Antimicrobial resistance in generic fecal *Escherichia coli* obtained from beef cattle on arrival at the feedlot and prior to slaughter, and associations with volume of total individual cattle antimicrobial treatments in one western Canadian feedlot

Sylvia L. Checkley, John R. Campbell, Manuel Chirino-Trejo, Eugene D. Janzen, John J. McKinnon

Abstract

A prospective observational study was carried out to examine antimicrobial resistance patterns of fecal *Escherichia coli* isolates of calves on arrival at the feedlot, and then evaluate the associations between the total volume of antimicrobial used for disease treatment and changes in antimicrobial resistance, during the feeding period. No macrolides or tetracyclines were administered in the feed during this study. On arrival, at the animal level, all 3 isolates obtained from 36.6% [95% confidence interval (CI): 29.0 to 44.8] of all cattle sampled (n = 153), were susceptible to all antimicrobials, while 5.9% (95% CI: 2.7 to 10.9) of cattle had at least 1 isolate that was resistant to ≥ 3 antimicrobials out of the 7 antimicrobials tested. The most frequent antimicrobials for which resistance was observed were sulphamethoxazole, ampicillin, and tetracycline where, of all cattle, 44.4% (95% CI: 36.4 to 52.7), 20.3% (95% CI: 14.2 to 27.5), and 17.7% (95% CI: 12.0 to 24.6), respectively had at least 1 resistant isolate. All cattle received antimicrobial metaphylaxis on arrival at the feedlot. Antimicrobial use was described for a cohort of 95 cattle. Antimicrobials were given to 42 of the 95 cattle during the feeding period, to treat disease. Amongst the 42 treated cattle, there were a total of 133 animal daily doses ($ADD_{Feedlot}$), where 1 $ADD_{Feedlot}$ represented 1 day of antimicrobial treatment received by a feedlot animal at the approved dose. Only 1 $ADD_{Feedlot}$ was given in the 100 days immediately prior to slaughter. There were no associations found between antimicrobial use and antimicrobial resistance in this study.

Résumé

*Une étude prospective observationnelle a été réalisée afin d'examiner les patrons de résistance aux antimicrobiens chez des isolats d'*Escherichia coli *d'origine fécale provenant de veaux lors de leur arrivée en parc d'engraissement, et ensuite évaluer les associations entre le volume total d'antimicrobiens utilisés pour traiter des maladies et les changements dans la résistance aux antimicrobiens, durant la période d'engraissement. Aucun macrolide ou tétracycline n'a été administré dans l'alimentation au cours de cette étude. À l'arrivée, au niveau de l'animal, les 3 isolats obtenus de 36,6 % [intervalle de confiance 95 % (CI) : 29,0 à 44,8] de tous les bovins échantillonnés (*n = *153) étaient sensibles à tous les agents antimicrobiens, alors que 5,9 % (CI95 % : 2,7 à 10,9) des bovins avaient au moins un isolat résistant à 3 antimicrobiens ou plus sur les 7 antimicrobiens testés. Les antimicrobiens pour lesquels de la résistance était observée le plus fréquemment étaient le sulfaméthoxazole, l'ampicilline et la tétracycline, où à partir de tous les animaux testés, respectivement, 44,4 % (CI 95 % : 36,4 à 52,7), 20,3 % (CI 95 % : 14,2 à 27,5) et 17,7 % (CI 95 % : 12,0 à 24,6) avaient au moins un isolat résistant. Tous les animaux ont reçu des antimicrobiens en métaphylaxie à leur arrivée en parc d'engraissement. L'utilisation d'antimicrobiens a été décrite pour une cohorte de 95 bovins. Des antimicrobiens ont été administrés à 42 des 95 bovins durant la période d'engraissement pour traiter des maladies. Parmi les 42 bovins traités, il y eut un total de 133 doses animales journalières ($ADD_{Feedlot}$) où 1 $ADD_{Feedlot}$ représentait 1 journée de traitement antimicrobien à la dose approuvée reçu par un animal en parc d'engraissement. Seulement 1 $ADD_{Feedlot}$ a été donnée dans les 100 jours précédents immédiatement l'abattage. Dans la présente étude, aucune association n'a été trouvée entre l'utilisation des antimicrobiens et de la résistance aux antimicrobiens.*

(Traduit par Docteur Serge Messier)

Food Safety Division, Alberta Agriculture and Food, 116B, 6909–116 Street, Edmonton, Alberta T6H 4P2 (Checkley); Department of Large Animal Clinical Sciences (Campbell), Department of Veterinary Microbiology (Chirino-Trejo), Western College of Veterinary Medicine, 52 Campus Drive, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5B4; Faculty of Veterinary Medicine, University of Calgary, HS G380, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1 (Janzen); Department of Animal and Poultry Science, College of Agriculture, 51 Campus Drive, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A8 (McKinnon).

Address all correspondence and reprint requests to Dr. Sylvia L. Checkley; telephone: (780) 427-8228; fax: (780) 422-3438; e-mail: sylvia.checkley@gov.ab.ca

This study was part of the PhD thesis of Dr. S.L. Checkley through the Department of Large Animal Clinical Sciences at the Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan.

