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Impact of the Direct Application of Therapeutic Agents to the Terminal Recta of Experimentally Colonized Calves on Escherichia coli O157:H7 Shedding

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Enterohemorrhagic Escherichia coli O157:H7 is an important intestinal pathogen of humans with a main reservoir of domesticated ruminants, particularly cattle. It is anticipated that the risk of human infection can be reduced by controlling the organism within its reservoir hosts. Several options for the control of E. coli O157:H7 in cattle have been proposed, but none have been demonstrated to be successful in the field. Here we describe a novel experimental method, based on the terminal-rectum-restricted colonization described previously, to eliminate fecal carriage of E. coli O157:H7. In experimentally challenged calves, direct application to the rectal mucosa of either of two therapeutic agents, polymyxin B or chlorhexidine, greatly reduced bacterial shedding levels in the immediate posttreatment period. The most efficacious therapeutic agent, chlorhexidine, was compared in orally and rectally challenged calves. The treatment eliminated high-level shedding and reduced low-level shedding by killing bacteria at the terminal rectum. A rapid-detection system based on the ability to identify E. coli O157:H7 from swabs of the rectal mucosa was also assessed. This test was sufficiently sensitive to identify high-level bacterial carriage. Thus, a combination of the detection method and treatment regimens could be used in the field to eliminate high-level fecal excretion of E. coli O157:H7, so greatly reducing its prevalence within this host and the risk of human infection.

To our knowledge, there is no method currently being applied to control the colonization by this microorganism in food-producing animals. However, several methods have been tested experimentally and these have been thoroughly reviewed (8, 30). Vaccination is potentially the most effective and economically viable option, and several candidate vaccines have been examined (14, 19, 34). Though a vaccine based on secreted proteins showed experimental promise (32), at field scale this did not perform satisfactorily (25). Other vaccines are being developed, but the possibility exists that none will be sufficiently protective to substantially reduce prevalence levels. Several groups have observed an effect on fecal shedding (determined either by prevalence, duration of shedding, or fecal concentration) from various biological controls (4, 5, 29, 31, 33, 35). Dietary manipulations have also been reported to alter fecal shedding of E. coli O157:H7 in cattle, but data from different studies are conflicting (17, 27). Chemical compounds with antimicrobial activity, esculin (15) and sodium chloride (7), have both been shown to reduce prevalence experimentally, but the latter has not been approved for use in food-producing animals due to potential mammalian toxicity. More-conventional antimicrobials, such as neomycin and oxytetracycline, had little effect on shedding levels in calves in one study (1), but in another study neomycin was shown to reduce shedding in calves (16). However, continuous use of antimicrobials to treat E. coli O157:H7 has been traditionally seen as unacceptable through fears of selecting for bacterial resistance. Since the maintenance of E. coli O157:H7 within a cattle population can be dependent upon a small proportion of cattle that are shedding high levels of the organism in feces (22), a

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control strategy that successfully targets this minority of positive animals should have a major impact on E. coli O157:H7 prevalence. Colonization of the terminal rectum (TR) is required for this high-level fecal shedding (21), and targeting of treatments to the TR (24) provides an excellent opportunity for a control method. The accessibility of the site means that antimicrobial agents can be applied directly to the TRs of food-producing animals in a single application. By the same principle, the effect of applying multiple doses of E. coli O157:H7 lytic bacteriophages to the TRs of colonized cattle has been recently assessed (29) and reduced but did not eliminate bacterial shedding. In this study, two compounds, polymyxin B and chlorhexidine, were assessed for the ability to reduce or eliminate E. coli O157:H7 from experimentally challenged calves by administration directly to the rectal mucosa. It has shown that a proportion of orally challenged calves have low levels of E. coli O157:H7 originating from regions other than the TR, as demonstrated by postmortem sampling of intestinal contents within this and previous studies (6, 11, 24).

