Effects of In-Feed Chlortetracycline Prophylaxis in Beef Cattle on Animal Health and Antimicrobial-Resistant Escherichia coli

Getahun E. Agga  
U.S. Department of Agriculture, Agricultural Research Service, getahun.agga@ars.usda.gov

John W. Schmidt  
USDA-ARS, john.w.schmidt@ars.usda.gov

Terrance M. Arthur  
USDA Meat Animal Research Center, terrance.arthur@ars.usda.gov

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U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U. S. Meat Animal Research Center, Clay Center, Nebraska, USA

ABSTRACT

Concerns have been raised that in-feed chlortetracycline (CTC) may increase antimicrobial resistance (AMR), specifically tetracycline-resistant (TET\(^\text{+}\)) *Escherichia coli* and third-generation cephalosporin-resistant (3GC\(^\text{-}\)) *E. coli*. We evaluated the impact of a 5-day in-feed CTC prophylaxis on animal health, TET\(^\text{+}\) *E. coli*, and 3GC\(^\text{-}\) *E. coli*. A control group of cattle (*n* = 150) received no CTC, while a CTC group (*n* = 150) received in-feed CTC (10 mg/lb of body weight/day) from the 5th to the 9th day after feedlot arrival. Over 25% (38/150) of the animals in the control group developed illnesses requiring therapeutic treatment with antimicrobials critically important to human medicine. Only two animals (1.3%) in the CTC group required such treatments. Fecal swab and pen surface occurrences of generic *E. coli* (isolated on media that did not contain antimicrobials of interest and were not isolated based on any specific resistance), TET\(^\text{+}\) *E. coli*, and 3GC\(^\text{-}\) *E. coli* were determined on five sampling occasions: arrival at the feedlot, 5 days posttreatment (5 dpt), 27 dpt, 75 dpt, and 117 dpt. On 5 dpt, TET\(^\text{+}\) *E. coli* concentrations were higher for the CTC group than the control group (*P* < 0.01). On 27 dpt, 75 dpt, and 117 dpt, TET\(^\text{+}\) *E. coli* concentrations did not differ between groups. 3GC *E. coli* occurrences did not differ between control and CTC groups on any sampling occasion. For both groups, generic, TET\(^\text{+}\), and 3GC\(^\text{-}\) *E. coli* occurrences were highest on 75 dpt and 117 dpt, suggesting that factors other than in-feed CTC contributed more significantly to antimicrobial-resistant *E. coli* occurrence.

IMPORTANCE

The occurrence of human bacterial infections resistant to antimicrobial therapy has been increasing. It has been postulated that antimicrobial resistance was inevitable, but the life span of the antimicrobial era has been prematurely compromised due to the misuse of antimicrobials in clinical and agricultural practices. Direct evidence relating the use of antimicrobials in livestock production to diminished human health outcomes due to antimicrobial resistance is lacking, and the U.S. Food and Drug Administration has taken an approach to maximize therapeutic efficacy and minimize the selection of resistant microorganisms through judicious use of antimicrobials. This study demonstrated that prophylactic in-feed treatment of chlortetracycline administered for 5 days to calves entering feedlots is judicious, as this therapy reduced animal morbidity, reduced the use of antimicrobials more critical to human health, and had no long-term impact on the occurrence of antimicrobial-resistant *E. coli*.

In-feed and in-water uses of medically important antimicrobials for the control and prevention of diseases have become controversial, because it is argued that they unnecessarily expose healthy animals to medically important antimicrobials and possibly increase the spread of antimicrobial resistance (AMR) (1–3). Restricting the use of medically important antimicrobials in food animal production has been suggested as a means to reduce AMR (1,3–5). Conversely, studies have reported only small quantitative risks of human-pathogenic bacteria acquiring AMR due the use of antimicrobials in food animal production (6–9). By the end of 2016, in-feed and in-water applications of medically important antimicrobials for growth promotion purposes will be banned in the United States (10). Henceforth, the U.S. Food and Drug Administration (FDA) will require a Veterinary Feed Directive for all in-feed and in-water applications of medically important antimicrobials (11). Regardless, concerns remain that restricting the prophylactic in-feed and in-water uses of medically important antimicrobials may negatively affect animal welfare, increase therapeutic veterinary uses of antimicrobials of higher importance to human medicine, and increase zoonotic pathogen load and transmission (12).