Introduction

Antimicrobial resistance (AMR) has been perceived as an important component of both animal health and food safety in the international community and specifically by countries that import beef (1–4). Health Canada has examined the use of antimicrobials in agriculture (5); however, the pathways by which antimicrobial use (AMU) in cattle could affect human health were not understood (5–7). More information was also needed about the extent to which the use of antimicrobials in animal agriculture was related to AMR in human infections (5,8,9). In Canada, the beef industry and the veterinarians serving that industry, were aware of these issues and thus developed prudent use guidelines for antimicrobials (10–12). The objectives of this study were to examine AMR patterns of fecal *Escherichia coli* isolates of auction market derived, newly weaned calves on arrival at the feedlot, and then evaluate the associations between the total volume of parenteral antimicrobials used for disease treatment and changes in antimicrobial resistance, during the feeding period.

Materials and methods

The University Committee on Animal Care and Supply approved this study and the guidelines of the Canadian Council on Animal Care were followed. This study was carried out at the same time as another trial described previously (13).

In the fall of 2001, backgrounding of 447 newly weaned, auction market derived steers was carried out at the University of Saskatchewan Research Feedlot (1 time capacity 800 head) with 12 adjacent pens of 37 or 38 steers. The steers were crossbred beef calves with an average weight of 249 kg (s = 17 kg). They were divided into weight groups and randomly allocated at processing to create pens of approximately equal weights. The routine vaccination, parasiticide, and implant strategy was described previously (13). A metaphylactic antimicrobial injection of long-acting oxytetracycline (Liquamycin LA-200; Pfizer Canada Animal Health Group, Kirkland, Quebec), 1 mL/10 kg bodyweight (BW), SC, was given to each animal with a body temperature < 41°C. The rest of the cattle received tilmicosin (Micotil; Provel, Division Eli Lilly Canada, Guelph, Ontario), 1 mL/30 kg BW, SC, for disease treatment. Booster vaccinations and the 2nd hormonal implant were given at 94 days on feed (DOF), 18 d before the steers moved to the finishing feedyard. The steers were finished in 2 adjacent pens in a large commercial feedlot with a 1 time capacity of approximately 30 000 head. These cattle remained in their respective study groups until slaughter.

During the study, all of the aforementioned animal health products were used according to label instructions and detailed individual animal records were maintained. Both feedlots were typical of those found in western Canada, with dirt floors, shared waterers, and a central alley for feeding. Feedlot staff checked all cattle daily for signs of disease. Any cattle that appeared depressed, gaunt, or distinctly different from their penmates were pulled and treated according to the standard treatment protocols in use at the feedlot. Cattle were weighed before treatments, and all medications were used at the approved dose per kg body weight. The feedlot staff was blinded as to the allocation of the treatment groups and to the specific objectives of the study. Steers were also individually weighed at the feedlot within 24 h of slaughter at 245 to 260 DOF.

Pens were randomly entered into 1 of 2 feeding programs, with different diet compositions and feeding methods, used at the University feedlot during the backgrounding period (13). Monensin sodium 3% (Rumensin; Elanco Animal Health, Guelph, Ontario) was fed to all cattle during the backgrounding period in the total mixed ration at 27–28 ppm DM. All cattle were fed a high grain diet ad libitum with decoquinate at a dose of 0.5 mg/kg BW (Deccox 6% Premix; Alpharma, Mississauga, Ontario) during the finishing period.

Calculations of sample size were performed for the 2 main objectives (Win Episcope v2; University of Edinburgh, Edinburgh, Scotland). Fecal samples from 150 steers were sufficient to characterize AMR in as few as 5% to 10% of cattle with 80% power and 95% confidence. Sample size for the cohort study depended on the total number of treated cattle during the study, estimated between 15% and 45%, based on previously reported bovine respiratory disease (BRD) treatment rates (14). Fifty cattle per group, based on 80% power and 95% confidence, were needed to detect a significant relative risk of 1.5 to 2.

On arrival, fecal samples were collected from the rectums of all cattle using a new obstetrical glove for each animal. The samples were placed into individual, clean foam cups with lids, labeled, and then transported to the laboratory for direct storage at −80°C. One hundred and fifty of the 447 mixed-breed steers were chosen randomly for characterization of AMR in fecal *E. coli* isolates on arrival. At the end of the study, a random sample of cattle that were treated with antimicrobials for disease during the feeding period, and a sample of those that were not treated, were chosen for inclusion in the cohort study. The arrival samples from these cattle were then retrieved from the frozen, stored samples and sent for laboratory analysis. A 2nd fecal sample was collected from the cohort of cattle within 24 h of slaughter. All preslaughter fecal samples were collected in the same manner as the arrival samples, and transported to the laboratory for culture the same day.

