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Temporal Prevalence of Antimicrobial Resistance in *Campylobacter* spp. from Beef Cattle in Alberta Feedlots†

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Antimicrobial resistance (AMR) was temporally assessed in campylobacters isolated from beef cattle (7,738 fecal samples from 2,622 animals) in four commercial feedlots in Alberta. All calves were administered chlortetracycline and oxytetracycline in feed, and a majority of the animals (93%) were injected with long-acting oxytetracycline upon arrival at the feedlot. Fecal samples from individual animals were collected upon arrival (i.e., entry sample), 69 days (standard deviation [SD] = 3 days) after arrival (i.e., interim sample), and 189 days (SD = 33 days) after arrival (i.e., exit sample) at the feedlot. In total, 1,586 *Campylobacter* isolates consisting of *Campylobacter coli* ($n = 154$), *Campylobacter fetus* ($n = 994$), *Campylobacter jejuni* ($n = 431$), *Campylobacter hyointestinalis* ($n = 4$), and *Campylobacter lanienae* ($n = 3$) were recovered and characterized. The administration of antimicrobials did not decrease carriage rates of campylobacters, and minimal resistance (≤4%) to azithromycin, ciprofloxacin, enrofloxacin, gentamicin, and meropenem was observed. In contrast, substantive increases in the prevalence of isolates resistant to tetracycline and doxycycline (56 to 89%) for *C. coli*, *C. fetus*, and *C. jejuni*, as well as in the number of animals (7 to 42%) from which resistant isolates were recovered, were observed during the feedlot period. Increased resistance to erythromycin (total isolates and carriages rates) was also observed in isolates of *C. coli* over the three isolation times. The majority of *C. fetus* isolates recovered were resistant to nalidixic acid, but this was independent of when they were isolated. A relatively limited number of multidrug-resistant isolates were recovered and consisted primarily of *C. coli* resistant to tetracyclines and erythromycin (10% of isolates). Over the course of the feedlot period, considerable increases in antimicrobial resistance were observed in *C. coli*, *C. fetus*, and *C. jejuni*, but with the exception of erythromycin resistance in *C. coli*, the administration of antimicrobial agents to beef cattle was found to have a minimal impact on resistance to macrolides and fluoroquinolones, the two classes of antimicrobials used to treat campylobacteriosis in humans. However, the widespread use of antimicrobial agents in beef production and the possible horizontal transfer of mobile genetic elements with antimicrobial resistance determinants among *Campylobacter* and other bacterial taxa emphasize the need to monitor AMR development in bacteria from beef cattle.

Alberta, Canada, possesses a very large beef cattle population (approximately 6 million head), and approximately 2 million of these animals are in finishing feedlots (Alberta Government website, http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sdd1492?opendocument). *Campylobacter* species are recognized as one of the most frequent causes of acute diarrheal disease in humans in North America (Centers for Disease Control and Prevention, U.S. Department of Agriculture, and Food and Drug Administration Collaborating Sites Foodborne Disease Active Survey Network [FoodNet]; Public Health Agency of Canada website, http://dsol-smed.phac-aspc.gc.ca/dsol-smed/ndis/index_e.html), and a large number of *Campylobacter* species are shed in the feces of beef cattle (15, 16, 17, 18, 24). Although the impact of cattle-borne campylobacters on human health has not been definitely determined, mounting evidence points toward cattle as a significant source of human-pathogenic campylobacters (6, 7, 27, 30, 33).

The impact of antimicrobial-resistant (AMR) campylobacters of livestock origin on human health is an emerging issue, and a number of studies have implicated the selection of AMR strains of *Campylobacter* species from livestock-administered antimicrobial agents (36). Limited research has investigated the impact that antimicrobial use in cattle may have on the selection for AMR campylobacters, despite the beef industry's reliance on antimicrobial agents (8, 14, 38). It is estimated that more than 2 million kg of antimicrobial agents is administered to beef cattle in North America each year as growth promoters and to prevent disease (23). Antimicrobials are typically administered to beef cattle in finishing feedlots in the diet, either continuously throughout the feeding period or at specific times of high disease risk. The continuous administration of antimicrobial agents at relatively low concentrations in confined feeding operations has been hypothesized to increase the likelihood of resistance development (20), and we confirmed that the administration of chlortetracycline selected for tetracycline-resistant isolates of *Campylobacter hyointestinalis* in beef cattle maintained in an experimental feedlot (19). Given the extensive use of antimicrobial agents in beef production in North America, the potential for selection of AMR campylobacters, and the large numbers of campylobacters that

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are released in feces, the overall objective of the current study was to measure the temporal occurrence of antimicrobial resistance in *Campylobacter* species obtained from a large number of beef cattle maintained in commercial feedlots under actual operating conditions. The particular emphasis was to focus on resistance to macrolides and/or to fluoroquinolones because of their importance as therapeutic antimicrobial treatments for humans afflicted with campylobacteriosis.

MATERIALS AND METHODS

Cattle and feedlots. Crossbred beef steer and heifer calves (ca. 6 to 8 months of age and weighing approximately 200 to 350 kg) were purchased from auction markets throughout western Canada. Animals were transported to one of four commercial feedlots by truck. The feedlots were located in south-central Alberta and possessed feeding capacities ranging from 16,000 to 32,000 animals. Upon arrival at the feedlot, each animal received an identification tag and was injected with the following: vaccines for infectious bovine rhinotracheitis virus and parainfluenza 3 virus, a multivalent clostridial bacterin-toxoid, a *Mannheimia haemolytica* bacterin-toxoid, and a *Haemophilus somnus* bacterin-toxoid. All animals were implanted with an anabolic growth implant and were treated topically for internal and external parasites, and the majority of bull calves were castrated. Animals were then assigned to pens based on sex, and pen assignments were recorded. All animals were housed in open-air, soil-floor pens arranged side by side with central feed alleys and 20% porosity wood-fence windbreaks. Each pen held approximately 200 to 300 animals. Initially, animals were maintained on a backgrounding diet consisting of barley silage (60 to 70%), rolled barley grain (25 to 35%), and a protein-vitamin-mineral supplement (4 to 5%). Animals were fed twice daily and allowed to feed and drink water ad libitum. Cattle were adapted to a high-barley grain finishing diet over a 50-day period (i.e., transition period) and remained on the high-grain diet (80 to 95%) until they were shipped from the feedlot.

