Antimicrobial resistance in fecal *Escherichia coli* and *Campylobacter* spp. from beef cows in western Canada and associations with herd attributes and antimicrobial use

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Abstract

The objectives of this study were to describe the frequency of antimicrobial resistance (AMR) in *Escherichia coli* and *Campylobacter* spp. isolates in fecal samples from beef cow-calf herds and to examine the associations between herd management practices, reported antimicrobial use, and AMR. Baseline prevalence data are needed to evaluate the effectiveness of antimicrobial stewardship programs. A pooled fecal sample, representing 20 cows, was collected from each of 107 herds during pregnancy testing. In the 305 recovered *E. coli* isolates (maximum 3 per herd), resistance to ≥ 1 antimicrobial was identified in 12 isolates [4%, 95% confidence interval (CI): 2% to 7%] from 105 herds (11%, 95% CI: 7% to 19%). The most common resistances identified in *E. coli* isolates were to tetracycline (3%) and to both streptomycin and sulfisoxazole (3%). Only 1 *E. coli* isolate was resistant to an antimicrobial of very high importance to human health — amoxicillin/clavulanic acid. However, 2 *E. coli* isolates had intermediate susceptibility to ciprofloxacin. Resistance to 1 antimicrobial was identified in 16 of 87 *Campylobacter* spp. isolates (18%, 95% CI: 11% to 28%) from 87 herds. Resistance to tetracycline was reported in 15% of *Campylobacter* spp. isolates and to nalidixic acid in 3.4%. Herds in which cows were treated with florfenicol were more likely to have *E. coli* resistance to ≥ 2 antimicrobials (OR 7.1, 95% CI: 1.1 to 57, $P = 0.03$). Herds with calf mortality of > 5% were more likely to have *E. coli* with resistance to streptomycin and sulfisoxazole [odds ratio (OR): 7.8, $P = 0.03$]. The results of this study are consistent with previous reports from western Canada and provide a starting point for designing an ongoing antimicrobial surveillance program.

Résumé

*Les objectifs de la présente étude étaient de décrire la fréquence de résistance antimicrobienne (RAM) chez des isolats d'*Escherichia coli *et de* Campylobacter *spp. provenant d'échantillons fécaux de troupeaux bovins de type vache-veau et d'examiner les associations entre les pratiques de conduite d'élevage, l'utilisation rapportée d'antimicrobiens, et la RAM. Des données de prévalence de base sont requises afin d'évaluer l'efficacité de programmes de gérance des antimicrobiens. Un échantillon fécal regroupé, représentant 20 vaches, fut prélevé de chacun des 107 troupeaux durant la vérification des gestations. Parmi les 305 isolats d'*E. coli *obtenus (maximum de 3 par troupeau), de la résistance envers ≥ 1 antimicrobien a été identifiée chez 12 isolats [4 %, intervalle de confiance (IC) 95 % : 2 % à 7 %] de 105 troupeaux (11 %, IC 95 % : 7 % à 19 %). Les résistances les plus fréquemment identifiées parmi les isolats d'*E. coli *était envers la tétracycline (3 %) ainsi que la streptomycine et le sulfisoxazole (3 %). Seulement un isolat d'*E. coli *était résistant à un antimicrobien de très haute importance en médecine humaine, soit à l'amoxicilline/acide clavulanique. Toutefois, deux isolats d'*E. coli *avaient une sensibilité intermédiaire au ciprofloxacin. De la résistance envers un antimicrobien fut identifiée chez 16 des 87 isolats de* Campylobacter *spp. (18 %, IC 95 % : 11 % à 28 %) de 87 troupeaux. La résistance à la tétracycline a été rapportée dans 15 % des isolats de* Campylobacter *spp. et à l'acide nalidixique chez 3,4 %. Les troupeaux parmi lesquels les vaches furent traitées avec du florfénicol étaient plus susceptibles d'avoir des isolats d'*E. coli *résistants à ≥ deux antimicrobiens [rapport de cotes (RC) : 7,1, IC 95 % : 1,1 à 57, P = 0,03]. Les troupeaux avec une mortalité des veaux > 5 % étaient plus susceptibles d'avoir des* E. coli *avec une résistance à la streptomycine et au sulfisoxazole (RC : 7,8, P = 0,03). Les résultats de la présente étude sont cohérents avec des rapports antérieurs provenant de l'ouest canadien et fournissent un point de départ pour concevoir un programme de surveillance de la résistance antimicrobienne.*

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Received April 27, 2018. Accepted July 22, 2018.