Laboratory analysis

The feces were thawed (arrival samples only) and cultured overnight on MacConkey's agar. Identification of *E. coli* was confirmed by standard biochemical tests. Three individual colonies from each animal, with the characteristic phenotype of *E. coli*, were chosen randomly for subculture on blood agar. From each blood agar plate, *E. coli* were inoculated into sterile phosphate-buffered saline (PBS) to make standard solutions of 0.5 MacFarland. This solution was delivered onto Mueller-Hinton agar using the replicator technique. The minimum inhibitory concentrations (MICs) of 7 antimicrobials were determined using the agar dilution method. The antimicrobials tested were ampicillin (AMP), enrofloxacin (ENR), tetracycline (TCY), gentamycin (GEN), sulphamethoxazole (SMX), trimethoprim/sulfanilamide (TMP/SSS), and trimethoprim (TMP). The Mueller-Hinton plates were cultured at 37°C and antimicrobial susceptibilities were read between 18 and 24 h. A control strain of *E. coli* ATCC 25922 was included with each plate. Antimicrobial breakpoints and interpretation were from the CLSI standards (15,16) (Table I). All laboratory procedures were carried out according to CLSI standards.

Table I. Concentration range and breakpoints of antimicrobials tested

Antimicrobial[a]	Breakpoint for resistance (μg/mL)	Concentration range measured (μg/mL)
AMP	≥ 32	< 4, 4, 8, 16, 32, > 32
ENR	≥ 2	< 0.5, 0.5, 1, 2, 4, > 4
GEN	≥ 16	< 4, 4, 8, 16, 32, > 32
SMX	≥ 512	< 125, 125, 256, 512, > 512
TCY	≥ 16	< 2, 2, 4, 8, 16, 32, > 32
TMP/SSS	≥ 4/76	< 1/19, 1/19, 2/38, 4/76, 8/152, > 8/152
TMP	≥ 16	< 4, 4, 8, 16, 32, > 32

[a] AMP — Ampicillin; ENR — Enrofloxacin; GEN — Gentamicin; SMX — Sulphamethoxazole; TCY — Tetracycline; TMP/SSS = Trimethoprim/sulfanilamide; TMP — Trimethoprim.

Statistical analysis

Data were entered into a commercial spreadsheet and descriptive statistics were generated (SPSS v. 15.0.0 for Windows; SPSS, Chicago, USA). An animal was considered resistant if it had at least 1 resistant isolate. Exact confidence intervals for animal-level prevalence estimates were calculated (PEPI v 4; Sagebrush Press, Salt Lake City, USA) (17). The pen allocation changed after 112 DOF from 12 pens to 2 pens. No adjustment for clustering was used as there was no clustered pen structure representative of the entire feeding period.

The measurement, $ADD_{Feedlot}$, was used to quantify the number of actual antimicrobial treatments given at the approved dose of the antimicrobial. This measurement accounted for the dosage and duration of action of the antimicrobial (Table II). The concept of $ADD_{Feedlot}$ was based on that of defined daily dose (DDD) used in human studies (18–21). Each antimicrobial treatment was described as 0, 1, 2, or 3 $ADD_{Feedlot}$ (Table II). An example calculation follows:

Example: An animal was treated with 27 cc of long acting oxytetracycline on November 9 (BW 270 kg) and 40 cc of oxytetracycline on January 19 (BW 400 kg).

20 mg/kg × 270 kg = 5400 mg, 5400 mg/(200 mg/mL) = 27 mL (actual dose given)

This was a long-acting treatment, equivalent to 3 $ADD_{Feedlot}$

20 mg/kg × 400 kg = 8000 mg, 8000 mg/(200 mg/mL) = 40 mL (actual dose given)

This was a long-acting treatment, equivalent to 3 $ADD_{Feedlot}$.

The total $ADD_{Feedlot}$ actually given to this animal, therefore, was 6.

Two dichotomous outcomes were examined: conversion to TCY resistance and conversion to AMP resistance. Resistance conversion was defined as an animal having more resistant isolates present preslaughter than at arrival. These outcomes were chosen because TCY is the antimicrobial class most commonly used in animals (22), and AMP resistance was thought to be associated with TCY resistance in previous feedlot work.

Table II. Antimicrobials used in this study and Animal Defined Dose ($ADD_{Feedlot}$) equivalent for a feedlot animal

Antimicrobials used in study — concentration	Dose equivalent (mg/kg BW)[a]	$ADD_{Feedlot}$ equivalent
Ceftiofur — 50 mg/mL; intramuscular injection (Excenel Sterile Powder; Pharmacia Animal Health, Orangeville, Ontario)	1	1
Oxytetracycline — 200 mg/mL; intramuscular injection (Liquamycin × LA-200; Pfizer Canada Animal Health Group, Kirkland, Quebec)	20	3
Tilmicosin — 300 mg/mL; subcutaneous injection (Micotil; Provel, Division Eli Lilly Canada, Guelph, Ontario)	10	3

[a] Actual body weight of the animal at the time of treatment.

Possible explanatory variables included Total $ADD_{Feedlot}$ (continuous), diet (dichotomous), antimicrobial treatment (dichotomous), conversion to AMP resistance for the outcome TCY resistance (dichotomous), and conversion to TCY resistance (dichotomous) as an explanatory variable for the outcome AMP resistance. Total $ADD_{Feedlot}$ was the sum of the actual doses received by the animal; this was a quantitative way to assess this potential association. Diet was examined to ensure the concurrent trial had no effect on the outcomes of this study. Antimicrobial treatment was a dichotomous variable representing whether the animal was treated or not. Tetracycline and AMP resistances were thought to be associated in previous feedlot studies, so AMP conversion was examined as an explanatory variable for the outcome TCY resistance, and TCY conversion was examined as a variable for AMP resistance.