Our experimental design therefore involved a comparison of treatment efficacy in both rectally and orally challenged calves in order to assess the impact on fecal shedding of non-TR sites of colonization. An initial experiment (experiment 1) involved a total of 54 weaned calves in three batches of 18 that were challenged with a nalidixic acid-resistant, Shiga toxin-negative E. coli O157:H7 strain (ZAP 198) (24). The initial objective was to compare E. coli O157:H7 shedding following rectal challenge to the established oral challenge route (24), as it was important to demonstrate that the two challenge routes created qualitatively and quantitatively similar patterns of colonization at the TR. Rectal challenge would then permit an assessment of the efficacy of subsequent treatment methods in the absence of bacteria originating from nonrectal sites.

The objective of experiment 2 was to assess the impact of two agents applied to the TR mucosa on E. coli O157:H7 shedding. In experiment 2, a total of 53 calves in six batches were successfully colonized (defined by fecal E. coli O157:H7 concentrations of greater than 10^8 CFU/g immediately prior to treatment) (initially, both the chlorhexidine and polymyxin protocols were highly successful in rectally challenged calves but the chlorhexidine treatment was selected for more extensive assessment.

Detection of rectally colonized E. coli O157:H7 by rectal swabbing of colonized cattle is a highly sensitive means of identifying positive animals when combined with enrichment culture (13, 26). An additional objective within this study was to combine rectal swabbing with an immunochromatographic test of sufficient sensitivity to create a rapid field method to detect high-level bacterial shedding. Such a procedure could offer many advantages when used in conjunction with the aforementioned treatment regimen.

MATERIALS AND METHODS

Animals and experimental challenge. Experimental calf challenges were performed at Morerun Research Institute (MRI) in containment level 2 housing facilities authorized by Home Office license number 60/3179. Ethical consent was obtained from the MRI Animal Experiments Committee. Calves were reared conventionally on the same farm of origin until at least 2 weeks postweaning and transported to MRI, where they were acclimatized for 1 week prior to challenge. Calves were tethered in individual pens separated by solid walls. They were fed twice daily on QRD pellets (BOCム Pauls, Renfrew, United Kingdom) and had ad libitum access to hay and water. Prior to challenge, fecal samples from each calf were confirmed to be negative for E. coli O157 by overnight enrichment culture in tryptic soy broth (Oxoid) containing 20 mg/liter novobiocin, followed by immunomagnetic separation with anti-O157 immunoglobulin-coated paramagnetic beads (IDG, United Kingdom) and culture on CT-SMAC plates (celfixime-tellurite sorbitol MacConkey; Oxoid). At the time of challenge, the mean ages were 114 days (experiment 1, orally challenged), 115 days (experiment 1, rectally challenged), 80 days (experiment 2, orally challenged), and 71 days (experiment 2, rectally challenged).

Bacterial challenge and enumeration. The challenge bacteria (nalidixic acid-resistant E. coli O157:H7 Walla J) were cultured overnight in L broth (Invitrogen) at 37°C with aeration. Each oral inoculum was created by adding 1 ml of undiluted cultural to 20 ml of phosphate-buffered saline (PBS), resulting in an approximately 10^9 CFU per dose. The oral inocula were administered to the calves via a stomach tube and washed down with 500 ml of sterile PBS. Rectal challenge was performed by smearing a large cotton swab (150-mm Jumbo cotton swab; Fisher Scientific) saturated with approximately 1 ml of an undiluted overnight bacterial culture (10^9 CFU/ml) over the rectal mucosa, a method similar to that described by Sheng et al. (28).

Feces were sampled on a daily basis and separated into surface and core components as previously described (24). There were some missing data; one calf died of unrelated causes, and three calves in one group became colonized with a nalidixic acid-resistant, sorbitol-fermenting E. coli strain that obscured the selective media used for enumeration. Stools were graded according to the ease with which the surface and core components were separated (A, separation likely to be poor due to feces being dry and pelletted; B, separation likely to be good; C, separation likely to be poor due to feces being too soft; D, separation impossible due to feces being liquid). Samples too liquid to be separated were processed as “whole feces.” Ten-gram quantities of feces were homogenized in 90 ml of sterile PBS and where appropriate were serially diluted in 10-fold steps in PBS. One hundred microliters of each dilution was spread onto sorbitol MacConkey agar plates containing 15 μg ml^-1 nalidixic acid (N-NSMAC; Oxoid). All inoculated media were incubated overnight at 37°C. Non-sorbitol-fermenting colonies were counted, and a colony from each sample was tested for O157 lipopolysaccharide (LPS) with a latex agglutination test kit (Oxoid). The concentration of E. coli O157:H7 in log, CFU per gram was calculated. When performed, enrichment cultures consisted of 1 ml of an undiluted fecal suspension added to 9 ml of L broth containing 15 μg ml^-1 nalidixic acid. Following incubation at 37°C for 24 h, the enrichment cultures were streaked onto N-NSMAC plates and these were incubated overnight at 37°C.