Introduction

Understanding the association between antimicrobial use (AMU) in livestock and antimicrobial resistance (AMR) is important for informing antimicrobial stewardship initiatives. Antimicrobial resistance is a growing threat to disease management within the livestock industry. While a number of research studies have investigated AMR in western Canadian feedlots and elsewhere in North America (1–5) and there are ongoing surveillance programs examining AMR in the Canadian swine and poultry industries (6), there are no recent reports describing AMR in Canadian cow-calf herds.

There is limited information on AMR in cow-calf herds in Canada and even less on factors that can be manipulated to manage AMR in this sector of the beef industry. The studies from western Canada published to date are more than a decade old and were limited to analysis of generic *Escherichia coli* cultures (7,8). In the first study of beef calves in the spring of 2002, resistance to at least 1 antimicrobial was identified in 49% of isolates, 62% of calves, and 91% of 91 herds (7). Resistance was lower in the fall, with AMR identified in 7% of isolates, 13% of calves, and 56% of 45 herds (8). Cows were not sampled in the fall, but resistance was identified in 10% of cow isolates, 15% of cows, and 61% of 69 herds in the spring (8).

In addition to the 2002 study from western Canada, fecal samples were collected in 13 Ontario cow-calf farms in 2001 (9); isolates from 11% of pooled cow samples and 19% of pooled calf samples were resistant to at least 1 antimicrobial. Similar to the study from western Canada, the analysis was limited to generic fecal *E. coli.* The United States, however, reported AMR for 173 cow-calf herds in the beef 2007–2008 report (10), which included AMR data for generic *E. coli, Campylobacter, Salmonella, Enterococcus,* and *Clostridium difficile* from cow fecal samples.

Baseline data on the prevalence of AMR in cow-calf herds are needed in order to plan future surveillance initiatives and to evaluate the effectiveness of stewardship programs. The importance of antimicrobial stewardship and increased AMR surveillance in human and veterinary medicine was highlighted in the Pan-Canadian Framework for Action released in August 2017 (11). Health Canada has also changed their regulations to strengthen veterinary oversight of antimicrobial use (12). The objectives of this study were to describe the frequency of antimicrobial resistance in *E. coli* and *Campylobacter* spp. isolates in fecal samples from beef cow-calf herds and to examine the association between herd management practices, reported antimicrobial use, and antimicrobial resistance.

Materials and methods

Producer recruitment and survey distribution

Participants were part of a cow-calf surveillance network recruited from Alberta, Saskatchewan, and Manitoba during the first quarter of 2014. Herd recruitment has been described in a previous report (13). Briefly, veterinarians from all 3 provinces were contacted to recruit spring calving cow-calf herds with at least 100 cows that kept basic herd records and pregnancy tested. From the prospective participant list provided by local veterinarians, 109 producers managing either moderate (100 to 300 cows) or large (> 300 cows) cow-calf operations were enrolled during the spring, summer, and fall of 2014 and were eligible for sample collection in fall 2014. A consent form and an initial recruitment survey were sent by mail at the time of enrollment to collect baseline management information and production data from the 2013 calving season. A second paper-based survey examining antimicrobial use was mailed out in July 2014 to the 104 producers enrolled at that time and captured product use from July 2013 to June 2014. Details of the survey development were reported elsewhere (13). The survey was accompanied by a booklet with color photographs that listed antimicrobials licensed for use in cattle in Canada as an aide to recall. Both the survey and booklet were pilot-tested before distribution. Herd owners received a small honorarium for completed surveys and collected samples. The study was approved by the University of Saskatchewan Behavioural Research Ethics Board (#14-07) and the Animal Research Ethics Board (#2014003).

Collection, transport, and processing of fecal samples

Enrolled producers chose whether to participate in the collection of biological samples in the fall of 2014. The herd veterinarian was provided with a cooler, ice packs, and shipping materials for transport to the University of Saskatchewan (U of S). Samples were collected from 20 cows on each farm using a systematic random collection strategy during pregnancy testing by the local veterinarian. A handful of feces with a volume of 30 to 50 mL was collected from the rectum of each selected cow. Feces were placed in a pre-labelled, 1-L plastic screw-top sterile container, which created 1 composite sample per herd. The veterinary clinics were advised to keep the samples cool before shipping, but not to freeze the samples.