The antimicrobial regimens were similar among the four feedlots and represented industry standard practices. In most instances (92%), animals were injected subcutaneously with long-acting oxytetracycline (LA 200; 20 mg kg^{-1} of body weight) upon arrival at the feedlot. A small number of animals were deemed sick upon arrival at the feedlot, 114 animals (11.9%) at feedlot 4 were injected subcutaneously with the macrolide tilmicosin (Micotil; 10 mg kg^{-1}), and 25 animals at the other three feedlots were injected with florfenicol (Nuflor; 40 mg kg^{-1}). The remainder of the antimicrobials were administered in feed. At all feedlots, diets were supplemented with oxytetracycline (Terramycin 200; 11 ppm) and chlortetracycline with sulfamethazine (Aureo S-700 G; 350 mg/head/day) in an attempt to control liver abscesses and bacterial pathogens and to serve as growth promoters. Terramycin 200 was fed throughout the feedlot period, whereas Aureo S-700 G was withdrawn from diets, on average, 53 days (standard deviation [SD]= 7 days) after the arrival of the calves. Diets were also amended with an ionophore to control coccidiosis and bloat; the ionophores, lasalocid (Bovatec), and/or monensin (Rumensin) were administered. Rumensin was fed throughout the feeding period in all instances. At feedlots 2 and 4, Bovatec was fed in conjunction with Aureo S-700 G, and this was the major difference in the nontherapeutic antimicrobial drug regimen among the four feedlots.

Throughout the study, care was taken to ensure that the appropriate restraint and animal handling procedures were applied, and the guidelines of the Canadian Council of Animal Care (29) were followed. Once or twice daily, experienced personnel checked the animals for signs of disease. Animals deemed to be "sick" were moved to an adjacent hospital facility, diagnosed, and treated in consultation with licensed veterinarians. In all instances, the dosages recommended by the drug manufacturer and approved by the Veterinary Drugs Directorate of Health Canada were used, and strict attention to withdrawal times prior to shipment to slaughter was exercised. All animal health events, including treatment date, presumptive diagnosis, drugs used, and doses administered, were recorded. If an animal died, a necropsy was conducted by a veterinarian.

Nearly one-half (49.1%) of the cattle in the study (n = 1,286) were treated therapeutically with at least one antimicrobial at some time during the backgrounding or finishing period. The antimicrobial agents used therapeutically included ceftiofur (n = 226), Cocci Bol-O-Tab (n = 4), florifenicol (n = 465), sulbactam and ampicillin (n = 2), trimethoprim and sulfadoxine (n = 97), tilmicosin (n = 281), oxytetracycline via intravenous injection (n = 5), and oxytetracycline via feed (n = 696). The majority of animals receiving antimicrobial agents therapeutically received these agents within 20 days of arrival at the feedlot, and only ceftiofur was administered after 130 days. The average duration of treatment for individual animals ranged from 1 to 5 days.

Sample collection. Animal enrollment began on 17 September 1999 and ended when there were no new fall calves arriving at the feedlot (November to December 1999). The animals enrolled in the study were a random sample of calves; a computer-generated randomization table was used to select the animals (10% of animals arriving at each feedlot on a given day). Each enrolled animal was given a study-specific identification tag. In total, 2,622 animals were sampled and consisted of 445 (2.7% of total incoming calves), 530 (4.0%), 686 (3.0%), and 961 (3.3%) animals in feedlots 1, 2, 3, and 4, respectively. At all four feedlots, the majority of animals were steer calves (93 to 95%). The remaining calves were heifers (4 to 7%) or bulls (0 to 0.9%). Of the 2,622 animals, 94.7% (n = 2,482) completed the feedlot study. In total, 61 animals died during their stay at the feedlot (2.3%), and 79 had incomplete follow-up information or lost identification tags.

Deep fecal samples were obtained per rectum using swabs (Accu-Culshure; Accu-Med Corporation, Pleasantville, NY). To obtain the fecal sample, the swab was inserted approximately 4 to 5 cm into the rectum of a constrained animal and rotated until it was covered with feces. Swabs were then placed in sterile tubes, placed on ice, and transported to the laboratory within 4 to 6 h of collection. Samples were obtained from cattle at three times during the course of the feedlot period: (i) within 1 day after arrival at the feedlot (i.e., entry sample), (ii) 69 days (SD = 3 days) after arrival (i.e., interim sample), and (iii) ca. 1 week before cattle were shipped (i.e., exit sample). The exit sample time was determined by the feedlot manager based on visual appraisal of when the cattle were finished for harvest. The exit sample was obtained, on average, 189 days (SD = 33 days) after the arrival of the animals at the feedlots. Any unfinished cattle that were removed from the feedlot for humane reasons were sampled prior to shipment, and those that died at the feedlots were sampled postmortem. Samples collected from animals that died or that were shipped prematurely before 100 days after arrival at the feedlot were deemed interim samples, whereas those collected from animals after 100 days were deemed exit samples. In total, 7,738 fecal samples were processed during the course of the study (2,621, 2,559, and 2,497 animals that were sampled at the entry, interim, and exit sample times, respectively). Sixty-one samples were obtained from animals that died during the course of the study.