Postmortem sampling. Postmortem sampling was performed on calves shedding E. coli O157:H7 beyond day 14 postchallenge in experiment 1 to confirm the TR-restricted colonization of rectally challenged calves and provide information on the possible degree of non-TR colonization of orally challenged calves. A full description of the methods and results of the postmortem sampling will be submitted for publication elsewhere. Essentially, 10-g samples of gut contents were obtained and E. coli O157:H7 bacteria were enumerated as described for fecal samples. For this paper, results from the large intestinal contents have been pooled. The proximal colon concentration was the mean of contents from three sites: the apex of the cecum, the illococcecolic valve, and the site just proximal to the narrowing where the proximal colon becomes the spiral colon. The distal colon concentration was the mean of the contents from the spiral colon (point of inflection) and the distal colon (midway between the spiral colon and rectum).

Application of therapeutic agents to the TR. In experiment 2, application of therapeutic agents was performed at 7 days postchallenge. This timing band was selected, on the basis of results of experiment 1 and previous experience with this model, to allow sufficient time for establishment of colonization and before the point at which E. coli O157:H7 shedding was expected to fall. Two treatment preparations were developed. One preparation included polymyxin B sulfite at a concentration of 2 mg/ml with sodium alginate at 10 mg/ml. This was applied via a syringe to the rectal mucosa in a volume of 100 ml. The second preparation contained chlorhexidine glucuronate at a concentration of 2% (wt/vol) in a total volume of 500 ml. The choices of therapeutic agents were based on minimal gut absorption, low host toxicity, bactericidal efficacy, and a low risk of generating bacterial resistance. Alginate was used in the polymyxin preparation to increase the time of contact with the mucosal surface. This was not used for the chlorhexidine preparation, as a larger volume was applied. Therapeutic concentrations were based upon in vitro determination of the concentration and time required to achieve 100% killing of the challenge strain (data not shown).