Beef cattle are susceptible to bovine respiratory disease (BRD) during weaning and transitioning into feedlots (13, 14). In-feed chlortetracycline (CTC) is an option for the management of BRD currently employed at U.S. cattle feedlots (15, 16). CTC, tetracycline (TET), and oxytetracycline compose the naturally occurring first-generation tetracycline antimicrobial class (here referred to as tetracyclines). In 2014, tetracyclines, considered important to human medicine, were the largest portion of antimicrobials sold in the United States for use in food-producing animals (17, 18). TET resistance in *Escherichia coli* is commonly associated with the presence of either *tet(A)* or *tet(B)* genes (19, 20). The third-generation cephalosporin (3GC) class of antimicrobials is considered critically important to human medicine (18). 3GC resistance in *E. coli* can be conferred by *bla*\(_{CMY-2}\) harbored by IncA/C plas-
mids, which also contain tet(A) (21). Thus, it is possible that in-feed CTC use in feedlot cattle may coselect and thereby increase the occurrence of 3GC resistance (22). The objective of this study was to evaluate the effect of a one-time 5-day in-feed CTC prophylaxis on animal health (morbidity and body weight gain), occurrence of tetracycline-resistant (TET+) E. coli, and occurrence of 3GC-resistant (3GC+) E. coli over a 4-month follow-up period.

MATERIALS AND METHODS

Experimental design. Three hundred weaned calves (150 steers and 150 heifers, born between 3 August 2014 and 30 September 2014) from the U.S. Meat Animal Research Center (USMARC) cow-calf production system were used for this trial. Calves arrived at the USMARC feedlot on 9 February 2015. Calves (blocked on birth date, arrival body weight, and sex) were randomly assigned to two groups (150 calves/treatment group). On 14 February 2015, 150 calves (5 pens with 30 calves/pen) received CTC-medicated feed (Aureomycin, chlorotetracycline complex equivalent to 10 mg/lb of body weight/day of chlorotetracycline; Zoetis, Kalamazoo, MI) (CTC group) for five consecutive days (last CTC-medicated feed was distributed on 18 February 2015). The remaining 150 calves were used as a control (5 pens with 30 calves/pen) and received feed without CTC throughout the experiment (control group). Empty pens separated the groups from each other and from nonstudy animals. Three empty pens were included in this study (empty group). The calves were fed standard diets typical of feedlot operations, according to the feedlot feeding protocol (see Table S1 in the supplemental material). Body weights were measured upon arrival and subsequently during sampling occasions. Common watering troughs were shared between adjacent pens in the same treatment group. Animals were monitored by cattle operations staff and received normal veterinary care as required. Animals that required any therapeutic antimicrobial treatment were removed from the experiment. The U.S. Meat Animal Research Center Institutional Animal Care and Use Committee approved this experiment (USMARC IACUC no. 3040-42000-014-07).

Sample collection and processing. Fecal swabs, pen surface material, feed, and water samples were collected on five occasions: at feedlot arrival (arrival, 9 February 2015), 5 days posttreatment (5 dpt, 23 February 2015), 27 days posttreatment (27 dpt, 17 March 2015), 75 days posttreatment (75 dpt, 4 May 2015), and 117 days posttreatment (117 dpt, 15 June 2015). Fecal swabs were collected directly from the rectum using a foam-tipped swab (Whirl-Pak; Nasco, Fort Atkinson, WI) in the trough, sponging the interior sides of the trough, including the water line, and then placing the sponge into a sterile bag. In addition, plastic bags were used to collect one fresh feed sample per pen immediately after feed was dispensed from the truck into the feed bunk, prior to contact by any animals. All samples were transported to the laboratory and were processed on the same day.