Isolation of campylobacters. Swabs were vortexed in 750 μl brain heart infusion broth (Difco, Sparks, MD) containing 20% glycerol (vol/vol). Tubes were agitated using a vortexer (high setting). Using a loop, the resultant slurry (~25 μl) was streaked onto each of three media: (i) *Campylobacter* blood-free selective agar base (modified charcoal cefoperazone desoxycholate agar [mCCDA]; Oxoid, Nepean, ON, Canada) containing a selective supplement, SR155; (ii) mCCDA amended with 2.0 μg ml^{-1} of ciprofloxacin (cCCDA; Sigma-Genosys, Oakville, ON, Canada); and (iii) mCCDA amended with 4.0 μg ml^{-1} erythromycin (eCCDA; Sigma-Genosys). Given that ciprofloxacin and erythromycin are primary antimicrobials used to therapeutically treat humans suffering from campylobacteriosis, a decision was made to utilize mCCDA containing one-half of the breakpoint concentration (CLSI [formerly NCCLS] or best recommendation) of these antimicrobials in an attempt to select for campylobacters with decreased sensitivity to these drugs. All media were used within 72 h of preparation to minimize the possibility of antimicrobial deterioration. All fecal samples were spread on cCCDA and eCCDA. As well, feces from ~10% of the cattle (selected across pens, using a computer-generated randomization method) at each feedlot and sample time were plated on mCCDA. All cultures were incubated at 42°C in an atmosphere consisting of 10% CO_2, 10% H_2, and 80% N_2; ambient atmosphere was allowed to enter the chamber. At 48 h, an arbitrarily selected colony representing the predominant colony morphology was transferred to mCCDA not containing the selective supplement and streaked for purity. In addition, cells from colonies distinct in appearance from the predominant colony type were collected. All cultures were incubated for 48 to 72 h, cells were transferred to brain heart infusion broth containing 20% glycerol, and isolates were stored at −80°C.

Identification of campylobacters. All isolates were identified to the species level based on physiological characters (26). Representatives of each physiological group (305 isolates in total) were further characterized using colony PCR for the *Campylobacter* species *C. coli*, *C. fetus*, *C. hyointestinalis*, *C. jejuni*, and *C. lanienae* (15); with the exception of seven isolates (three *C. lanienae* isolates and four *C. hyointestinalis* isolates), PCR-based identifications corresponded to those made by using physiological characters.

Antimicrobial susceptibility testing. The MICs to azithromycin, ciprofloxacin, doxycycline, enrofloxacin, erythromycin, gentamicin, meropenem, nalidixic acid, and tetracycline hydrochloride were determined using the agar dilution methodology according to the CLSI, with the exception of the incubation atmosphere

used (10% CO_2, 10% H_2, and 80% N_2). The medium used was Mueller-Hinton II agar (Difco, Sparks, MD) containing 5% defibrinated horse blood. Cells were harvested from the surface of the medium after 24 h of growth at 42°C. Cells were suspended in sterile saline (0.075% NaCl), and cell density was adjusted to a 0.5 McFarland turbidity standard. Aliquots (450 μl) of the saline suspension were pipetted into the seeding wells of a Cathra replicator (Oxoid, Inc.). Freshly prepared plates of Mueller-Hinton agar amended with antimicrobial agents were then inoculated using 1-mm pins in the inoculating head of the replicator. Cultures were incubated at 42°C for 48 h, and the MIC was defined as the lowest concentration resulting in complete inhibition of visible growth on the medium. *Campylobacter jejuni* (ATCC 33560) was utilized as a quality control strain.

We applied the following breakpoint values defined by the CLSI for *Enterobacteriaceae*: 4 μg ml^{-1} for ciprofloxacin, 16 μg ml^{-1} for doxycycline, 32 μg ml^{-1} for nalidixic acid, and 16 μg ml^{-1} for tetracycline. For meropenem, the CLSI suggested a much lower breakpoint for campylobacters than for *Enterobacteriaceae*, and we employed a breakpoint of 0.25 μg ml^{-1} for this antimicrobial agent. The breakpoints selected for azithromycin (2 μg ml^{-1}) and erythromycin (8 μg ml^{-1}) were specified by the National Antimicrobial Resistance Monitoring System (FDA/USDA/CDC 1999). For enrofloxacin, a breakpoint of 2 μg ml^{-1} was used (1).

Data analysis. The carriage rates and prevalences of antimicrobial-resistant strains (i.e., on mCCDA and cCCDA) were very similar among the four feedlots (data not presented), and data were combined across feedlots for analyses. For example, the mean carriage rates ± standard errors of *C. jejuni* determined on mCCDA at the entry, interim, and exit sample times were 18.4% ± 1.9%, 40.5% ± 4.6%, and 46.0% ± 1.8%, respectively. The mean prevalences ± standard errors of tetracycline-resistant *C. jejuni* shed in feces on mCCDA were 1.4% ± 0.5%, 38.9% ± 4.9%, and 43.1% ± 2.1% at the entry, interim, and exit sample times, respectively. Since so few isolates were recovered on eCCDA, data obtained from this medium were not subjected to analyses independent of the other media. Analyses were conducted using Statistical Analyses System software (31). In order to determine whether significant count changes occurred among the three sampling times at the various MICs, the Genmod procedure from SAS was used to perform a log-linear analysis for each antimicrobial agent for the mCCDA and cCCDA isolation media, using frequency counts as the dependent variable and MIC, sampling time, and their interaction as the factors in the model; only MICs that had at least one frequency for each sampling time were used in these analyses. To determine whether significant differences in frequency distributions of MICs occurred between media, a log-linear analysis was also conducted but medium was included in the model; to account for differences in numbers of isolates recovered on the two media, natural log transformations of the numbers were used as an offset variable for total counts. When a significant treatment effect was observed, contrast statements were used to evaluate differences among means of interest. Median MICs (MIC_{50}) for each species, antimicrobial agent, and medium were calculated from cumulative susceptibility data. A log-linear analysis was also performed to determine whether significant differences existed in resistance (i.e., as defined by breakpoints) to each antimicrobial between the sampling times for isolates of *C. coli*, *C. fetus*, and *C. jejuni*; a natural log transformation was also used as an offset variable for total counts, and contrast statements were used to evaluate differences among means of interest when a significant treatment effect was observed. The frequency procedure of SAS was used to perform the chi-square test with the Fisher exact test in order to assess the relationship among categorical variables at the interim and exit sample times for combined *Campylobacter* species and antimicrobial resistance occurrence; analyses were restricted to antimicrobial resistance to doxycycline, erythromycin, and tetracycline ($P \leq 0.05$).