Swab recovery and E. coli O157 detection. The rectal mucosas of calves to be treated with therapeutic agents in experiment 2 were swabbed with large cotton swabs that were then vortexed in 5 ml of PBS. The presence of E. coli O157 was
which were then incubated for 1 h. Pre- and postchallenge serum samples from and standard sera from 1 in 12.5 to 12,800 were applied to the coated plates, carbonate buffer, and incubated overnight at 4°C. Twofold dilution series of test assay. Microtiter plates (Immulon 2 HB; Thermo Electron) were coated at the (12) and used as a coating antigen for an indirect enzyme-linked immunosorbent Biological Laboratories) was conjugated to polymyxin B as previously described calves were collected prior to challenge and at postmortem. Purified O157 (List sample diluent. A second O157 Coli-Strip was then immersed in the suspension centrifuged (1,500/f11003H11006). Quantification of serum anti-O157 LPS IgG. Serum samples of experiment 1 calves were collected prior to challenge and at postmortem. Purified O157 (List Biological Laboratories) was conjugated to polymyxin B as previously described (12) and used as a coating antigen for an indirect enzyme-linked immunosorbent assay. Microtiter plates (Immulon 2 HB; Thermo Electron) were coated at the optimum coating concentration, determined to be 1 µg per well in 100 µl of carbonate buffer, and incubated overnight at 4°C. Twofold dilution series of test and standard sera from 1 in 12.5 to 12,800 were applied to the coated plates, which were then incubated for 1 h. Pre- and postchallenge serum samples from the same calves were always tested on the same plate. After washing, the secondary antibody (sheep anti-bovine immunoglobulin G [IgG] conjugated with horseradish peroxidase; Serotec) diluted 1 in 800 was added for 1 h of incubation and then the plates were washed and developed with substrate (Sigma-Fast OPD; Sigma-Aldrich). Plates were read in a Dynex Revelation 3.04 enzyme-linked immunosorbent assay plate reader at 492 nm. Endpoint titers were determined, and the fold increase in the postcolonization samples relative to the prechallenge serum titer was calculated for each calf. Data analysis. Unless otherwise stated, bacterial counts are expressed as the \( \log_{10}(\text{count plus one}) \) to permit samples with zero plate counts to be expressed as zero. However, it is possible that a sample expressed as zero may have contained bacteria at levels below the limit of detection, in theory, \( \log_{10} 1.5 \). A repeated-measures model was fitted to the log counts for the surface samples for the first 14 days postchallenge; after day 14, high-shedding calves were selectively culled. Calf group and route of challenge were included as factors in the model. Because the data set contained some missing observations, the correlation between observations on the same animal was modeled with a power model. Additional statistics were used to summarize the colonization ability and shedding patterns of the challenge strain administered by the rectal and oral routes. These statistics were calculated from \( E. coli \) O157:H7 counts from surface feces (or whole feces when separation was not possible). The area under the curve (AUC) for shedding for the first 14 days postchallenge was calculated by the trapezoidal method. Uptake was the proportion of challenged calves that became colonized initially (AUC, >10). The peak level was the highest \( E. coli \) O157:H7 concentration observed within the shedding period. Persistence was the proportion of calves that were initially colonized with \( E. coli \) O157:H7 still detectable by direct plating at day 14 postchallenge. The distribution of anti-O157 LPS IgG serum titers was positively skewed, and the results are expressed as \( \log_{10} \) relative titers. Pre- and postcolonization titers were compared with a one-sample \( t \) test. The graphs in Fig. 1 feature mean surface and core fecal \( E. coli \) O157:H7 concentrations calculated from day 1 postchallenge by using all samples where separation was possible (grades A, B, and C) in all animals with confirmed uptake (AUC, >10). In experiment 2, calves with surface fecal concentrations of at least \( 10^3 \) CFU/g on the day of treatment were included in the analyses of the treatment effect. In order to estimate the changes in fecal concentration in response to treatment, a general linear mixed model was fitted to the change in the number of CFU per gram between the immediate pretreatment and 1 day posttreatment (PT) samples. The type of intervention and route of challenge were included as fixed effects, and calf batch was included as a random effect. Approximate 95% confidence intervals for the mean change were calculated as the mean plus or minus two times the appropriate standard error. Further analysis was performed on the bacterial counts for several time points before and after intervention for the chlorhexidine and control groups by a repeated-measures model fitted to the data. The lack of independence between counts made on the same individual calves was modeled with a power model. Type of intervention (chlorhexidine or control) and route (oral or rectal) were fitted as fixed effects. All statistical calculations were performed in Genstat 8th edition; the parameters of the repeated-measures and general linear mixed models were estimated with the restricted maximum-likelihood directive.

RESULTS

Comparison of challenge routes. Several parameters were used to compare the oral and rectal challenge routes in experiment 1 (Table 1). These included uptake, mean peak count, mean AUC, persistence, and mean serum IgG anti-O157 LPS responses to colonization. No statistically significant differences were demonstrated for these parameters between the

![FIG. 1. Mean daily fecal \( E. coli \) O157:H7 concentrations (log10 CFU per gram) for orally (a) and rectally (b) challenged calves within experiment 1. Surface (SF) and core (CF) fecal compartments were calculated separately, allowing comparison on the log10 scale. Data include all samples where separation of fecal compartments was possible within the first 14 days postchallenge. Error bars indicate the standard errors of the means.](image)

**TABLE 1.** Comparison of the two challenge routes in experiment 1 calves

<table>
<thead>
<tr>
<th>Challenge route</th>
<th>Uptake (no. of calves colonized/total)</th>
<th>Persistence (no. of calves shedding/total)</th>
<th>Mean peak count (log10 CFU/g) ± SD (CF)</th>
<th>Mean serum IgG LPS (log, relative titer) ± SD (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal</td>
<td>22/24</td>
<td>16/22</td>
<td>5.8 ± 0.6 (5.5–6.0)</td>
<td>2.5 ± 1.9 (1.5–3.5)</td>
</tr>
<tr>
<td>Oral</td>
<td>24/30</td>
<td>15/20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5 ± 0.7 (5.1–5.8)</td>
<td>2.3 ± 1.9 (1.3–3.3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> CI, 95% confidence interval for the mean.

