 7, higher magnitude recovery both distally and within mucus was typically observed. This indicated that there was not just transient passage of the bolus, but that a stable colonization of bacteria in the mcosa of the distal portion of the intestine, accompanied by apparent replication, was occurring. Furthermore, the highest magnitude of bacterial recovery was typically in the mcosa of the terminal portion of the rectum, lending additional support to the finding of Naylor et al. that the terminal rectal mcosa is a site of tropism for *E. coli* O157:H7. However, the consistent recovery of *E. coli* O157:H7 from ileal mcosa or feces was partially at odds with the previous study, although cattle in that study were younger and euthanized later, at 3 to 8 weeks after colonization. The mcosa of the terminal portion of the rectum and ileum is rich in lymphoid tissue, and this may be important for the confirmed and suspected areas of tropism for *E. coli* O157:H7. Recovery from the gall bladder of the input strain was accomplished in 3 cattle in the study reported here, a finding that corroborates previous work by Stoffregen et al.

Although models of *E. coli* O157:H7 colonization in adult cattle are most relevant to the shedding and food contamination problem, use of adult cattle is costly. The purchase price and maintenance costs of adult cattle are substantially higher than similar costs of studies with young calves, and such costs could result in small sample sizes. Because sample sizes are generally smaller, this places greater emphasis on consistency to achieve appropriate statistical power. A further disadvantage of this model is its labor intensity, chiefly caused by the daily streptomycin treatment, which is not administered in cattle in other colonization models. However, the chief compensation for these disadvantages was the
consistently reliable and reproducible colonizations of adult cattle, which allowed testing of a hypothesis with only 5 animals. The model is not presented as a replacement for existing models, but provides a useful alternative for other E. coli O157:H7 investigations, especially those in which competitive exclusion, vaccine strategies, or single-gene knockouts are being tested or used.

References