RESULTS

Isolation of campylobacters. All samples were streaked onto cCCDA and eCCDA, whereas 878 (11.3%) randomly selected samples were also streaked onto mCCDA. In total, 1,586 *Campylobacter* isolates were recovered. Most of the isolates were *C. coli*, *C. fetus*, or *C. jejuni* isolates, and a small number of *C. hyointestinalis* ($n = 4$) and *C. lanienae* ($n = 3$) isolates were also recovered (Table 1). The amendment of the basal isolation medium with ciprofloxacin or erythromycin had a profound effect on the isolation of campylobacters. Overall, recovery rates from cattle were 50.3, 14.0, and 0.5% on mCCDA, cCCDA, and eCCDA, respectively. Although *C. coli*, *C. fetus*, and *C. jejuni* were isolated on all three media, the media differentially affected the recovery of specific taxa. For example, the majority of isolates recovered on cCCDA were *C. fetus* (85%) isolates, whereas most isolates (66%) recovered on mCCDA were *C. jejuni* isolates. For samples in which fecal material from the same animal was streaked on both mCCDA and cCCDA and campylobacters were recovered on both media ($n = 98$ samples), the same taxon was isolated on both media 46.9% of the time. For 43.9% of the other samples, *C. fetus* was recovered on cCCDA whereas *C. jejuni* was isolated on mCCDA.

TABLE 1. Carriage rates of *C. coli*, *C. fetus*, and *C. jejuni* in feedlot cattle by isolation medium and sample time[a]

Sample and species[d]	No. (%) of positive animals by indicated isolation medium[b]		
	mCCDA	cCCDA[c]	eCCDA
Entry			
C. coli	9 (3.4)	5 (0.2)	1 (0.04)
C. fetus	15 (5.6)	258 (9.8)	2 (0.08)
C. jejuni	47 (17.7)	97 (3.7)	0 (0)
C. lanienae	0 (0)	1 (0.04)	0 (0)
All species	71 (26.7)	361 (13.8)	3 (0.1)
Interim			
C. coli	54 (19.8)	12 (0.5)	13 (0.5)
C. fetus	8 (2.9)	111 (4.3)	0 (0)
C. jejuni	110 (40.3)	18 (0.7)	0 (0)
C. hyointestinalis	1 (0.4)	0 (0)	0 (0)
All species	173 (63.4)	141 (5.5)	13 (0.5)
Exit			
C. coli	18 (6.5)	7 (0.3)	10 (0.4)
C. fetus	29 (10.4)	550 (22.0)	0 (0)
C. jejuni	130 (46.8)	9 (0.4)	5 (0.2)
C. hyointestinalis	0 (0)	2 (0.08)	1 (0.04)
C. lanienae	0 (0)	2 (0.08)	0 (0)
All species	177 (63.7)	570 (22.8)	16 (0.6)
Dead			
C. coli	13 (21.3)	5 (8.2)	5 (8.2)
C. fetus	2 (3.3)	5 (8.2)	0 (0)
C. jejuni	6 (9.8)	2 (3.3)	0 (0)
All species	21 (34.4)	12 (19.7)	5 (8.2)
All samples	442 (50.3)	1,084 (14.0)	37 (0.5)

[a] In total, 7,738 fecal samples from individual animals were plated on cCCDA and eCCDA, whereas 878 samples were plated on mCCDA. See Materials and Methods for details on isolation media.

[b] Percent values were based on the total number of animals sampled. For mCCDA, 266, 273, and 278 fecal samples were processed at the entry, interim, and exit sample times, respectively. For cCCDA and eCCDA, 2,621, 2,559, and 2,497 fecal samples were processed at the entry, interim, and exit samples times. In addition, 61 samples were obtained from animals that died during the feedlot period. The total number of isolates recovered was 1,586 (154 for *C. coli*, 994 for *C. fetus*, 431 for *C. jejuni*, 4 for *C. hyointestinalis*, and 3 for *C. lanienae*).

[c] An additional 23 isolates were obtained from cCCDA (i.e., secondary cultures) and are not shown in the table.

[d] Samples were collected upon the arrival of the cattle at the feedlot (i.e., entry sample), ca. 70 days thereafter (i.e., interim sample), and just before cattle were shipped for slaughter (i.e., exit sample). If an animal died or was shipped prematurely, a sample was also obtained.

Carriage rates of campylobacters. Over the feedlot period, isolation frequencies of campylobacters from beef cattle differed on mCCDA (chi-square = 55.7; df = 6; $P < 0.001$) and cCCDA (chi-square = 188.8; df = 6; $P < 0.001$) (Table 1). For both *C. jejuni* and *C. coli* isolates on mCCDA, fewer ($P < 0.001$) isolates were recovered from animals at the entry rela-

tive to later sample times. In contrast, carriage rates of *C. fetus* determined using mCCDA did not change ($P \geq 0.23$) among the three sample times. On cCCDA, more ($P < 0.001$) isolates of *C. fetus* were recovered from animals at the exit sample time, whereas there was no difference ($P \geq 0.09$) among the three sample times in carriage rates of *C. coli* and *C. jejuni* isolates.

Antimicrobial resistance. MICs of azithromycin, ciprofloxacin, doxycycline, enrofloxacin, erythromycin, gentamicin, meropenem, nalidixic acid, and tetracycline were determined for each isolate of *C. coli*, *C. fetus*, and *C. jejuni*, and susceptibilities were then based on the breakpoint values specified previously. A high percentage of the isolates recovered were resistant to doxycycline and tetraycline for *C. coli* (84 and 89%), *C. fetus* (39%), and *C. jejuni* (50 and 64%) over the course of the feedlot period (Table 2). In addition, considerable resistance to erythromycin in *C. coli* isolates (83%) and to nalidixic acid in *C. fetus* isolates (97%) was observed over the three sample times. For the remainder of the antimicrobial agents tested, limited (≤4%) resistance was observed. For all antimicrobial agents, significant differences in frequency distribution (chi-square = 10.2 to 73.1; df = 4 to 8; $P \leq 0.045$) of total counts among MICs and sample times occurred between mCCDA and cCCDA (data not shown). This difference was most pronounced for doxycycline and tetracycline, to which isolates recovered on mCCDA were generally more resistant, and this was reflected in the higher median MICs for isolates recovered on this medium (Table 2). Furthermore, a bimodal distribution of resistance to doxycycline and tetracycline was observed for *C. fetus* and *C. jejuni* and, to a lesser extent, for *C. coli*. While less conspicuous than for tetracyclines, significant overall differences in the frequency distribution of MICs of nalidixic acid, enrofloxacin, and ciprofloxacin were also observed between mCCDA and cCCDA; this was due primarily to the higher MICs observed for *C. fetus* isolates recovered on cCCDA (Table 2). In six instances (doxycycline and tetracycline resistance for *C. coli*, tetracycline resistance for *C. jejuni*, and nalidixic acid resistance for *C. fetus*), the median MICs were greater than the breakpoint concentration for the corresponding antimicrobial agent.