<sup>b</sup> The records for four calves were incomplete.
two challenge routes. The daily mean levels of bacterial shedding generated by the two challenge routes throughout the 14-day observation period are compared in Fig. 1a and b by using mean fecal surface and core Enterobacteriaceae concentrations in separate columns. Samples negative by direct plating are in the region below the broken line labeled NEG.

It was demonstrated previously (24) that although the majority of fecal Escherichia coli O157:H7 bacteria originate at the TR, a proportion of individuals carry low levels at other sites. These levels typically arise in the proximal colon, though numbers of E. coli O157:H7 bacteria in gut contents do not increase significantly until the TR, where those originating from higher intestinal colonization sites are obscured by much higher concentrations at the rectal mucosa. To confirm the TR-restricted colonization of rectally challenged calves and provide information on the possible degree of non-TR colonization of orally challenged calves, postmortem sampling was performed on calves in experiment 1 still shedding beyond day 14 postchallenge. The large-intestinal-content concentrations of E. coli O157:H7 at postmortem are shown in Fig. 2. The proximal-colon contents were all negative for rectally challenged calves. E. coli O157:H7 within the core samples of these calves therefore originated from the surface feces rather than non-TR sites. E. coli O157:H7 concentrations were highest in the surface feces, the difference being at least 10-fold and often considerably higher where reliable separation of the surface and core was achieved. For the orally challenged calves, a third (4 of 12) of the mid- and distal-colon contents contained detectable E. coli O157:H7 but target bacterial numbers in surface fecal samples were always much higher and the core fecal samples therefore contained a mixture of E. coli O157:H7 from non-TR and TR sources.

### Treatment effects in the immediate PT period
A total of 22 rectally challenged calves were colonized. Of these, 4 were left as untreated controls, 6 were treated with polymyxin, and 12 were treated with chlorhexidine. Thirty-one orally challenged calves were successfully colonized, and of these, 6 were untreated controls, 2 were treated with polymyxin, and 23 were treated with chlorhexidine. Treatments were applied at 7 days after challenge, and daily fecal samples were taken up to this point and for 7 further days for enumeration of E. coli O157:H7 bacteria. On day 7 PT, the fecal samples, if negative by direct plating, were subjected to enrichment culture to confirm the absence of the challenge strain. The 11 calves in the final group were maintained for 3 weeks after treatment to monitor for reacquisition of the challenge strain.

A summary of the bacterial shedding by calves before and after treatment with chlorhexidine and the same values obtained for untreated controls are shown in Table 2. The most direct means of assessing the effect of treatment is to compare the E. coli O157:H7 concentrations in feces immediately before and 1 day after treatment application. Table 2 shows the mean change in log_{10} CFU per gram for the chlorhexidine

### Table 2. Comparison of chlorhexidine-treated and untreated groups

<table>
<thead>
<tr>
<th>Challenge route and treatment</th>
<th>Pretreatment peak concn</th>
<th>Immediate pretreatment concn</th>
<th>Day 1 PT concn</th>
<th>Change in concn</th>
<th>Peak/day 7 PT concn</th>
<th>No. of calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Chl</td>
<td>4.8</td>
<td>4.3</td>
<td>1.5</td>
<td>−2.8 (−3.3, −2.3)</td>
<td>2.1/0.7</td>
<td>23</td>
</tr>
<tr>
<td>None</td>
<td>4.6</td>
<td>3.8</td>
<td>2.9</td>
<td>−0.9 (−2.0, 0.1)</td>
<td>3.4/1.5</td>
<td>6</td>
</tr>
<tr>
<td>Rectal Chl</td>
<td>4.9</td>
<td>4.5</td>
<td>0.5</td>
<td>−4.0 (−4.7, −3.3)</td>
<td>1.4/0.4</td>
<td>12</td>
</tr>
<tr>
<td>None</td>
<td>4.9</td>
<td>4.7</td>
<td>4.0</td>
<td>−0.7 (−2.0, 0.6)</td>
<td>4.5/1.9</td>
<td>4</td>
</tr>
</tbody>
</table>

*a* The values shown are log_{10} CFU/g.