There were no biological reasons to force any variables into the model. First, explanatory variables were each screened individually using unconditional logistic regression (SPSS v. 15.0.0 for Windows, SPSS). Variables with a statistical association at a level $P < 0.15$ were considered for entry into a multivariable model. When no variables were found to be significant through unconditional associations, the variables were also screened for entry into a multivariable model using a backwards stepwise approach. This was done because the effect of an important explanatory variable could be masked in the unconditional association due to uncontrolled confounding. The liberal *P*-value, $P < 0.15$, for entry into the model, was also used for this reason. A value of $P < 0.05$ was considered statistically significant and was necessary for a variable to stay in the model.

Results

Missing data

Three of the total 150 arrival samples had only 2 *E. coli* isolates cultured, and another 3 had only 1 isolate cultured. An extra 3 arrival samples were chosen randomly to be part of the study. This gave

Table III. Antimicrobial resistance in fecal *Escherichia coli* isolates from steers on arrival at the feedlot

Antimicrobial resistance	Count resistant (% resistant) n = 153	Exact lower confidence limit (%)	Exact upper confidence limit (%)
Resistance to antimicrobials[a]			
AMP[b] resistance	31 (20.3)	14.2	27.5
SMX resistance	68 (44.4)	36.4	52.7
TCY resistance	27 (17.6)	12.0	24.6
TMP/SSS resistance	7 (4.6)	1.9	9.2
TMP resistance	1 (0.7)	0.02	3.6
Multidrug Resistance[c]			
Resistant to 0 antimicrobials	56 (36.6)	29.0	44.8
Resistant to 1 antimicrobial	71 (46.4)	38.3	54.6
Resistant to ≥ 1 antimicrobials	97 (63.4)	55.2	71.0
Resistant to ≥ 2 antimicrobials	17 (11.1)	6.6	17.2
Resistant to ≥ 3 antimicrobials	9 (5.9)	2.7	10.9

[a] An animal was considered "resistant" if 1 or more isolate was resistant to the antimicrobial.
[b] AMP — Ampicillin, SMX — Sulphamethoxazole, TCY — Tetracycline, TMP/SSS — Trimethoprim/sulfanilamide, TMP — Trimethoprim.
[c] The number of antimicrobials to which an animal was resistant, was characterized by the isolate with resistance to the most number of antimicrobials.

a total of 450 isolates from 153 cattle on arrival and represented an *E. coli* recovery rate of 3 isolates per animal from 96.1% of animals.

Fifty treated and 50 untreated cattle were chosen to be in the cohort analysis; 5 substitutes were also chosen if needed. Of the 55 treated cattle, 13 were excluded from the analysis; 8 lost their individual identification, and 5 died or were euthanized during the study due to chronic disease conditions. Of the 55 untreated cattle, 2 were excluded because they lost their individual identification preslaughter. The 3 extra, untreated cattle were left in the analysis. Overall, 42 treated cattle and 53 untreated cattle (n = 95) cattle were included in the cohort analysis.

Arrival

The most frequent antimicrobials for which resistance was observed were SMX, AMP, and TCY where, of all cattle, 44.4% (95% CI: 36.4% to 52.7%), 20.3% (95% CI: 14.2% to 27.5%), and 17.7% (95% CI: 12.0% to 24.6%), respectively had at least 1 resistant isolate (Table III). All isolates from an animal obtained from 36.6% (95% CI: 29.0% to 44.8%) of all cattle sampled (n = 153), were susceptible to all antimicrobials, while 5.9% (95% CI: 2.7% to 10.9%) of cattle had at least 1 isolate that was resistant to 3 or more antimicrobials out of the 7 antimicrobials tested (Table III).

Antimicrobial use

Sixty-six of the 447 cattle (14.8%) were given antimicrobials for disease treatment during the feeding period. Of the 447 cattle, 420 (94.0%) received a metaphylactic injection of long-acting oxytetracycline (Pfizer Canada Animal Health Group) on arrival. The other 27 (6.0%) were treated with tilmicosin (Division Eli Lilly Canada) on arrival due to undifferentiated fever or other early symptoms of disease.

Overall, amongst the 42 treated cattle used in the analysis, there was a total of 133 $ADD_{Feedlot}$ antimicrobials used for disease treatment; only 1 $ADD_{Feedlot}$ was administered during the last 100 d immediately prior to slaughter. There were 81 $ADD_{Feedlot}$ of tilmicosin (Micotil; Provel, Division Eli Lilly Canada) given to 26 cattle, 49 $ADD_{Feedlot}$ of ceftiofur (Excenel Sterile Powder; Pharmacia Animal Health, Orangeville, Ontario) given to 20 cattle, and 3 $ADD_{Feedlot}$ of oxytetracycline (Pfizer Canada Animal Health Group) given to 1 animal. Some cattle received treatment with more than 1 antimicrobial.