Fecal swabs were homogenized by vortexing for 30 s, after which a 1-ml aliquot was removed for enumeration. The remaining fecal suspension was enriched at 42°C for 8 h and then held at 4°C until secondary enrichments were performed the following day. Water trough sponge samples were homogenized by hand massaging the sample bags for 15 s, and a 1-ml aliquot was removed for enumeration. Homogenized water trough sponge samples were combined with 80 ml of TSB-PO₄ and then incubated at 25°C for 2 h, 42°C for 6 h, and held at 4°C until secondary enrichments were performed the following day. For pen surface materials and feed samples, 10 g of the samples was transferred to filter barrier bags, and 90 ml of TSB-PO₄ was added. From each sample suspension, a 1-ml aliquot was removed and used for enumeration. The remaining pen surface and feed sample suspensions were then enriched at 25°C for 2 h, 42°C for 6 h, and held at 4°C until secondary enrichments were performed the following day.

Enumeration and detection of generic, 3GC, and TET+ E. coli. Generic (isolated on media that did not contain antimicrobials of interest and were not isolated based on any specific resistance), 3GC, and TET+ E. coli bacteria were each enumerated and detected as previously described (23, 24). Briefly, 50 µl of the enumeration aliquot or an appropriate dilution of the enumeration aliquot was spiral plated using Autoplate 4000 (Spiral Biotech, Norwood, MA) onto CHROMagar E. coli (DRG International, Mountainside, NJ) with no supplemental antimicrobials (CEC), CEC supplemented with 2 mg/liter cefotaxime (CEC + CTX), or CEC supplemented with 32 mg/liter tetracycline (CEC + TET). Antimicrobials were obtained from Sigma-Aldrich Corp., St. Louis, MO, and the antimicrobial concentrations were based on previous reports (19, 23, 24). Tetracycline was used in agar medium at 32 mg/liter, rather than the 16 mg/liter resistance breakpoint used by the National Antimicrobial Resistance Monitoring System (25), as in our experience, 16 mg/liter tetracycline in agar medium results in large numbers of false-positive colonies. Agar plates were incubated at 37°C for 24 h. Blue colonies were considered presumptive E. coli colonies and were enumerated with an automated colony counter (ProtoCOL 3; Symbiosis, Frederick, MD) (19, 23, 24).

Secondary enrichments were prepared by inoculating 0.5 ml of the primary enrichment cultures into 2.5 ml of MacConkey broth (Becton Dickinson, Sparks, MD) with no supplemental antimicrobials (MCB), 2.5 ml of MCB supplemented with 2.4 mg/liter cefotaxime (MCB + CTX), or 2.5 ml of MCB supplemented with 38.4 mg/liter tetracycline (MCB + TET), as previously described (19, 23, 24). After incubation at 42°C for 18 h, MCB, MCB + CTX, and MCB + TET secondary enrichment cultures were streaked on CEC, CEC + CTX, and CEC + TET agar plates, respectively, and incubated at 37°C for 18 h to detect generic, 3GC, and TET+ E. coli, respectively. From each plate, up to two presumptive colonies were inoculated into TSB and incubated overnight at 37°C. Colony lysates were prepared from overnight enrichment cultures using BAX DNA buffer (DuPont Qualicon, Inc., Wilmington, DE), according to the manufacturer’s instructions. The colony lysates were used to confirm presumptive E. coli by using multiplex PCR targeting lacY, lacZ, cyd, and uidA genes (26).

Statistical analysis. The effect of CTC prophylaxis on body weight was evaluated by multilevel mixed-effects linear regression with a maximum likelihood estimation method in which treatment and sampling date were modeled as fixed effects, while repeated measures of animals over time and pen were modeled as random effects. The impact of CTC prophylaxis on the morbidity of the animals was assessed by survival analysis with Cox proportional hazards regression model, and hazard ratio (HR) was calculated to compare the hazard of morbidity between the CTC and control groups (27). Times to event of morbidity (in days) were recorded as they occurred and later used in the survival analysis. End of the study was considered the last day of the follow-up period.