*b* The values in parentheses are approximate 95% confidence intervals for the mean changes in concentration.
TABLE 3. Day 1 PT surface and core fecal E. coli O157:H7 levels of orally challenged, chlorhexidine-treated calvesa

<table>
<thead>
<tr>
<th>Challenge route, TR/non-TR colonization category, and calf IDa no.</th>
<th>SFc</th>
<th>CFd</th>
<th>S/C ratioe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Likely/unlikely</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>659</td>
<td>3.8</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>663</td>
<td>2.4</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>732</td>
<td>1.5</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>666</td>
<td>2.4</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>686</td>
<td>1.8</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Unlikely/likely</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>602</td>
<td>1.5</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>641</td>
<td>2.4</td>
<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td>651</td>
<td>2.6</td>
<td>2.8</td>
<td>0.5</td>
</tr>
<tr>
<td>679</td>
<td>4.6</td>
<td>4.3</td>
<td>1.8</td>
</tr>
<tr>
<td>689</td>
<td>2.8</td>
<td>2.7</td>
<td>1.3</td>
</tr>
<tr>
<td>604</td>
<td>2.7</td>
<td>2.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Likely/possible</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>635</td>
<td>3.6</td>
<td>1.8</td>
<td>53.7</td>
</tr>
<tr>
<td>726</td>
<td>2.5</td>
<td>1.8</td>
<td>4.4</td>
</tr>
<tr>
<td>Rectal Likely/unlikely</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>661</td>
<td>3.9</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>684</td>
<td>2.2</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

a Excludes calves with no detectable E. coli O157:H7.

b ID, identification.

c SF, surface fecal concentration.

d CF, core fecal concentration.

e S/C ratio, ratio of surface to core fecal concentrations (not log10 transformed).

treatment groups together with approximate 95% confidence intervals estimated from the general linear mixed model. A general linear model was used to estimate significant differences between the change in the mean E. coli O157:H7 concentration for treatment type and challenge route. The reduction in mean bacterial concentration of the treated groups was significantly greater than that of the untreated groups (P < 0.001). Both polymyxin and chlorhexidine produced a significant reduction in log10 CFU per gram (mean changes of −3.4 and −3.2, respectively) relative to the untreated groups (mean change of −0.8), but there was no evidence that one was more effective than the other. There was evidence of a difference in treatment effects for calves challenged by different routes. The rectal route had a significantly greater (P = 0.016) reduction in log10 CFU per gram than the oral route (mean changes of −4.0 and −2.8, respectively, for the chlorhexidine group).

Factors confounding treatment success. Within the rectally challenged group, the treatment was highly successful, with the majority (10/12) being cleared of any detectable fecal E. coli O157:H7. This gives an indication of the direct effect of the treatment at eliminating the target organism at the TR. The success of treatment of the orally challenged group was inferior to that of the rectally challenged group, but this difference is only partially accounted for by E. coli O157:H7 originating from nonrectal sites. Examination of the surface-to-core ratios 1 day after treatment (Table 3) suggests that the failure to kill bacteria at the TR occurred in a greater proportion of the orally challenged group (7/23) than in the rectally challenged group (2/12). However, a Fisher’s exact test of these proportions yielded a mid-P value of 0.209, indicating that the difference was insignificant. Of the 13 orally challenged calves with >10^11 CFU/g in the day 1 PT feces, 7 exhibited evidence of TR colonization, with 5 having E. coli O157:H7 only in the surface feces and 2 having surface-to-core ratios of 4 or greater. Eight of the orally challenged calves had E. coli O157:H7 in the core feces at day 1 PT, six of which had surface-to-core ratios of less than 2, indicating likely nonrectal colonization. The fecal E. coli O157:H7 concentrations in all but one of these were less than 10^9 CFU/g.