Conversion of cattle from nonresistant to resistant between arrival and preslaughter was described for each antimicrobial. Resistance conversion was stratified by the number of treated (ADD ≥ 1) and untreated cattle (ADD ≤ 1) (Table IV), and by the number of $ADD_{Feedlot}$ per animal (Table V). Tetracycline and AMP had the highest levels of resistance conversion during the study at 72/95 (75.8%, 95% CI: 65.9% to 84.0%) and 46/95 (48.4%, 95% CI: 38.0% to 58.9%) cattle, respectively (Table IV). The SMX conversion occurred in 11/95 (11.6%, 95% CI: 5.9% to 19.8%) cattle (Table IV). Overall, 34 of the 72 cattle (47.2%, 95% CI: 35.3% to 59.3%) that showed TCY conversion were treated for disease during the feeding period with a total of 107 $ADD_{Feedlot}$ (between 2 and 9 $ADD_{Feedlot}$ each). Thirty-eight of these 72 cattle (52.8%, 95% CI: 40.7% to 64.7%) that showed TCY conversion during the feeding period had no antimicrobial treatments during the feeding period, but all received oxytetracycline for metaphylaxis on arrival at the feedlot (Table V). Similarly, 24 of the 46 cattle with positive AMP conversion (52.2%, 95% CI: 36.9% to 67.1%) were treated with a total of 70 $ADD_{Feedlot}$ during the feeding period and 22 (47.8%, 95% CI: 32.9% to 63.1%) were not, other than metaphylaxis (Table V).

Table IV. Antimicrobial treatments stratified by whether animals converted to a resistant status to specific antimicrobials during the feeding period

Antimicrobial resistance to:	Treatment during feeding period	Resistance conversion[b]	No conversion	Total cattle
AMP[a]	No	22	31	53
	Yes	24	18	42
	Total	46	49	95
TCY	No	38	15	53
	Yes	34	8	42
	Total	72	23	95
SMX	No	6	47	53
	Yes	5	37	42
	Total	11	84	95
TMP/SSS	No	2	51	53
	Yes	0	42	42
	Total	2	93	95
TMP	No	3	50	53
	Yes	0	42	42
	Total	3	92	95

[a] AMP — Ampicillin; SMX — Sulphamethoxazole; TCY — Tetracycline; TMP/SSS — Trimethoprim/sulfanilamide; TMP — Trimethoprim.
[b] Resistance conversion was defined as an animal having more resistant isolates present preslaughter than at arrival.

Table V. Number of $ADD_{Feedlot}$ per animal level stratified by whether animals converted to a resistant status to specific antimicrobials during the feeding period

Change in antimicrobial resistance pattern	$ADD_{Feedlot}$ total						Grand total
	0	2	3	5	6	9	
No TCY[a] conversion	15	1	6	0	1	0	23
TCY conversion	38	8	22	2	1	1	72
No AMP conversion	31	4	10	2	1	1	49
AMP conversion	22	5	18	0	1	0	46
No SMX conversion	47	6	27	2	1	1	84
SMX conversion	6	3	1	0	1	0	11
No TMP/SSS conversion	51	9	28	2	2	1	93
TMP/SSS conversion	2	0	0	0	0	0	2
No TMP conversion	50	9	28	2	2	1	92
TMP conversion	3	0	0	0	0	0	3
Grand total	53	9	28	2	2	1	95

[a] AMP — Ampicillin; SMX — Sulphamethoxazole; TCY — Tetracycline; TMP/SSS — Trimethoprim/sulfanilamide; TMP — Trimethoprim.

Associations between antimicrobial use and antimicrobial resistance

No statistically significant associations were found between the outcomes and explanatory variables described (Table VI); therefore, no multivariable analyses were undertaken.

Discussion

Escherichia coli was chosen as the indicator organism in this study because it was a commensal bacterium in cattle that was ubiquitous, easy to culture, and one of the major carcass contaminants at slaughter (23). *Escherichia coli* was considered a potential reservoir of resistance genes that could transfer resistance to other zoonotic or commensal organisms that might cause disease in cattle or people (24–27). It was therefore judged important to ensure that the last fecal sample was collected during the last 24 h prior to shipping for slaughter, to be representative of bacteria at the stage of production that might ultimately affect the consumer.

There were few published studies on AMU and AMR in feedlot cattle. Direct comparison of results between species or even between different stages in the production cycle was not appropriate as AMU and management norms were extremely different (28). In general, calves arrive at the feedlot with relatively low proportions of resistant bacterial isolates. In 2005, surveillance of generic *E. coli* isolates from colon samples at Canadian abattoirs suggested that the prevalence of isolates resistant to ≥ 1 antimicrobials was about 27% in beef cattle, 85% in swine, and 77% in chickens (29). Numbers in this study suggested a slightly higher prevalence, but only because it was calculated for animals not isolates, where an animal was considered resistant if it had ≥ 1 resistant isolates. Even in comparable studies in feedlot cattle, differences may exist between methodologies for sample collection, culture, and determination of antimicrobial susceptibilities, analysis and presentation of results due to differing underlying purposes or interests (30). The use of veterinary specific antimicrobial susceptibility methods and standards from the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing, in this study and in the Canadian and American national surveillance systems, narrows some of these differences. However, it is important to keep in mind that CLSI methods and interpretive criteria were developed for approved indications of the antimicrobial for specific pathogens, but have been applied to surveillance of commensal bacteria (29,31).