Temporal occurrence of AMR campylobacters. Total numbers of *C. coli*, *C. fetus*, and *C. jejuni* isolates resistant to tetracycline and doxycycline and the number of animals from which resistant isolates were isolated on mCCDA and cCCDA increased at later sample times (chi-square = 117.8 to 147.6; df = 2; $P < 0.001$) (Tables 2 and 3). Upon entry, numbers of *C. coli*, *C. jejuni*, and *C. fetus* isolates resistant to doxycycline and tetracycline were relatively low (≤11%) and increased by 32 to 100% over the course of the feedlot period (Table 2). Similarly, less than 2% of the animals shed resistant campylobacters at the entry sample period, and this rate increased from 7.2 to 42.0% at subsequent sample times for isolates recovered on mCCDA. On cCCDA, less conspicuous differences in carriage rates of isolates resistant to doxycycline and tetracycline were observed between the entry (≤ 0.3%), interim (0.4 to 1.4%), and exit (0.3 to 12.7%) sample times.

An increase in numbers of erythromycin-resistant isolates (43 to 50%) recovered on both mCCDA and cCCDA and in the rate of shedding (15%) of erythromycin-resistant campylobacters on mCCDA was observed during the feedlot period, but only for *C. coli* (chi-square = 6.4 to 42.0; df = 2; $P \leq 0.042$) (Tables 2 and 3). The majority (97%) of *C. fetus* isolates recovered were resistant to nalidixic acid, and carriage of resistant strains differed among the three sample times for both mCCDA (chi-square = 11.7; df = 2; $P = 0.003$) and cCCDA (chi-square = 301.6; df = 2; $P < 0.001$); a significantly greater ($P \leq 0.002$) number of cattle that carried nalidixic acid-resistant isolates was observed for the exit sample time (Table 2). For all other antimicrobial agents tested, no increase or minimal increases in resistance were observed while animals were in the feedlot.

Multidrug resistance. Resistance to two or more classes of antimicrobial agents was infrequently observed, and the majority of *Campylobacter* isolates that exhibited multidrug resistance were *C. coli* resistant to tetracyclines and erythromycin (9.8%). Only 3.1% of *C. jejuni* isolates exhibited multidrug resistance; 11 isolates were resistant to tetracyclines and erythromycin, and 2 isolates were resistant to nalidixic acid and fluoroquinolones.

Impact of categorical variables on antimicrobial resistance. In general, the low counts of AMR *Campylobacter* isolates compromised the power of the analyses conducted. The categorical variables tested were as follows: antimicrobial treatment (not prophylactically), Aureomycin S-700 treatment, Aureomycin S-700 and Bovatec treatment, Cocci Bol-O-Tab treatment, ceftiofur treatment, florfenicol treatment, oxytetracyline treatment, ampicillin and sulbactam, timethoprim and sulfadoxine treatment, and tilmicosin treatment. In no instance were any of the variables evaluated associated with significant acquisition of resistance ($P \leq 0.05$).

DISCUSSION

The primary objective of the current study was to determine what impact, if any, the administration of antimicrobial agents to feedlot cattle had on the occurrence of antimicrobial resistance. To achieve this, we monitored 2,622 individual cattle in four commercial feedlots in southern Alberta. More than 1,500 isolates of *Campylobacter* were recovered, and their sensitivities to eight antimicrobial agents were quantified. Overall, we observed a high percentage of isolates resistant to doxycycline and tetracycline for *C. coli* (83 to 89%), *C. fetus* (39%), and *C. jejuni* (50 to 64%). In two recent snapshot surveys, Bae et al. (4) observed moderate rates of resistance to doxycycline (~31%) in 59 isolates of *C. jejuni* and *C. coli* obtained from 98 fecal pats in two Washington State feedlots, and Englen et al. (10) observed that 52% of 231 isolates of *C. jejuni* and *C. coli* obtained from 1,029 fecal pats and 73 feedlots across the United States were resistant to tetracycline. These studies neither monitored antimicrobial exposure in these cattle nor addressed the impact that antimicrobial administration within feedlots had on resistance development. In all four feedlots that we monitored, extensive administrations of tetracyclines (both chlortetracycline and oxytetracycline) were made to cattle prophylactically and/or metaphylactically; in North America, such antimicrobial use is the industry standard (NebGuide, University of Nebraska, Lincoln [http://ianrpubs.unl.edu/beef/g761.htm]).

To determine the impact that antimicrobial administration had on temporal development of resistance in feedlots, we

TABLE 2. Resistant *C. coli*, *C. fetus*, and *C. jejuni* isolates by isolation medium and sample time[a]