Further analysis was performed on the bacterial counts for several time points before and after intervention for the chlorhexidine and control groups with a repeated-measures model fitted to the data. Group means and standard errors estimated from this model are displayed in Fig. 3. There was very strong evidence of a difference in shedding pattern over time between the two groups (time × intervention interaction, P < 0.001). In the chlorhexidine-treated groups, there was a marked decrease in the mean number of CFU per gram after intervention. There was also evidence of an effect of the route on the pattern of responses (route × intervention interaction, P = 0.019). In the oral control group, the mean counts decreased fairly steadily after the time of intervention (7 days postchallenge), whereas the decrease appeared to be delayed in the rectal group; however, these observations are based on small numbers of animals and the means have high estimated standard errors. For animals treated with chlorhexidine, the mean decrease in counts was more pronounced for the rectally challenged group than for the orally challenged group.

For rectally challenged calves, an increase in shedding beyond day 1 PT was substantial (an increase of >10^1 CFU/g from the zero level) in only 1 out of 12 individuals. Five of the 23 orally challenged calves had substantially increased shedding beyond day 1 PT. Analysis of the day 1 PT surface-to-core ratios of these individuals (Table 3) indicates examples in which recovery was likely to have originated from TR surviving bacteria (calf 663) and non-TR colonization.
tion of those testing positive after processing was 3.3 CFU/ml or greater within the preprocessed swab suspension be unacceptably high. For example, it did not identify all calves force of

ited marked recovery of (calf 604), but the source of recovery was unclear for the other

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levels of active by direct plating (data not shown).

was no evidence of recolonization once the levels became neg-

maintained for 3 to 4 weeks PT. Although occasional samples

recovery over a longer time, the final group of 11 calves was

Coli-Strip immunochemical detection kit. Although designed

procedure yielded a positive result for samples with 10^2

immediate test were filtered and concentrated by centrifuga-

absence of bacteria originating from nonrectal sites. Impor-

was used to assess the efficacy of the treatment methods in the

Differences in the early stages after challenge reflect the phys-

firming the previous findings of Sheng et al. (28). Both

challenge routes resulted in similar levels of uptake, E. coli

O157 shedding levels, persistence, and surface-to-core ra-

tios that indicated colonization primarily occurred at the TR.

the digesta originating from nonrectal colonization sites, (iii)

recolonization of the TR by bacteria surviving the treatment at

the TR, and (iv) recolonization of the TR by bacteria originat-

from nonrectal sites of orally challenged calves. As an oral challenge may present the bacteria to sites such as the Peyer’s patch, the colonization of host tissues may result in different humoral immune responses between challenge routes and potentially influence the clearance of the challenge bacte-

ria. Serum anti-O157 LPS antibody responses were therefore

used to compare the possible degree of exposure and re-

sponses for the two challenge routes. Although the serum

anti-O157 LPS IgG titers increased significantly in response to

colonization, the mean responses did not differ significantly

between challenge groups.

The objective of the second part of this study (experiment 2)

was the testing of two treatments applied to the TR in exper-

imentally challenged calves. Our model generates groups of

calves composed primarily of high shedders, and only those

shedding >10^5 CFU/g at the time of intervention were used in

the analyses. In orally challenged individuals, high numbers of

inoculated bacteria increase the potential for non-TR coloni-

zation. Thus, this experiment represents a more stringent test

of the treatment than may be expected for treatment of natu-

rally colonized cattle, in which the majority of positive individ-

uals shed very low levels (22). It is likely that high-level-shed-

ding individuals would be the most resistant to a single

treatment and are also the most important to target (18, 22).

Overall, the direct treatments were successful in reducing E.

coli O157:H7 carriage and bacterial numbers were brought

below the thresholds suggested by Matthews et al. (22) as

necessary to eliminate effective transmission of the organism.

Initially, two treatments were compared in rectally challenged

calves and shedding parameters were determined before and

after treatment. Though the difference in change between

treatment types was small, the chlorhexidine treatment was

chosen for further study. This choice was justified by the fact

that an antiseptic such as chlorhexidine does not have the

perceived risk of generating bacterial resistance associated

with an antibiotic. In addition, the lower cost of chlorhexidine

allows larger volumes to be applied, maximizing mucosal dis-

tribution, and is especially important when treating large

numbers of large animals.