The AMR profiles of generic *E. coli* isolated from cattle feces on arrival at the feedlot in western Canada have not been previously characterized. Similarly, baseline levels of resistant bacteria in the feces of newly weaned, auction market derived calves on arrival at the feedlot in western Canada are not well documented. The AMR in fecal *E. coli* on arrival at the feedlot represents the antimicrobial resistant strains of bacteria that the calves were carrying prior to antimicrobial treatment at the feedlot, and may be related to resistance patterns and AMU in their herd of origin. In this study, there was no resistance found to ENR (a fluoroquinolone) and GEN (an aminoglycoside), both of which represented drug classes used in human medicine. Both ENR and GEN were not approved for use in cattle in Canada at the time of this study, but were evaluated due to concerns about cross-resistance and coresistance, mechanisms by which resistance to 1 antimicrobial may be associated with resistance to another, related or unrelated, antimicrobial (22). Enrofloxacin (ENR) has now been licensed for use in cattle for the treatment of bovine respiratory disease. Another feedlot study in the USA also found no resistance to GEN or ciprofloxacin, a fluoroquinolone like ENR (32). The proportion of TCY resistant *E. coli* isolates was similar to that found in newly weaned calves on pasture in an American

Table VI. Univariate associations for antimicrobial resistance in cattle that had an increase in resistant isolates to tetracycline preslaughter

Variable	Variable (categorical)[a]	β	SE	Wald	df	*P*
TCY[b]	Treatment group	−0.517	0.497	1.082	1	0.298
	Diet	−0.374	0.482	0.600	1	0.439
	Total $ADD_{Feedlot}$ (overall)	—	—	1.411	2	0.494
	AMP conversion	−0.497	0.488	1.039	1	0.308
AMP	Treatment group	−0.631	0.418	2.273	1	0.132
	Diet	0.128	0.411	0.097	1	0.756
	Total $ADD_{Feedlot}$ (overall)	—	—	2.284	2	0.319
	TCY conversion	0.497	0.488	1.039	1	0.308

[a] β — Beta coefficient; SE — standard error of Beta coefficient; Wald — test statistic; df — degrees of freedom; *P* — probability value.
[b] AMP — Ampicillin; TCY — Tetracycline.

study where 13 to 17% of fecal samples contained *E. coli* resistant to TCY in at least 1 of the 5 isolates per fecal sample (33).

This study examined individual animal antimicrobial usage in the absence of feed antimicrobials other than coccidiostats. The use of feed antimicrobials for disease prophylaxis and treatment was a common practice in feedlots in western Canada, and elsewhere in North America; growth promotion was not always the primary objective of this use (34,35). There were no published studies on the quantities of antimicrobials used in western Canadian feedlots, but regional feedlot consultants describe their use of feed antimicrobials, other than ionophores, as primarily for disease prophylaxis (35). In western Canadian feedlots, use of antimicrobials by approved instructions was the operating norm (35). Coccidiostats, including ionophores, were antimicrobials but they have not been used in human medicine and the importance of these agents with respect to AMR remains unclear.

There were concerns that DDD in humans do not represent the prescribed daily dose (PDD) in humans at the national level (18,36). This was not a valid concern for the use of $ADD_{Feedlot}$ in this study where each $ADD_{Feedlot}$ was calculated for the actual antimicrobial dose received.

Recent studies in western Canada examined associations between AMR and AMU in *Campylobacter* spp. (37,38). In one of the studies, the authors suggest that the increased proportion of cattle with tetracycline resistant isolates of *Campylobacter* spp. preslaughter might be associated with the use of oxytetracycline or chlortetracycline in the feed (38). Other attempts to evaluate the association between individual animal AMU and AMR have been confounded by the concurrent use of antimicrobials in the feed (39,40). Therefore, the opportunity to evaluate associations in this study, in the absence of feed antimicrobials, such as oxytetracycline or tylosin, was considered important.

In the cohort of cattle examined, there were treated and untreated cattle with no resistant isolates on arrival, but ≥ 1 resistant isolates preslaughter. There were also cattle that had at least 1 resistant isolate on arrival but none preslaughter. No associations were found in this study between individual animal AMU and AMR. This was somewhat different than findings from other studies where associations were found between AMR in fecal *E. coli* isolates in pigs and cattle and individual AMU when feed antimicrobials were also used (39,40). In 1 study, individual animal use of GEN was associated with the farm-level prevalence of GEN resistance in fecal *E. coli* isolates in pigs (39). In another study, the use of injectable oxytetracycline in individual cattle receiving chlortetracycline in the feed was associated with increased prevalence of resistance to chloramphenicol and sulfisoxazole in fecal *E. coli* isolates (40). In both these cases, associations were found with antimicrobials (GEN, chloramphenicol and sulfisoxazole) not used for mass medication of cattle in the feed. It was suggested that an association of AMR with feed medication might obscure the relationship between individual animal antimicrobial treatment and AMR if this relationship did indeed exist (39,40). Studies in pre-weaned dairy calves have shown associations between AMR and AMU. In the 1 study, calves treated within 5 d prior to sampling were more likely to have multiple drug resistant fecal *E. coli* than those not treated during that time frame (41). This may indicate that individual antimicrobial treatment has a transient effect that would no longer be present in the current study, at the time immediately preslaughter when the 2nd sample was taken. Only 1 animal was treated in the 100 d immediately preslaughter. Again, the focus of this study was the change in AMR over the feeding period to understand the effect of antimicrobial agents over that time. It should also be noted that AMR does occur in the absence of AMU pressure (24,42).