Samples were refrigerated immediately on arrival at the laboratory. Fecal samples were thoroughly mixed by hand using a sterile instrument and 2 subsamples were removed and retained at the University of Saskatchewan. The 1-L container with the remaining fecal matter was then couriered overnight on ice to the Public Health Agency of Canada (PHAC), National Microbiology Laboratory (NML) in St. Hyacinthe, Quebec.

Laboratory methods

All samples were processed by the PHAC, NML, Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) laboratory using previously reported standard protocols (14). The field sample was thoroughly stirred with a sterile instrument and then a 25-gram subsample of feces was removed and mixed with 225 mL of buffered peptone water before homogenizing at 230 rpm for 30 s. Each sample was then cultured for *E. coli, Campylobacter,* and *Salmonella.* A maximum of 3 *E. coli,* 1 *Campylobacter,* and 1 *Salmonella* (when identified) isolates were retained per sample and tested for susceptibility using automated broth microdilution (Sensititre; TREK Diagnostic Systems, Oakwood Village, Ohio, USA) and the National Antimicrobial Resistance Monitoring System (NARMS) Gram-negative panel. All testing was done in accordance with Clinical Laboratory Standards Institute (CLSI) standards. Data were reported as minimum inhibitory concentrations (MICs), which were then classified into either susceptible (susceptible and intermediate) or resistant (resistant) categories using

Table I. Breakpoints used for categorizing resistance (14).

Panel type: CMV3AGNF	Resistance breakpoints (μg/mL) *Escherichia coli*		
Antimicrobial	Susceptible	Intermediate	Resistant
Amoxicillin/Clavulanic acid[a]	≤ 8	16	≥ 32
Ampicillin[a]	≤ 8	16	≥ 32
Azithromycin[b]	≤ 16	—	≥ 32
Cefoxitin[a]	≤ 8	16	≥ 32
Ceftiofur[c]	≤ 2	4	≥ 8
Ceftriaxone[a]	≤ 1	2	≥ 4
Chloramphenicol[a]	≤ 8	16	≥ 32
Ciprofloxacin[a]	≤ 0.06	0.12 to 0.5	≥ 1
Gentamicin[a]	≤ 4	8	≥ 16
Kanamycin[a]	≤ 16	32	≥ 64
Nalidixic acid[a]	≤ 16	—	≥ 32
Streptomycin[b]	≤ 32	—	≥ 64
Sulfisoxazole[a]	≤ 256	—	≥ 512
Tetracycline[a]	≤ 4	8	≥ 16
Trimethoprim/Sulphamethoxazol[a]	≤ 2	—	≥ 4
Panel type: Campy	*Campylobacter jejuni*, *Campylobacter coli*		
Antimicrobial	Susceptible	Intermediate	Resistant
Gentamicin[b]	≤ 2	4	≥ 8
Telithromycin[b]	≤ 4	8	≥ 16
Clindamycin[b]	≤ 2	4	≥ 8
Azithromycin[b]	≤ 2	4	≥ 8
Erythromycin[d]	≤ 8	16	≥ 32
Florfenicol[b]	≤ 4	—	—
Ciprofloxacin[d]	≤ 1	2	≥ 4
Nalidixic acid[b]	≤ 16	32	≥ 64
Tetracycline[d]	≤ 4	8	≥ 16

[a] CLSI M100-S24, Vol. 34 No. 1, January 2014.

[b] No CLSI breakpoints. Breakpoints were based on distribution of MICs and were harmonized with NARMS.

[c] CLSI VET01-S2, Vol. 33 No. 8, July 2013.

[d] CLSI M45-A2, Vol. 26 No. 18, August 2010.

CLSI — Clinical and Laboratory Standards Institute; MICs — minimum inhibitory concentrations; NARMS — National Antimicrobial Resistance Monitoring System.

CLSI breakpoints (Table I). *Camplyobacter* isolates were speciated and *Salmonella* isolates were serotyped.

Data management and statistical analysis

Surveys were entered into a spreadsheet program (Microsoft Office Excel; Microsoft Corporation, Redmond, Washington, USA) and merged using a commercial database program (Microsoft Office Access; Microsoft Corporation). Herds were categorized by the following attributes: whether there were > 300 cows in the herd on January 1, 2014; if any purebred animals were sold; and if they started calving before March 1, 2014.