In order to analyze the data sets, zero values were replaced using the following criteria. The convention was to assign concentration values to samples that were prevalence positive (above the lower limit of detection [LLOD] for the prevalence assay) but enumeration negative (below the lower limit of enumeration [LLOE] for the enumeration assay). For samples that fell into this category, the midpoint between detection limits for each sample type was used to fill the zero values. Samples that were below the LLOD for the prevalence assay were assigned a value 1 log less than the LLOD for the prevalence assay for each sample type.

Fecal swab sample enumeration plate counts were converted to log₁₀ CFU per swab value. For fecal swab samples, the LLOE was 2.00 log₁₀ CFU/swab, and the theoretical LLOD was 0.00 log₁₀ CFU/swab. Fecal swab samples with no E. coli colonies on enumeration plates but with
**RESULTS**

**Description of animal characteristics.** At arrival, mean body weight, age, and prior antimicrobial treatments did not significantly ($P > 0.05$) differ between the two treatment groups (Table 1). Out of the 12 calves that received antimicrobial treatments prior to the study, the most common (9/12) antimicrobial administered was ceftiofur sodium (Naxcel; Zoetis).

**Mean body weight, morbidity, and therapeutic antimicrobials administered.** Mean body weights did not significantly differ ($P > 0.05$) between the treatment groups across all sampling occasions (see Fig. S1 in the supplemental material). Cumulative morbidities were 38 and 2 animals in the control and CTC groups, respectively, and survival analysis showed that cattle in the control group were 28 times more likely to become sick than those in the CTC group (HR, 27.9; 95% confidence interval [CI], 6.7 to 116.5; $P < 0.001$) (Fig. 1). Pneumonia (37/40 animals) was the most common (38/40 animals) therapeutic antimicrobial treatment given (see Table S2 in the supplemental material).

**Fecal swab occurrences of generic, TET*, and 3GC* E. coli.** Arrival fecal swab generic E. coli concentrations did not differ ($P = 0.98$) between the control (4.73 log$_{10}$ CFU/swab) and CTC (4.81 log$_{10}$ CFU/swab; Fig. 2A) groups. Similarly, arrival fecal swab TET* E. coli concentrations did not differ ($P = 0.76$) between the control (3.12 log$_{10}$ CFU/swab) and CTC (3.34 log$_{10}$ CFU/swab) groups (Fig. 2B). At 5 dpt, CTC group fecal swab generic E. coli (4.70 log$_{10}$ CFU/swab) and TET* E. coli (4.25 log$_{10}$ CFU/swab) concentrations were higher ($P < 0.01$) than their respective control group concentrations (generic E. coli, 3.51 log$_{10}$ CFU/swab; TET* E. coli, 1.86 log$_{10}$ CFU/swab). The control group 5 dpt fecal swab generic and TET* E. coli concentrations were lower ($P < 0.01$) than the arrival concentrations. Conversely, the CTC group 5 dpt fecal swab generic E. coli concentration did not differ ($P = 0.99$) from the arrival concentration, while the 5 dpt TET* E. coli concentration was higher ($P < 0.01$) than that during arrival. At 27 dpt, fecal swab generic E. coli concentrations did not differ ($P = 0.93$) between groups, nor did concentrations differ from their respective arrival concentrations (control group, $P = 1.00$; CTC group, $P = 0.69$). Similarly, 27 dpt fecal swab TET* E. coli concentrations did not differ between groups ($P = 0.99$) and did not differ (control group, $P = 0.35$; CTC group, $P = 0.79$) from their respective arrival concentrations (Fig. 2A and B).