A weakness in this study was that metaphylactic antimicrobial injections were given to all cattle; there were several reasons for this. The goal of the study was evaluation of associations between the volume of individual animal AMU and AMR. In western Canada, metaphylactic usage of antimicrobials was estimated at 20% to 50% of calves on arrival at the feedlot across all groups, based on the risk profiles of each group of cattle on arrival (28). Metaphylaxis use in this study was representative of commercial feedlot management; only 1 AMU variable was removed, the in-feed use of oxytetracycline or tylosin. It is likely that metaphylaxis was associated with AMR in this study; considerable TCY and AMP conversion occurred in both groups in the cohort study, not associated with the individual animal antimicrobial disease treatments. The volume of individual animal antimicrobial doses was a logical way to evaluate antimicrobial use quantified beyond that used for metaphylaxis. Using the variable Total $ADD_{Feedlot}$, not $ADD_{Feedlot}$ calculated for specific antimicrobials, could have masked associations between AMU of

specific antimicrobials and AMR (43). This study was not designed examine these specific associations.

In conclusion, calves arrived at the feedlot with relatively low numbers of resistant bacteria compared with other species (29). Basic description of AMU in 1 group of calves in a western Canadian commercial feedlot was given. Understanding of resistance patterns existing on arrival at the feedlot as well as AMU during the feeding period was essential to understanding the changing patterns of antimicrobial resistant isolates at the feedlot and interpreting resistance patterns preslaughter. No association was found between total AMU for individual animal disease treatment and AMR in fecal *E. coli* isolates. This study allowed a unique opportunity to evaluate these associations in cattle that were not fed antimicrobials, other than a coccidiostat, during the entire feeding period. The results suggest that metaphylaxis and AMR patterns should be evaluated further, and that the evaluation of conversion to resistance during the feeding period is important when assessing associations of AMR attributable to AMU during the feeding period.

Acknowledgments

The authors thank the management and staff of the University of Saskatchewan Feedlot and PoundMaker AgVentures for their cooperation during this study. The Saskatchewan Agriculture Development Fund and Novartis Animal Health Canada are also thanked for their support of this study.

References

1. Angulo FJ, Nunnery JA, Bair HD. Antimicrobial resistance in zoonotic enteric pathogens. Rev Sci Tech 2004;23:485–496.
2. Khachatourians GG. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. CMAJ 1998;159:1129–1136.
3. McGeer AJ. Agricultural antibiotics and resistance in human pathogens: Villain or scapegoat? CMAJ 1998;159:1119–1120.
4. OIE, Paris, France. OIE International Standards on Antimicrobial Resistance, 2003. [homepage on the Internet]. Available from http://www.oie.int/eng/publicat/ouvrages/A_119.htm Last accessed January 14, 2008.
5. Veterinary Drug Directorate, Health Canada. Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health. 2002. [homepage on the Internet]. Available from http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html Last accessed September 27, 2007.
6. Linton AH. Antibiotic resistance in veterinary practice. Vet Record, In practice 1982;14:11–13.
7. McKellar QA. Antimicrobial resistance: A veterinary perspective. BMJ 1998;317:610–611.
8. Conly J. Antimicrobial resistance in Canada. CMAJ 2002;167: 885–891.
9. Bywater RJ. Identification and surveillance of antimicrobial resistance dissemination in animal production. Poultry Science 2005;84:644–648.
10. Canadian Cattlemen's Association and Beef Information Centre (2006). Antibiotics in the Beef Cattle Industry, 2006. [factsheet on the Internet]. Available from http://www.cattle.ca/factsheets/Antibiotics.pdf Last accessed March 25, 2007.
11. Crandall J, Van Donkersgoed J. Canadian Cattlemen Quality Starts Here Recommended Operating Procedures for Feedlot Animal Health. Calgary: Canadian Cattlemen's Association, 2000.
12. Canadian Veterinary Medical Association. Prudent use guidelines, 2001. [homepage on the Interenet]. Available from http://canadianveterinarians.net/ShowText.aspx?ResourceID=86 Last accessed October 7, 2007.
13. Checkley SL, Janzen ED, Campbell JR, McKinnon JJ. Efficacy of vaccination against *Fusobacterium necrophorum* infection for control of liver abscesses and footrot in feedlot cattle in western Canada. Can Vet J 2005;46:1002–1007.
14. Kelly AP, Janzen ED. A review of morbidity and mortality rates and disease occurrence in North American feedlot cattle. Can Vet J 1986;27:496–500.
15. Clinical and Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial Susceptibility Testing; 16th Informational Supplement. (Use for Humans). CLSI document M100-S16, 2006.
16. NCCLS. Performance standards for Antimicrobial Disk and Dilution Susceptibility Tests for bacteria Isolates from Animals; Information Supplement M32-S1, 2004.
17. Abramson JH, Gahlinger PM. Computer programs for epidemiologists: PEPI version 4.0. Salt Lake City, Utah: Sagebrush Pr, 2001.
18. Grave K, Jensen VF, McEwen S, Kruse H. Monitoring of antimicrobial drug usage in animals: Methods and applications. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington DC: ASM Pr, 2006:380–385.
19. Austin DJ, Kakehashi M, Anderson RM. The transmission dynamics of antibiotic-resistant bacteria: The relationship between resistance in commensal organisms and antibiotic consumption. Proc Biol Sci 1997;264:1629–1638.
20. Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci USA 1999;96:1152–1156.
21. Baquero F. Trends in antibiotic resistance of respiratory pathogens: An analysis and commentary on a collaborative surveillance study. J Antimicrob Chemother 1998;38(Suppl A): 117–132.
22. Guardabassi L, Courvalin P. Antimicrobial resistance in bacteria of animal origin. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington, DC: ASM Pr, 2006, 5, 7.
23. Stopforth JD, Lopes M, Shultz JE, Miksch RR, Samadpour M. Microbiological status of fresh beef cuts. J Food Prot 2006;69: 1456–1459.
24. Donaldson SC, Straley BA, Hegde NV, Sawant AA, DebRoy C, Jayarao BM. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. App Environ Epidem 2006;72:3940–3948.
25. Hoyle DV, Yates CM, Chase-Topping ME, et al. Molecular epidemiology of antimicrobial-resistant commensal *Escherichia coli*