Producers reported which antimicrobials were used and the proportion of animals treated at least once with a particular antimicrobial (< 5%, 6% to 30%, 31% to 70%, > 70%). Antimicrobial use (AMU) was also grouped by importance to human health (15) and by route of administration. The prevalence of resistance was summarized at the isolate and herd level for *E. coli* and for *Campylobacter* spp. with 95% confidence intervals (95% CI) (16).

The association between herd management, AMU, and the presence or absence of the most commonly identified types of resistance within each herd was investigated for both *E. coli* and *Campylobacter* spp. using exact logistic regression (Stata SE, Version 14.1; StataCorp, College Station, Texas, USA). The outcomes examined were limited by the frequency of AMR identified in the samples. The resistance outcomes examined for *E. coli* included resistance to ≥ 1 or ≥ 2 antimicrobials, resistance to sulfisoxazole and/or streptomycin, and intermediate or resistant to tetracycline. The resistance outcomes examined for *Campylobacter* spp. included resistance to ≥ 1 antimicrobial and resistance to tetracycline.

All potential risk factors were screened based on unconditional or univariable analysis; factors with $P < 0.2$ were considered for inclusion in a final multivariable model. Continuous predictors were

Table II. Antimicrobials used at least once in cows or calves before weaning from July 2013 through June 2014 in 98 cow-calf herds.

Cows	Number of herds	Percent	Calves	Number of herds	Percent
Antimicrobials used (category of importance to human health[b])					
Aminoglycosides (II)	1	1%			
Aminoglycosides (topical agents) (III)	2	2%			
Cephalosporins (1st generation) (II)	3	3%			
Cephalosporins (3rd generation) (I)	7	7%	Cephalosporins (3rd generation) (I)	14	14%
			Fluoroquinolones (I)	8	8%
Florfenicol (III)	27	28%	Florfenicol (III)	79	81%
Lasalocid (IV)	2	2%	Lasalocid (IV)	1	1%
Macrolides (II)	19	19%	Macrolides (II)	23	24%
Monensin (IV)	10	10%	Monensin (IV)	6	6%
			Neomycin-sulfamethazine (II)	11	11%
Penicillin (II)	36	37%	Penicillin (II)	19	19%
Polymyxins[a] (I)	14	14%	Polymyxins[a] (I)	1	1%
Sulfa boluses (III)	5	5%	Sulfa boluses (III)	43	44%
			Sulphonamides (III)	2	2%
Tetracyclines (III)	78	80%	Tetracyclines (III)	54	55%
Trimethoprim-sulfadoxine (II)	7	7%	Trimethoprim-sulfadoxine (II)	30	31%
Any antimicrobial use	93	95%	Any antimicrobial use	93	95%
Importance to human health[b]					
Category I	20	20%	Category I	19	19%
Category II	49	50%	Category II	55	59%
Category III	85	87%	Category III	93	95%
Category IV	11	11%	Category IV	7	7%
Route of administration					
In-feed products	14	14%	In-feed products	9	9%
Injectable products	92	94%	Injectable products	92	93%
Intra-mammary product	22	22%	Intra-mammary product	1	1%
Intra-uterine bolus	6	6%	Oral boluses	51	52%
Topical wound spray	2	2%	Water-soluble product	5	5%

[a] Contained in intra-mammary preparation (Special Formula 17900-Forte Suspension).

[b] Importance to human health as categorized by Health Canada (15). Category I includes drugs of very high importance to human health, Category II includes drugs of high importance, Category III are medium importance, and Category IV are low importance.

examined to determine if they were linearly associated with the logit of the disease outcome. Manual step-wise backward selection was used, retaining only variables significant at $P < 0.05$. Multivariable models were reported only when at least 2 variables were retained in the final model. Risk factors removed from the full model were then evaluated to see if they changed effect estimates of interest by $> 20\%$ and, assuming that they were not intervening variables, were retained as confounders. Biologically reasonable first-order interactions were considered and retained in the final model and reported only if $P < 0.05$. Residuals of the final models were examined for outliers.

Results

Study population

Of the 109 herd owners recruited to the Western Canadian Cow-Calf Surveillance Network who were offered the opportunity to collect fecal samples, 2 chose not to participate and 107 provided fecal samples. This included 98 of 100 herds for which AMU data were available. At least 1 organism of interest was recovered from each herd. *Escherichia coli* was recovered from 105 of the possible 107 samples (98%, 95% CI: 93% to 100%), *Campylobacter* spp. were

Table III. Antimicrobial resistance results from 305 *E. coli* isolates from composite fecal samples from cows (*n* = 20 per herd) in 105 cow-calf herds.