Species and drug	MIC breakpoint ($\mu g\ ml^{-1}$)	% (no.) of resistant isolates on mCCDA in indicated sample				MIC_{50} ($\mu g\ ml^{-1}$) on mCCDA	% (no.) of resistant isolates on cCCDA in indicated sample				MIC_{50} ($\mu g\ ml^{-1}$) on cCCDA	Total % (no.) of resistant isolates[b]
		Entry	Interim	Exit	Dead		Entry	Interim	Exit	Dead		
C. coli												
Azithromycin	2	0 (0)	1.9 (1)	0 (0)	7.7 (1)	0.56	0 (0)	0 (0)	0 (0)	0 (0)	0.47	3.9 (6)
Ciprofloxacin	4	0 (0)	0 (0)	0 (0)	0 (0)	0.18	0 (0)	0 (0)	0 (0)	0 (0)	0.18	0 (0)
Doxycycline	16	11.1 (1)	90.7 (49)	88.9 (16)	100 (13)	20.5	0 (0)	75.0 (9)	100 (7)	100 (5)	12.0	83.8 (129)
Enrofloxacin	2	0 (0)	0 (0)	0 (0)	0 (0)	0.12	0 (0)	0 (0)	0 (0)	0 (0)	0.12	0 (0)
Erythromycin	8	44.4 (4)	83.3 (45)	94.4 (17)	100 (13)	5.8	40.0 (2)	83.3 (10)	57.1 (4)	100 (5)	5.2	82.5 (127)
Gentamicin	16	0 (0)	0 (0)	0 (0)	0 (0)	0.50	0 (0)	0 (0)	0 (0)	0 (0)	0.40	0 (0)
Meropenem	0.25	0 (0)	0 (0)	0 (0)	0 (0)	0.023	0 (0)	0 (0)	0 (0)	0 (0)	0.02	0 (0)
Nalidixic acid	32	0 (0)	0 (0)	0 (0)	15.4 (2)	11.0	0 (0)	8.3 (1)	0 (0)	0 (0)	7.7	1.9 (3)
Tetracycline	16	11.1 (1)	100 (54)	88.9 (16)	100 (13)	178.0	0 (0)	100 (12)	100 (7)	100 (5)	82.0	89.0 (137)
C. fetus												
Azithromycin	2	0 (0)	0 (0)	0 (0)	0 (0)	0.18	0 (0)	0 (0)	0 (0)	0 (0)	0.18	0 (0)
Ciprofloxacin	4	0 (0)	0 (0)	3.4 (1)	0 (0)	0.46	0.4 (1)	0 (0)	0.7 (4)	0 (0)	0.51	0.6 (6)
Doxycycline	16	0 (0)	62.5 (5)	69.0 (20)	50.0 (1)	3.0	0.8 (2)	32.4 (36)	57.5 (316)	60.0 (3)	0.46	39.0 (388)
Enrofloxacin	2	0 (0)	0 (0)	3.4 (1)	0 (0)	0.38	0.4 (1)	0 (0)	0.9 (5)	0 (0)	0.43	0.7 (7)
Erythromycin	8	0 (0)	0 (0)	0 (0)	0 (0)	0.95	0 (0)	0.9 (1)	0 (0)	0 (0)	0.90	0.1 (1)
Gentamicin	16	0 (0)	0 (0)	0 (0)	0 (0)	0.58	0 (0)	0 (0)	0 (0)	0 (0)	0.60	0 (0)
Meropenem	0.25	6.7 (1)	0 (0)	0 (0)	0 (0)	0.02	0 (0)	0 (0)	0 (0)	0 (0)	0.02	0.1 (1)
Nalidixic acid	32	100 (15)	100 (8)	100 (29)	100 (2)	159.0	100 (258)	99.1 (110)	94.6 (520)	100 (5)	184.0	96.9 (963)
Tetracycline	16	0 (0)	75.0 (6)	69.0 (20)	50.0 (1)	2.20	0.8 (2)	33.3 (37)	57.1 (314)	60.0 (3)	0.41	39.0 (388)
C. jejuni												
Azithromycin	2	0 (0)	0 (0)	0 (0)	0 (0)	0.12	0 (0)	0 (0)	0 (0)	0 (0)	0.13	0.2 (1)
Ciprofloxacin	4	0 (0)	0 (0)	0 (0)	0 (0)	0.15	2.0 (2)	0 (0)	0 (0)	0 (0)	0.11	0.5 (2)
Doxycycline	16	8.5 (4)	60.0 (66)	82.3 (107)	66.7 (4)	10.0	6.1 (6)	83.3 (15)	77.8 (7)	100 (2)	0.11	50.1 (216)
Enrofloxacin	2	0 (0)	0 (0)	0 (0)	0 (0)	0.06	2.1 (2)	0 (0)	0 (0)	0 (0)	0.07	0.5 (2)
Erythromycin	8	0 (0)	2.7 (3)	0.8 (1)	0 (0)	1.28	1.0 (1)	0 (0)	22.2 (2)	0 (0)	1.39	2.8 (12)
Gentamicin	16	0 (0)	0 (0)	0 (0)	0 (0)	0.39	0 (0)	0 (0)	0 (0)	0 (0)	0.36	0 (0)
Meropenem	0.25	0 (0)	0.9 (1)	0 (0)	0 (0)	0.004	0 (0)	0 (0)	0 (0)	0 (0)	0.01	0.2 (1)
Nalidixic acid	32	0 (0)	0 (0)	0 (0)	0 (0)	0.49	5.1 (5)	0 (0)	11.1 (1)	0 (0)	0.48	1.4 (6)
Tetracycline	16	8.5 (4)	94.6 (104)	93.1 (121)	83.3 (5)	38.0	8.2 (8)	100 (18)	77.8 (7)	100 (2)	0.12	63.6 (274)

[a] Percent values were based on the total number of isolates recovered for each species on each medium and for each sample time (see Table 1). Samples were collected upon the arrival of the cattle at the feedlot (i.e., entry sample), ca. 70 days thereafter (i.e., interim sample), and just before cattle were shipped for slaughter (i.e., exit sample). Only one isolate was recovered per sample. See Materials and Methods for details on isolation media.

[b] Combined data for mCCDA, cCCDA, and eCCDA.

TABLE 3. Animals carrying antimicrobial-resistant *C. coli*, *C. fetus*, and *C. jejuni* by isolation medium and sample time[a]