strains in a cohort of newborn calves. Appl Environ Microbiol 2005;71:6680–6688.
26. Linton AH. Antibiotic resistance in bacteria associated with animals and their importance to man. J Antimicrob Chemother 1985;15:385–386.
27. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp ID, Doern GV. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates form food animals and humans. Antimicrob Agents Chemother 2001;45:2716–2722.
28. Radostits OM. Control of infectious diseases of food-producing animals. In: Radostits OM, ed. Herd Health: Food Animal Production Medicine, 3rd ed. Philadelphia: WB Saunders, 2001:151.
29. Public Health Agency of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2005 — Final Report, 2005. [homepage on the Internet]. Available from http://www.phac-aspc.gc.ca/cipars-picra/2005_e.html#sum. Last accessed October 7, 2007.
30. McEwen SA, Aarestrup FM, Jordan D. Monitoring of antimicrobial resistance in animals: Principles and practices. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington DC: ASM Pr, 2006:398–399.
31. Watts JL, Lindeman CJ. Antimicrobial susceptibility testing of bacteria of veterinary origin. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington DC: ASM Pr, 2006:30, 33–34.
32. Wagner BA, Dargatz DA, Salman MD, Morley PS, Wittum TE, Keefe TJ. Comparison of sampling techniques for measuring the antimicrobial susceptibility of enteric *Escherichia coli* recovered from feedlot cattle. Am J Vet Res 2002;63:1662–1670.
33. Huston CL, Bailey RH, Best TF, Huston JE, Evans RR. Antimicrobial resistance of enteric *E. coli* in beef cattle treated with antibiotics. The AABP Proceedings 2003;36:156–157.
34. USDA. Part III: Health Management and Biosecurity in US Feedlots, 1999. USDA:APHIS:VS: CEAH, National Animal Health Monitoring System. Fort Collins, CO. #N336.1200.
35. Booker CW, Guichon PT, Schunicht OC, Wildman BK, Jim GK. Economic impact of antimicrobial use in feedlots. Bovine Proceedings 1999;32:111–112.
36. Monnet DL, Molstad S, Cars O. Defined daily doses of antimicrobial reflect antimicrobial prescriptions in ambulatory care. J Antimicrob Chemother 2004;53:1109–1111.
37. Inglis GD, McAllister TA, Busz HW, et al. Effects of subtherapeutic administration of antimicrobial agents to beef cattle on the prevalence of antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter hyointestinalis*. Appl Environ Microbiol 2005;71:3872–3881.
38. Inglis GD, Morck DW, McAllister TA, et al. Temporal prevalence of antimicrobial resistance in *Campylobacter* spp. from beef cattle in Alberta feedlots. Appl Environ Microbiol 2006;72:4088–4095.
39. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev Vet Med 1998;34:283–305.
40. O'Connor AM, Poppe C, McEwen SA. Changes in the prevalence of resistant *Escherichia coli* in cattle receiving subcutaneously injectable oxytetracycline in addition to in-feed chlortetracycline compared with cattle receiving only in-feed chlortetracycline. Can J Vet Res 2002;66:145–150.
41. Berge ACB, Atwill ER, Sischo WM. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. Prev Vet Med 2005;69:25–38.
42. Hoyle DV, Davison HC, Knight HI, et al. Molecular characterisation of bovine faecal *Escherichia coli* show persistence of defined ampicillin resistant strains and the presence of class 1 integrons on an organic beef farm. Vet Microbiol 2006;115:250–257.
43. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. Charlottetown: AVC Inc., University of Prince Edward Island, 2003.