Resistant to	Resistant isolates	Isolate prevalence	Herds with resistant isolates	Herd prevalence
At least 1 antimicrobial	12	4%	10	10%
At least 2 antimicrobials	9	3%	8	8%
At least 3 antimicrobials	7	2%	6	6%
At least 4 antimicrobials	3	1%	3	3%
At least 5 antimicrobials	1	0.3%	1	1%
Amoxicillin-clavulanic acid[a]	1	0.3%	1	1%
Ampicillin	1	0.3%	1	1%
Azithromycin	0	0%	0	0%
Ceftiofur[a]	0	0%	0	0%
Ceftriaxone[a]	0	0%	0	0%
Chloramphenicol	3	1%	3	3%
Ciprofloxacin[a]	0	0%	0	0%
Cefoxitin	0	0%	0	0%
Gentamicin	0	0%	0	0%
Nalidixic acid	0	0%	0	0%
Streptomycin	8	3%	7	7%
Sulfisoxazole	8	3%	7	7%
Tetracycline	10	3%	8	8%
Trimethoprim-sulfamethoxazole	1	0.3%	1	1%
Aminoglycosides	8	3%	7	7%
Beta-lactams	1	0.3%	1	1%
Folic acid inhibitors	8	3%	7	7%
Macrolides	0	0%	0	0%
Phenicols	3	1%	3	3%
Quinolones	0	0%	0	0%
Tetracyclines	10	3%	8	8%

[a] Category I — antimicrobials of very high importance to human health (15).

recovered from 89 samples (83%, 95% CI: 75% to 90%), and *Salmonella* sp. from 1 sample (1%, 95% CI: 0.1% to 5%).

Data on AMU from July 2013 through June 2014 were available for 92% (98/107) of these herds: 48 from Alberta, 31 from Saskatchewan, 18 from Manitoba, and 1 from British Columbia. For the 98 herds with complete data, the median number of cows present in January 2014 was 228 [interquartile ratio (IQR): 159 to 354] and 31% (30/98) of herd owners reported having > 300 cows to calve in the spring of 2014. The herds started calving between December 2013 and May 2014; 41% (40/98) started calving before March and 33% (32/98) finished calving within 4 cycles (84 d). Of the producers who provided calf loss data, 17% (15/87) reported pre-weaning calf mortality of > 5%.

Of the survey respondents, 95% (93/98) identified having commercial cattle and 23% (23/98) sold at least some purebred cattle. Calves were retained and backgrounded after weaning in 37% (36/98) of herds and 9% (9/98) reported having a feedlot. Most participating herd owners had a veterinarian pregnancy test their cows (92%, 90/98) and examine their bulls for breeding soundness (89%, 87/98).

The average age of the person(s) making the day-to-day decisions for the herd was 47 y (IQR: 37 to 56 y). Two people were identified as the main decision-makers for 4 participating herds. At least 1 primary decision-maker was < 40 y in 33% (33/98) of herds.

The 98 composite herd fecal samples with AMU data were collected in: October, 23 (23%); November, 37 (38%); December, 27 (28%); January, 8 (8%); and February, 3 (3%). Most fecal samples [73% (72/98)] were collected after the 2014 calf crop had been weaned. Antimicrobial use by drug class, category of importance to use in humans, and route of administration for the herds that provided fecal samples is summarized in Table II.

Antimicrobial resistance findings

Escherichia coli isolates

From the 105/107 herd composite samples submitted in which *E. coli* was identified (98% recovery rate), 306 isolates of the 315 expected (105 × 3) were recovered. There were 3 samples from which only 1 *E. coli* isolate were recovered and 3 samples from which only 2 *E. coli* isolates were recovered. One additional isolate from the initial culture could not be recovered for AMR testing.

In the 305 isolates tested for AMR, resistance to ≥ 1 antimicrobial was identified in 12 isolates (4%, 95% CI: 2% to 7%) examined from

105 herds (11%, 95% CI: 7% to 19%) (Table III). Tetracycline (3%) was the most common type of resistance identified (Table III). Eight isolates (3%) were resistant to both streptomycin and sulfisoxazole, either alone or in combination with other antimicrobials.