Species and drug[b]	% (no.) of animals carrying species on mCCDA in indicated sample				% (no.) of animals carrying species on cCCDA in indicated sample			
	Entry (n = 266)	Interim (n = 273)	Exit (n = 278)	Dead (n = 61)	Entry (n = 2,621)	Interim (n = 2,559)	Exit (n = 2,497)	Dead (n = 61)
C. coli								
Azithromycin	0 (0)	0.4 (1)	0 (0)	1.6 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Doxycycline	0.4 (1)	18.0 (49)	5.8 (16)	21.3 (13)	0 (0)	0.4 (9)	0.3 (7)	8.2 (5)
Enrofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Erythromycin	1.5 (4)	16.5 (45)	6.1 (17)	21.3 (13)	0.1 (2)	0.4 (10)	0.2 (4)	8.2 (5)
Meropenem	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nalidixic acid	0 (0)	0 (0)	0 (0)	3.3 (2)	0 (0)	<0.1 (1)	0 (0)	0 (0)
Tetracycline	0.4 (1)	19.8 (54)	5.8 (16)	21.3 (13)	0 (0)	0.5 (12)	0.3 (7)	8.2 (5)
C. fetus								
Azithromycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	0 (0)	0.4 (1)	0 (0)	<0.1 (1)	0 (0)	0.2 (4)	0 (0)
Doxycycline	0 (0)	1.8 (5)	7.2 (20)	1.6 (1)	0.1 (2)	1.4 (36)	12.7 (316)	4.9 (3)
Enrofloxacin	0 (0)	0 (0)	0.4 (1)	0 (0)	<0.1 (1)	0 (0)	0.2 (5)	0 (0)
Erythromycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	<0.1 (1)	0 (0)	0 (0)
Meropenem	0.4 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nalidixic acid	5.6 (15)	2.9 (8)	10.4 (29)	3.3 (2)	9.8 (258)	4.3 (110)	20.8 (520)	8.2 (5)
Tetracycline	0 (0)	2.2 (6)	7.2 (20)	1.6 (1)	0.1 (2)	1.4 (37)	12.6 (314)	4.9 (3)
C. jejuni								
Azithromycin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (2)	0 (0)	0 (0)	0 (0)
Doxycycline	1.5 (4)	24.2 (66)	38.5 (107)	6.6 (4)	0.2 (6)	0.6 (15)	0.3 (7)	3.3 (2)
Enrofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (2)	0 (0)	0 (0)	0 (0)
Erythromycin	0 (0)	1.1 (3)	0.4 (1)	0 (0)	<0.1 (1)	0 (0)	0.1 (2)	0 (0)
Meropenem	0 (0)	0.4 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nalidixic acid	0 (0)	0 (0)	0 (0)	0 (0)	0.2 (5)	0 (0)	<0.1 (1)	0 (0)
Tetracycline	1.5 (4)	38.1 (104)	43.5 (121)	8.2 (5)	0.3 (8)	0.7 (18)	0.3 (7)	3.3 (2)

[a] Percent values were based on the total number of animals sampled for each medium and sample time. Only one primary isolate was recovered per sample (i.e., animal). Samples were collected upon the arrival of the cattle at the feedlot (i.e., entry sample), ca. 70 days thereafter (i.e., interim sample), and just before cattle were shipped for slaughter (i.e., exit sample). In addition, animals that died were sampled. See Materials and Methods for details on isolation media.

[b] No resistance to gentamicin was observed for campylobacters isolated on mCCDA or cCCDA.

isolated campylobacters at three times during the feedlot period. Relative to antimicrobial resistance in isolates obtained from calves upon entry into the feedlot, we observed a substantively higher prevalence of *C. coli* (≥83% increase), *C. fetus* (≥38% increase), and *C. jejuni* (≥50% increase) isolates that were resistant to both tetracycline and doxycycline after ~70 to 190 days in the feedlot. Only a small number of *Campylobacter* isolates resistant to doxycycline and tetracycline (≤2%) were isolated from calves upon entry into the feedlot, which supports the hypothesis that the administration of tetracyclines throughout the feeding period in feedlots exerts considerable pressure, thereby selecting for AMR bacteria residing within the intestinal tracts of calves on pasture. Beef calves and cows typically have limited exposure to antimicrobial drugs in Alberta. This observation is also consistent with the findings of a previous study in which beef cattle were fed tetracycline in an experimental feedlot and antimicrobial resistance was longitudinally monitored in *C. jejuni* and *C. hyointestinalis* (19). In dairy cattle, Sato et al. (32) detected high rates of resistance to tetracycline (45%) in campylobacters but observed no difference in resistance between a conventional farm (i.e., cattle received therapeutic applications of antimicrobials) and an organic farm, suggesting that resistance to tetracycline occurs naturally in the populations of *Campylobacter* and/or that nonantimicrobial selection pressures are involved in resistance to tetracycline. Given the low number of resistant isolates recovered from calves upon entry at the feedlots, our results do not support the above conclusion concerning beef cattle. However, Bae et al. (4) detected substantial resistance (81%) to doxycycline in 26 isolates of *C. jejuni* recovered from beef calves in Washington State. Although they provided no information on antimicrobial exposure in these animals, this observation, relative to the findings of the current study, raises questions regarding what role antimicrobial administrations have in the selection and/or persistence of AMR strains of *Campylobacter* in beef cattle.

Tetracycline has been suggested as a treatment for humans infected with *C. jejuni* and *C. coli* (25). However, the extensive development of resistance to tetracyclines in countries such as Canada has led to a decrease in their clinical use. For example, Gaudreau and Gilbert (12) observed a significant increase in the number of *C. jejuni* isolates resistant to tetracycline in Québec; resistance rates were 19% in 1985 to 1986 compared with 56% in 1995 to 1997. Similarly, resistance to tetracycline in clinical isolates of *C. jejuni* in Alberta has increased from ~8 to 50% over the past 20 years (13). This observation is mirrored in other *Campylobacter* species, such as *C. fetus*. Tremblay et al. (37) observed that 34% of 111 isolates of *C. fetus* subsp. *fetus* obtained from patients in Québec were resistant to tetracycline. Although *C. jejuni* and, to a lesser extent, *C. coli*,

are recognized as important causes of gastroenteritis in humans (34), the significance of cattle as a source of human-pathogenic strains has not been fully ascertained. However, evidence now points toward *C. jejuni* from cattle as an important source of infectious strains (6, 7, 27, 30, 33). Given the increasing reliance on confined feeding operations for beef production, the extensive use of tetracyclines during this period of production and the high rates of tetracycline resistance development in campylobacters may be contributing to the increasing prevalence of tetracycline-resistant strains of *Campylobacter* encountered in diagnostic facilities in Canada.