Various reasons for the failure of a treatment directly ap-

plied to the TR were anticipated. These included (i) failure of

the treatment to kill bacteria at the TR, (ii) bacteria present in

the digesta originating from nonrectal colonization sites, (iii)

recolonization of the TR by bacteria surviving the treatment at

the TR, and (iv) recolonization of the TR by bacteria originat-

ing from nonrectal sites. The difference in treatment effect

between challenge routes was significant, and detailed analysis

of the surface-to-core ratios in the PT feces of the chlorhexi-

DISCUSSION

An initial objective of this study was to assess different chal-

lenge routes in establishing persistent colonization of calves

(experiment 1). In addition to the conventional oral challenge

route, a rectal challenge protocol with a methodology and

resultant colonization similar to those reported previously (28)

was used to assess the efficacy of the treatment methods in the

absence of bacteria originating from nonrectal sites. Impor-

tantly, the two challenge routes created qualitatively and quan-

titatively similar patterns of E. coli O157:H7 colonization, con-

firming the previous findings of Sheng et al. (28). Both

challenge routes resulted in similar levels of uptake, E. coli

O157:H7 shedding levels, persistence, and surface-to-core ra-

tios that indicated colonization primarily occurred at the TR.

Differences in the early stages after challenge reflect the phys-

cical differences between the two routes, as a rectal challenge

deposits bacteria immediately onto the TR mucosa. We there-

fore conclude that, beyond the initial period of establishment

of colonization, the rectal challenge method effectively repro-

duced the TR-restricted colonization pattern observed after an

oral challenge.

It was anticipated from previous data (24) that low E. coli

O157:H7 levels could originate from nonrectal regions after an

oral challenge, and this was confirmed by postmortem sam-

pling. The challenge strain was not recovered from the gut

contents of rectally challenged calves, but relatively low con-

centrations were demonstrated in the core feces, indicating

that separation of the two fecal components was rarely absolu-

tute. Incomplete separation of the two components when the

surface concentration was high would therefore obscure any

low concentrations of E. coli O157:H7 present in the core feces

originating from nonrectal regions of orally challenged calves.

...
dine-treated calves suggests that this was partially a result of bacteria originating from nonrectal regions in orally challenged calves (6 out of 23 calves) with the remaining difference being due to less efficacious bacterial killing at the TR in the orally challenged, relative to the rectally challenged, calves. The latter difference could be accounted for by random variability, as it was statistically insignificant and postmortem sampling (experiment 1) did not demonstrate any obvious difference between challenge routes in the pattern of colonization within the rectum (data not shown). However, the possibility that a hypothetical physiological effect, such as a stress response or a change in gene expression following passage through the gastrointestinal tract, reduced the bactericidal efficacy of the treatment in orally challenged calves cannot be ruled out.

The ability to identify high shedders with a rapid field test offers many advantages for use in conjunction with the treatment methods. As the direct test can be performed on the farm offers many advantages for use in conjunction with the treatment prior to transport. Since a single treatment would have the greatest effect when applied at certain critical points—for example, maintenance of clean herds by screening of introduced animals, treatment and removal of high shedders within an infected establishment or to limit transmission to other farms, markets, or slaughterhouses by implementation prior to transport. Since a single treatment was successful in the majority of individuals and reduced shedding levels below the threshold suggested by Matthews et al. (22), a large-scale experiment or field trial should be conducted in which the maintenance of E. coli O157 is dependent upon natural routes of transmission, to allow an accurate assessment of the efficacy of these experimental detection and treatment regimens. The applicability of this approach to the control of other EHEC strains originating from cattle would depend upon these strains also exhibiting a TR tropism. There is no evidence from published experimental challenges that this is the case, although the control of E. coli O157:H7 would lead to a considerable reduction in EHEC-mediated human disease in the majority of developed countries.

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REFERENCES


cattle diets on the transmission of pathogenic *Escherichia coli* to humans. Microbes Infect. 2:45–53.