There was 1 isolate that was resistant to 5 antimicrobials: streptomycin, sulfisoxazole, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole. The other identified resistance patterns included streptomycin, sulfisoxazole, tetracycline, and chloramphenicol in 2 isolates; streptomycin, sulfisoxazole, and tetracycline in 4 isolates; and streptomycin and sulfisoxazole in 1 isolate. The only other resistance pattern was amoxicillin-clavulanic acid and ampicillin in 1 isolate. Three isolates were only resistant to tetracycline.

In addition to the isolates that were classified as resistant, 3 isolates were considered intermediate rather than susceptible to chloramphenicol, 2 were intermediate for tetracycline, and 2 were intermediate for ciprofloxacin. All isolates were susceptible to the category-I antimicrobials (15) ceftiofur, ceftriaxone, and ciprofloxacin and 1 isolate was resistant to amoxicillin/clavulanic acid.

***Campylobacter* spp. isolates**

From the 89/107 herd composite samples in which *Campylobacter* spp. was initially identified (83% recovery rate), 87 isolates were subsequently recovered for AMR testing. Two isolates were not regrown.

Resistance to 1 antimicrobial was identified in 16 of 87 isolates from the 87 herds (18%, 95% CI: 11% to 28%). Resistance to 1 antimicrobial was identified in 10 (14%) of 73 *C. jejuni* isolates, 3 (27%) of 11 *C. coli* isolates, and 3 of 3 untyped *Campylobacter* spp. isolates.

Tetracycline resistance was reported in 13 isolates from 87 herds (15%) (10 *C. jejuni* and 3 *C. coli* isolates) and nalidixic acid resistance in 3 others (3%) (3 *Campylobacter* spp.). None of the 73 *C. jejuni*, 11 *C. coli*, or 3 *Campylobacter* spp. isolates were resistant to azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, gentamicin, or tulathromycin.

***Salmonella* sp.**

One *Salmonella enterica* serovar Typhimurium (antigen: 4,5:i:1,2, phage type: 40) was identified from 107 composite herd samples. It was susceptible to all antimicrobials tested, including amoxicillin-clavulanic acid, ampicillin, azithromycin, chloramphenicol, ciprofloxacin, ceftriaxone, cefoxitin, gentamicin, nalidixic acid, sulfisoxazole, streptomycin, trimethoprim-sulfamethoxazole, tetracycline, and ceftiofur. The 3 *E. coli* isolates and the 1 *Campylobacter* spp. isolated from the same herd were also susceptible to all antimicrobials tested. The sample was from a commercial herd with < 300 cows, $< 5\%$ calf mortality in 2014, and $< 5\%$ of calves treated for diarrhea.

Escherichia coli — Risk factors for resistance to ≥ 1 or ≥ 2 antimicrobials

Antimicrobial resistance (AMR) results were available for 278 isolates from 96 of the 98 herds with AMU information; no *E. coli* were recovered from 2 herds with AMU data. In samples from 9 herds that did not provide AMU data, 27 *E. coli* isolates were recovered and no AMR was identified. Resistance to at least 1 antimicrobial was identified in 12 of the 278 isolates (4.3%) from herds with AMU data.

There were no significant ($P < 0.05$) associations between either herd management or AMU and resistance to ≥ 1 or ≥ 2 antimicrobials in at least 1 *E. coli* isolate per herd (Table IV). After accounting for herds with $> 5\%$ preweaning calf mortality (OR 6.4, 95% CI: 0.91 to 52, $P = 0.06$), herds that treated cows with florfenicol were more likely to have *E. coli* resistance to ≥ 2 antimicrobials (OR 7.1, 95% CI: 1.1 to 57, $P = 0.03$) in the final multivariable model.

Escherichia coli — Risk factors for the most commonly observed types of AMR

The unconditional associations between herd management practices and AMU and resistance to sulfisoxazole and/or streptomycin and intermediate or resistant status to tetracycline are summarized in Table IV. Sulfisoxazole and streptomycin were considered together as all isolates resistant to 1 antimicrobial were also resistant to the other. The factor most likely to be associated ($P < 0.20$) with herd-level measures of AMR in *E. coli* was whether calf mortality before weaning in 2014 was $> 5\%$ (Table IV). After considering all risk factors, herds with calf mortality of $> 5\%$ were more likely to have ≥ 1 *E. coli* isolate with resistance to streptomycin and sulfisoxazole (OR: 7.8, 95% CI: 1.2 to 61, $P = 0.03$). Only 1 of the 2 herds with an isolate with intermediate susceptibility for ciprofloxacin reported using enrofloxacin and that was limited to $< 5\%$ of unweaned calves.