For both groups, 75 dpt fecal swab generic E. coli concentra-

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**TABLE 1** Animal characteristics at feedlot arrival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>CTC group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>150</td>
<td>150</td>
<td>0.001*</td>
</tr>
<tr>
<td>No. of females</td>
<td>60</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>No. that received prior antimicrobial treatment</td>
<td>8</td>
<td>4</td>
<td>0.234*</td>
</tr>
<tr>
<td>Oxazepamide</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime and tildipirosin</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime and tildipirosin</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Florfenicol</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* $P$ values are based on likelihood ratio test comparing the proportions between the two groups.

**FIG 1** Kaplan-Meier survival plot for the evaluation of the effect of chlorotet-

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<table>
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<tr>
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<th>CTC group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>150</td>
<td>150</td>
<td>0.001*</td>
</tr>
<tr>
<td>No. of females</td>
<td>60</td>
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<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Florfenicol</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* $P$ values are based on two sample t test comparing the mean values between the two groups.

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In-Feed CTC Effect on Animal Health and AMR

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<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>CTC group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>150</td>
<td>150</td>
<td>0.001*</td>
</tr>
<tr>
<td>No. of females</td>
<td>60</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Body wt (mean ± SE) (kg)</td>
<td>181.3 ± 2.3</td>
<td>180.9 ± 2.3</td>
<td>0.893*</td>
</tr>
<tr>
<td>Age (mean ± SE) (mo)</td>
<td>5.6 ± 0.0</td>
<td>5.5 ± 0.0</td>
<td>0.678*</td>
</tr>
</tbody>
</table>

* $P$ values are based on two sample t test comparing the mean values between the two groups.

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E. coli colonies on prevalence plates were judged prevalence positive and enumeration negative (PP-EN) and were assigned a concentration of 1.00 log$_{10}$ CFU/swab, because these samples were assumed to have concentrations between 0.00 and 2.00 log$_{10}$ CFU/swab. Fecal swab samples with no E. coli colonies on enumeration and prevalence plates were judged prevalence negative (PN) and were assigned a concentration of $-1.00$ log$_{10}$ CFU/swab; because these samples were assigned to have concentrations of $<0.00$ log$_{10}$ CFU/swab.

Pen surface and feed sample enumeration plate counts were converted to log$_{10}$ CFU per gram of sample values. For pen surface and feed samples, the LLOE was 2.30 log$_{10}$ CFU/g, and the LLOD was $-1.00$ log$_{10}$ CFU/g. Pen surface and feed PP and EN samples were assigned a concentration of 0.65 log$_{10}$ CFU/g, since these samples were assumed to have concentrations between $-1.00$ and 2.30 log$_{10}$ CFU/g. Pen surface and feed PN samples were assigned a concentration of $-2.00$ log$_{10}$ CFU/g, since these samples were assumed to have concentrations of less than $-1.00$ log$_{10}$ CFU/g.

Each water trough sponge sample was assumed to contain 20 ml of liquid. Water trough sample enumeration plate counts were converted to log$_{10}$ CFU per milliliter of water values. For water trough samples, the LLOE was 1.30 log$_{10}$ CFU/ml, and the LLOD was $-1.30$ log$_{10}$ CFU/ml. Water trough PP-EN samples were assigned a concentration of 0.00 log$_{10}$ CFU/ml, since these samples were assumed to have concentrations between $-1.30$ and 1.30 log$_{10}$ CFU/ml. Water trough PN samples were assigned a concentration of $-2.30$ log$_{10}$ CFU/ml, since these samples were assumed to have concentrations of less than $-1.30$ log$_{10}$ CFU/ml.