The macrolide erythromycin was the first antimicrobial agent used to treat *Campylobacter* infections in humans, and it remains the treatment of choice for patients with uncomplicated enteritis in many countries, including Canada (34). Macrolides are frequently used as veterinary drugs, and considerable resistance in campylobacters to erythromycin (up to 50%) has been reported in some countries (34). In Canada, resistance to this antimicrobial agent in clinical isolates of *C. jejuni* has remained consistently low (13). Consistent with that report, we observed that only 12 (3%) *C. jejuni* isolates and 1 (0.1%) *C. fetus* isolate obtained from feedlot cattle exhibited resistance to erythromycin. Similarly, only one isolate of *C. jejuni* and six isolates of *C. coli* exhibited resistance to the macrolide azithromycin. In contrast to *C. jejuni* and *C. fetus*, 83% of *C. coli* isolates were resistant to erythromycin, apparently independent of macrolide selection pressure, given that macrolides were not administered subtherapeutically in any of the four feedlots in the current study; erythromycin resistance in *C. coli* is common relative to that in *C. jejuni* (9, 11). The prevalence of erythromycin resistance that we observed is substantially higher than that observed by Englen et al. (10) for *C. coli* (3%). The macrolide tylosin is used subtherapeutically to prevent liver abscesses in beef cattle, but tylosin and other macrolides (e.g., tilmicosin and erythromycin) may be used therapeutically to treat cattle with respiratory disease and foot rot (28). The impact of tylosin and/or tilmicosin administration to beef cattle on the development of macrolide resistance in campylobacters has not been empirically investigated to our knowledge. However, it is well documented that erythromycin resistance is readily selected for in streptococci and staphylococci within the intestinal tracts of animals ingesting feed supplemented with tylosin (20). Although they did not observe the development of resistance to erythromycin in beef cattle that were administered tylosin, Inglis et al. (19) did observe significant resistance to erythromycin in *C. hyointestinalis* isolates obtained from cattle that were administered chlortetracycline. Whether resistance to erythromycin is influenced by tetracycline treatment, and the mechanisms by which this may occur, is uncertain.

As an alternative to erythromycin, the quinolone ciprofloxacin is often used to treat humans suffering from campylobacteriosis. However, in some countries, extensive resistance to ciprofloxacin has adversely affected its efficacy as a treatment for campylobacteriosis (35). Resistance frequencies in *C. jejuni* to ciprofloxacin have not apparently increased in Alberta over the past 20 years; only 2% of clinical isolates of *C. jejuni* were found to be resistant according to a recent survey of clinical isolates (13). Similarly, only 5% of clinical isolates of *C. fetus* subsp. *fetus* from Québec were resistant to ciprofloxacin (37). We observed minimal resistance to ciprofloxacin ($\leq$1%) in *C. coli*, *C. fetus*, and *C. jejuni* isolates obtained from feedlot cattle. Although fluoroquinolones are not registered for subtherapeutic use in beef cattle in Canada, recently enrofloxacin was licensed for therapeutic use. The impact of enrofloxacin administration in beef cattle on the development of resistance to fluoroquinolones in campylobacters and other bacteria is currently uncertain.

We amended mCCDA with one-half of the breakpoint concentration of either erythromycin or ciprofloxacin in an attempt to select for campylobacters with decreased sensitivity to these antimicrobials given that these drugs are generally advocated as first- and second-line drugs for antimicrobial treatment of campylobacteriosis (9). The inclusion of erythromycin and ciprofloxacin in the isolation medium provided mixed results. For example, the amendment of media with these antimicrobials profoundly affected isolation efficacy. Overall, isolation rates on cCCDA and eCCDA were 14% and 0.5%, respectively, compared to 50% on mCCDA. However, a significant shift in the frequency distribution of susceptibility to ciprofloxacin was observed for isolates recovered on cCCDA relative to those recovered on mCCDA, although the majority of isolates recovered on cCCDA possessed an MIC of less than 2 $\mu g\ ml^{-1}$ (i.e., the concentration of ciprofloxacin used in the isolation medium). While *C. coli* and *C. jejuni* were isolated 29 and 21 times less frequently on cCCDA relative to mCCDA, respectively, *C. fetus* was isolated two times more frequently on cCCDA. Consistent with this observation, the same taxon (i.e., *C. coli*, *C. jejuni*, or *C. fetus*) was recovered on both media (i.e., when the same sample was processed on both) 47% of the time, whereas isolation of *C. fetus* relative to other taxa on cCCDA occurred 51% of the time. One possible reason for the enhanced isolation of *C. fetus* on cCCDA (12%) relative to that on mCCDA (6%) is this taxon's inherent tolerance to quinolones (5), and it may be possible to utilize quinolones or fluoroquinolones to selectively isolate *C. fetus* from microbiologically complex substrates such as feces. Although we did not use direct PCR in the current study, the shedding rates that we observed for *C. fetus* on cCCDA are consistent with findings from previous reports using culture-independent detection methods for *C. fetus* in bovine feces (17, 18). *Campylobacter fetus* subsp. *fetus* is a rarely reported pathogen of humans (37) relative to *C. jejuni* and *C. coli*. However, thermotolerant *C. fetus* can cause gastroenteritis (2, 21), although it is more frequently involved in bloodstream or extraintestinal infections (22), often in immunocompromised individuals. A possible link between thermotolerant *C. fetus* isolates shed in bovine feces and human health is uncertain.

In conclusion, 1,586 isolates of *Campylobacter* were recovered from beef cattle maintained in four commercial feedlots in Alberta, and their susceptibilities to nine antimicrobial agents were quantitatively assessed. The most prevalent taxa that we isolated were *C. coli*, *C. fetus*, and *C. jejuni*. For all three species, substantive resistance to tetracycline and doxycycline was observed, a result that likely arose because all animals were administered chlortetracycline and oxytetracycline throughout the feedlot period. A significant occurrence of resistance to erythromycin in *C. coli* was also observed. The emergence of erythromycin resistance may be linked to tetracycline use through a presently undefined mechanism. A min-

imal occurrence of resistance to azithromycin, ciprofloxacin, enrofloxacin, gentamicin, meropenem, and nalidixic acid was observed. Although no resistance to ciprofloxacin and erythromycin in *C. jejuni* was observed, the beef industry's reliance on prophylactic and metaphylactic administrations of antimicrobial agents which cause the proliferation of AMR campylobacters in feedlots emphasizes the need to monitor antimicrobial resistance development in campylobacters from beef cattle. Furthermore, the mechanisms of transmission of mobile genetic elements possessing antimicrobial resistance determinants among *Campylobacter* and other bacterial taxa within the feedlot environment and to meat, and the possible threat this poses to human health, warrant investigation.

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