Campylobacter spp. — Risk factors for AMR

Antimicrobial resistance (AMR) results were available for isolates from 80 of the 98 herds that provided both fecal samples and AMU data. In the samples from 9 herds with no AMU data, 6 *C. jejuni* and 1 *C. coli* isolates were recovered; no AMR was identified. Resistance to 1 antimicrobial was identified in 16 of 80 herds with management and use data (20%). There were no significant associations between either herd management or AMU and either resistance to ≥ 1 antimicrobial or resistance to tetracycline in *Campylobacter* spp. (Table V). The highest proportion of the cow herd in which antimicrobials were used for any reason, whether cows were treated with florfenicol, and sampling date were the only factors where the association with either resistance to ≥ 1 antimicrobial or resistance to tetracycline in *Campylobacter* spp. resulted in $P < 0.20$.

Discussion

The prevalence of resistance to at least 1 antimicrobial was relatively low for *E. coli* isolates (4%), but slightly higher in *Campylobacter* spp. (18%) from cow fecal samples in the fall of 2014. The only identified published study of AMR in cows in western Canada described the prevalence of resistance in *E. coli* to at least 1 antimicrobial to be 10% of isolates in the spring of 2002 and 6% in 2003 (8). The prevalence of AMR in fecal *Campylobacter* spp. has not been previously reported for beef cows from western Canada. The prevalence of AMR in both *E. coli* and *Campylobacter* spp. from cows in the present study was lower than that reported for samples collected in cow-calf herds in the United States in 2008 (17% and 44%, respectively) (10).

While *E. coli* and *Campylobacter* spp. were recovered from 98% and 83% of herd composite samples, only 1 *Salmonella* isolate was identified. *Salmonella* is not a good sentinel organism for AMR in cow-calf herds in western Canada as the frequency of isolation is so low. This differs from the beef 2007–2008 study in the United States, which found that 9% of operations had ≥ 1 *Salmonella* positive cow and 0.5% of cows were positive (10). Although *E. coli* recovery was

Table IV. Unconditional associations between herd management and antimicrobial use practices and antimicrobial resistance in at least 1 *E. coli* isolate from a composite fecal sample collected from 20 cows per herd at fall pregnancy testing (*n* = 96 herds).