For each sampling occasion, pen-level generic, TET*, and 3GC* E. coli mean and 95% confidence intervals were determined for fecal swab and pen surface sample concentrations from multilevel mixed-effects linear regression models, as described above. For each sampling occasion, pen-level generic, TET*, and 3GC* E. coli fecal swab and pen surface mean prevalences (%) were determined from multilevel mixed-effects logistic regression models. Multiple comparisons were adjusted for by Bonferroni method. In this experiment, our experimental unit was pen, as CTC was administered was ceftiofur sodium (Naxcel; Zoetis).
tions (control group, 6.13 log_{10} CFU/swab; CTC group, 5.94 log_{10} CFU/swab) increased compared to earlier sampling occasions (P < 0.05) but did not differ from each other (P = 0.63). Likewise, 75 dpt fecal swab TET^T E. coli concentrations (control group, 5.22 log_{10} CFU/swab; CTC group, 5.04 log_{10} CFU/swab) were higher than earlier sampling occasions (P < 0.05) but did not differ from each other (P = 0.90). At 117 dpt, the control group fecal swab concentration of generic E. coli was higher (P = 0.01) than that of the CTC group concentration, but the TET^T E. coli concentration did not differ (P > 0.05) between groups. Both generic E. coli and TET^T E. coli concentrations were higher (P < 0.05) than their respective earlier sampling occasions (Fig. 2).

For 3GC^C E. coli, fecal swab prevalences (%) were analyzed, because enumerable concentrations (≥2.00 log_{10} CFU/swab) were present in only 0.1% (2 of 1,352 swabs) of the fecal swabs (data not shown). Fecal swab 3GC^C E. coli prevalences did not differ (P ≥ 0.90) between the control and CTC groups during any sampling occasion (Fig. 3). For both groups, fecal swab 3GC^C E. coli prevalences on 75 dpt and 117 dpt were >70% and were higher (P < 0.01) than those during arrival, 5 dpt, and 27 dpt (<10%).

Pen surface occurrences of generic, TET^T, and 3GC^C E. coli. Arrival pen surface generic E. coli concentrations in the control (1.14 log_{10} CFU/g) and CTC (1.32 log_{10} CFU/g) groups did not differ (P = 0.97, Fig. 4A). Arrival pen surface TET^T E. coli concentrations in the control (1.29 log_{10} CFU/g) and CTC (1.24 log_{10} CFU/g) groups did not differ (P = 1.00, Fig. 4B). At 5 dpt, pen surface generic E. coli concentrations for the control (2.53 log_{10}
CFU/g) and CTC (3.47 log₁₀ CFU/g) groups did not differ (P = 0.13) from each other but were higher (P < 0.05) than their respective arrival concentrations (Fig. 4A). At 5 dpt, the CTC group pen surface TET⁺ E. coli concentration (3.23 log₁₀ CFU/g) was higher (P < 0.01) than the control group concentration (1.56 log₁₀ CFU/g; Fig. 4B). The CTC group 5 dpt TET⁺ E. coli concentration was (P < 0.01) higher than its arrival concentration, while the control group 5 dpt TET⁺ E. coli concentration did not differ (P = 1.00) from its arrival concentration (Fig. 4B).

Control and CTC group pen surface generic E. coli concentrations on 27 dpt, 75 dpt, and 117 dpt ranged from 4.75 to 5.71 log₁₀ CFU/g and did not differ (P ≥ 0.49) from each other within a sampling occasion (Fig. 4A). However, these generic E. coli concentrations were higher (P ≤ 0.01) than their respective arrival and 5 dpt concentrations. Similarly, control and CTC group pen surface TET⁺ E. coli concentrations on 27 dpt, 75 dpt, and 117 dpt ranged from 4.75 to 5.71 log₁₀ CFU/g and did not differ (P ≥ 0.06) from each other within a sampling occasion, but they were higher (P < 0.01) than their respective arrival and 5 dpt concentrations (Fig. 4B).

Arrival empty group pen surface generic E. coli (1.53 log₁₀ CFU/g) and TET⁺ E. coli (1.24 log₁₀ CFU/g) concentrations did not differ (P ≥ 0.85) from their respective concentrations for the control and CTC groups (Fig. 4A). On all subsequent sampling occasions, empty pen surface generic E. coli concentrations ranged from 1.45 to 2.03 log₁₀ CFU/g, while TET⁺ E. coli concentrations ranged from 0.65 to 1.32 log₁₀ CFU/g (Fig. 4). None of these empty pen group concentrations differed (P > 0.05) from their respective empty pen group arrival concentrations. On 5 dpt, empty group pen surface generic E. coli and TET⁺ E. coli concentrations did not differ (P ≥ 0.58) from their respective control group concentrations but were lower (P = 0.02) than their respective CTC group concentrations. On 27 dpt, 75 dpt, and 117 dpt, generic and TET⁺ E. coli concentrations from pen surface samples in the empty pens were lower (P < 0.01) than their respective control and CTC group pen surface concentrations (Fig. 4).

3GC⁺ E. coli pen surface prevalences were analyzed because only 1.25% (3 of 260 samples) of the pen surface samples had enumerable (≥ 2.30 log₁₀ CFU/g) 3GC⁺ E. coli concentrations (data not shown). Control and CTC group pen surface 3GC⁺ E. coli prevalences did not differ (P ≥ 0.18) on any sampling occasion (Fig. 5). Pen surface 3GC⁺ E. coli prevalences did not differ (P ≥ 0.18) between control (60%), CTC (30%), and empty (58%) group pens at arrival. On 75 dpt and 117 dpt, 3GC⁺ E. coli prevalences in the control and CTC group pens were higher (P ≤ 0.01) than in empty pens (Fig. 5).

Occurrences of generic, TET⁺, and 3GC⁺ E. coli in feed and water troughs. At arrival, 5 dpt, and 27 dpt feed generic E. coli concentrations ranged from 2.53 to 3.62 log₁₀ CFU/g (see Table S3 in the supplemental material). Notably, for both groups, the feed generic E. coli concentrations on 75 dpt and 117 dpt were higher, between 5.01 and 5.13 log₁₀ CFU/g. Within a sampling occasion, similar feed generic E. coli concentrations were present in the two groups except on 27 dpt. However, this difference is due to a single prevalence-positive enumeration-negative sample in the CTC group (see Table S3). Feed TET⁺ E. coli concentrations at arrival, 5 dpt, and 27 dpt ranged from 0.98 to 1.91 log₁₀ CFU/g. For both groups, feed TET⁺ E. coli concentrations on 75 dpt and 117 dpt were between 3.79 and 3.88 log₁₀ CFU/g, higher than those on their preceding sampling occasions. 3GC⁺ E. coli was not detected in feed during arrival, 5 dpt, 27 dpt, and 75 dpt sampling occasions. Interestingly, on the 117 dpt sampling occasion, 3GC⁺ E. coli was prevalent in 100% of the feed samples (see Table S3).

At arrival, 5 dpt, and 27 dpt, water trough generic E. coli concentrations ranged from −0.33 to 0.45 log₁₀ CFU/ml (see Table S4 in the supplemental material). On 75 dpt and 117 dpt, water trough generic E. coli concentrations were higher than those on their preceding sampling occasions, ranging from 1.83 to 3.01 log₁₀ CFU/ml. Similarly, at arrival, 5 dpt, and 27 dpt, water trough TET⁺ E. coli concentrations ranged from −0.77 to 0.00 log₁₀ CFU/ml, while on 75 dpt and 117 dpt, TET⁺ E. coli concentrations were higher, ranging from 0.59 to 1.81 log₁₀ CFU/ml. 3GC⁺ E. coli was not enumerable from any water trough sample. 3GC⁺ E. coli was not detected in water trough samples during sampling occasions at arrival, 5 dpt, and 27 dpt. 3GC⁺ E. coli was prevalent in 2 of 7 water trough samples from 75 dpt and 5 of 7 water trough samples from 117 dpt (see Table S4).

DISCUSSION

To our knowledge, two experimental studies have previously investigated the impact of in-feed CTC applications at beef cattle feedlots on fecal TET⁺ E. coli levels. Studies published in 2008 by Platt et al. (28) and in 2013 by Kanwar et al. (22) found that in-feed CTC increased fecal TET⁺ E. coli detection in the short term. Similarly, in the current study, the TET⁺ E. coli concentration from the fecal swab samples at 5 dpt for the CTC group was 2.39 log₁₀ CFU/swab higher than the control group concentration (Fig. 2B). We found that the 5 dpt CTC group fecal swab generic E. coli concentration was significantly (P < 0.01) higher than the 5 dpt control group concentration. A graph of fecal swab concentrations (Fig. 2B) clearly demonstrates that in-feed CTC administration increased the fecal swab TET⁺ E. coli concentration immediately after treatment but that this concentration did not differ from the control group concentration by 27 dpt. Figure 2 also indicates that the fecal swab TET⁺ E. coli concentration increase that occurred between arrival and 5 dpt was responsible for the CTC group 5 dpt fecal swab generic E. coli concentration remaining the same as the arrival concentration (Fig. 2A).
Platt et al. (28) reported a transient reduction in the prevalence of fecal 3GC E. coli following in-feed CTC treatment, while Kanwar et al. (22) reported increased 3GC E. coli occurrence when a veterinary 3GC, ceftiofur, was administered followed by in-feed CTC administration. In contrast to both studies, we found that fecal swab 3GC E. coli prevalences did not differ significantly (P ≥ 0.90) between groups on any sampling occasion (Fig. 3). More importantly, our study provides substantial original evidence that factors other than in-feed CTC treatment had a greater impact on TET and 3GC E. coli shed by feedlot cattle. For both groups, 75 dpt and 117 dpt fecal swab TET E. coli concentrations were significantly higher than the 5 dpt CTC group concentration (Fig. 2). Similarly, 75 dpt and 117 dpt 3GC E. coli fecal swab prevalences (>70%) were significantly higher than arrival, 5 dpt, and 27 dpt prevalences (<10%, Fig. 3) in both groups.

Concerns also have been raised that in-feed CTC could increase the occurrence of antimicrobial resistance in pen surface soil, possibly re inoculating cattle with resistant bacteria or increasing the occurrence of antimicrobial resistance in manure, which may impact crops or the environment (29–32). To our knowledge, the impact of in-feed CTC on beef cattle feedlot pen surface generic, TET, and 3GC E. coli occurrences has not been reported. We conclude that factors other than in-feed CTC administration had a greater impact on pen surface TET E. coli. While the CTC group 5 dpt pen surface TET E. coli concentration was significantly higher (P < 0.01) than the 5 dpt control group concentration, 27 dpt, 75 dpt, and 117 dpt pen surface TET E. coli concentrations for both control and CTC groups were all significantly higher than the 5 dpt CTC group pen surface TET E. coli concentration (Fig. 4). Graphing each group’s pen surface generic and TET E. coli concentrations (Fig. 4) led us to conclude that the deposition of manure (feces and urine) in occupied pens (control and CTC groups) was the principal factor contributing to the increase in TET E. coli concentrations compared to the empty pens. We note that for all three groups, TET E. coli concentrations were proportional to the generic E. coli concentrations (Fig. 4). We postulate that the temporal increase in E. coli concentrations in occupied pens was due to the deposited E. coli populations themselves, increased nutrients from the deposited manure, or a combination of these factors. These findings and postulations are consistent with recent studies that found antimicrobial-resistant bacteria and antimicrobial resistance genes increased in soils from pens holding cattle that had not received antibiotics and soils fertilized with manure from dairy cattle that had not received antibiotics (30, 33).

We can only speculate on the factors driving the observed increases in TET and 3GC E. coli occurrence as sampling occasions progressed. Generic, TET, and 3GC E. coli occurrences in feed may be a factor because as sampling occasions progressed, generic, TET, and 3GC E. coli occurrences in the feed samples also increased (see Table S3 in the supplemental material). A similar temporal increase in generic, TET, and 3GC E. coli occurrences was observed in water trough samples as well (see Table S4 in the supplemental material). We note that the feed and water trough samplings were limited (10 feed samples and seven water trough samples were obtained at each sampling occasion). We also note that E. coli is known to be present in feed and that E. coli on the cattle head and in the oral cavity could contaminate water troughs (34–37). Furthermore, we also noted that the ration fed to study animals at times included the antimicrobials tylosin and monen-