	Resistant to at least 1 antimicrobial		Resistant to at least 2 antimicrobials		Resistant to sulfisoxazole and streptomycin		Intermediate or resistant to tetracycline	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Sample collection date		**0.11**		0.35		0.44		**0.11**
November: October[a]	**0.22 (0.02 to 1.5)**	**0.16**	0.41 (0.03 to 3.9)	0.61	0.64 (0.04 to 9.5)	0.99	**0.22 (0.02 to 1.5)**	**0.16**
December: October	**0.14 (0.003 to 1.4)**	**0.13**	0.26 (0.005 to 3.6)	0.49	0.41 (0.01 to 8.1)	0.88	**0.14 (0.003 to 1.4)**	**0.13**
January and February: October	0.81 (0.06 to 6.2)	0.99	1.5 (0.11 to 15)	0.99	2.3 (0.14 to 36)	0.78	0.81 (0.06 to 6.2)	0.99
Samples collected after weaning	0.85 (0.17 to 5.5)	0.99	0.59 (0.11 to 4.1)	0.74	0.47 (0.07 to 3.4)	0.57	0.85 (0.17 to 5.5)	0.99
Producer age (years)	1.0 (0.96 to 1.1)	0.60	1.0 (0.95 to 1.1)	0.73	1.0 (0.95 to 1.1)	0.64	1.0 (0.96 to 1.1)	0.66
Produces purebred cattle for sale	1.5 (0.23 to 7.4)	0.82	0.46 (0.01 to 3.9)	0.82	0.54 (0.01 to 4.9)	0.99	1.5 (0.23 to 7.4)	0.82
Total cows calved 2014 > 300	0.22 (0.005 to 1.7)	0.23	0.29 (0.01 to 2.5)	0.44	0.35 (0.01 to 3.1)	0.59	0.52 (0.05 to 2.9)	0.68
Number of cows to calf in 2014	**0.996 (0.989 to 1.000)**	**0.17**	0.997 (0.989 to 1.001)	0.25	0.997 (0.988 to 1.001)	0.30	0.998 (0.992 to 1.001)	0.30
Calves retained after weaning	0.04 (0.06 to 3.7)	0.86	0.22 (0.005 to 1.8)	0.25	0.26 (0.01 to 2.3)	0.37	0.69 (0.11 to 3.3)	0.88
Feedlot operation	0.65 (0.00 to 4.6)	0.71	0.83 (0.0 to 6.2)	0.88	0.97 (0.0 to 7.3)	0.98	1.1 (0.02 to 9.9)	0.99
Calving started before March 2014	1.6 (0.34 to 7.5)	0.70	1.6 (0.27 to 9.1)	0.79	1.2 (0.16 to 7.3)	0.99	1.0 (0.20 to 4.7)	0.99
Calf mortality > 5%	**3.8 (0.68 to 19)**	**0.14**	**5.8 (0.94 to 36)**	**0.06**	**7.8 (1.2 to 61)**	**0.03**	2.2 (0.32 to 12)	0.49
Antimicrobials provided to cows in feed	2.9 (0.42 to 15)	0.32	2.1 (0.19 to 14)	0.66	0.97 (0.02 to 9.1)	0.99	**4.9 (0.87 to 25)**	**0.07**
Injectable antimicrobials used on cows	0.98 (0.13 to ∞)	0.99	0.76 (0.10 to ∞)	0.99	0.65 (0.08 to ∞)	0.99	0.98 (0.13 to ∞)	0.99
Highest % of cows in which antimicrobials were used for any reason	1.0 (0.99 to 1.0)	0.31	1.0 (0.97 to 1.0)	0.65	1.0 (0.94 to 1.0)	0.99	**1.0 (1.0 to 1.1)**	**0.07**
Antimicrobials used in cows								
Aminoglycosides (not topical)	8.6 (0.0 to 335)	0.99	11 (0.0 to 429)	0.99	13 (0.0 to 496)	0.99	8.6 (0.0 to 335)	0.99
1st generation cephalosporins	2.2 (0.0 to 22)	0.99	2.9 (0.0 to 29)	0.99	3.3 (0.0 to 34)	0.99	2.2 (0.0 to 22)	0.99
Ceftiofur	1.0 (0.0 to 7.9)	0.99	1.3 (0.0 to 10)	0.99	1.5 (0.0 to 12)	0.99	1.0 (0.0 to 7.9)	0.99
Florfenicol	2.9 (0.60 to 14)	0.22	**4.9 (0.87 to 34)**	**0.08**	**3.8 (0.59 to 28)**	**0.19**	2.9 (0.60 to 14)	0.22
Lasalocid	9.0 (0.11 to 750)	0.40	12 (0.14 to 986)	0.32	14 (0.16 to 1163)	0.29	9.0 (0.11 to 750)	0.40
Macrolides	0.30 (0.0 to 2.0)	0.26	0.39 (0.0 to 2.7)	0.39	0.46 (0.0 to 3.3)	0.49	0.30 (0.0 to 2.0)	0.26
Monensin	2.8 (0.24 to 19)	0.47	1.4 (0.03 to 14)	0.99	0.97 (0.0 to 7.3)	0.98	**5.5 (0.74 to 34)**	**0.10**
Penicillin	1.9 (0.39 to 8.8)	0.54	1.1 (0.15 to 5.8)	0.99	1.3 (0.18 to 8.4)	0.99	**2.9 (0.64 to 15)**	**0.20**
Polymyxins[b]	0.46 (0.0 to 3.2)	0.49	0.6 (0.0 to 4.3)	0.66	0.69 (0.0 to 5.1)	0.76	0.46 (0.0 to 3.2)	0.49
Sulfa boluses (including intra-uterine)	2.3 (0.04 to 26)	0.86	3.0 (0.05 to 36)	0.72	1.9 (0.0 to 16)	0.99	2.3 (0.04 to 26)	0.86
Tetracyclines	0.6 (0.13 to 4.1)	0.76	0.43 (0.08 to 3.1)	0.48	0.34 (0.05 to 2.6)	0.35	0.62 (0.13 to 4.1)	0.76
Trimethoprim-sulfadoxine	1.5 (0.03 to 15)	0.99	1.9 (0.04 to 20)	0.94	2.3 (0.04 to 25)	0.84	1.5 (0.03 to 14)	0.99
Category-I drugs used in cow herd[c]	**0.26 (0.0 to 1.8)**	**0.19**	0.34 (0.0 to 2.4)	0.32	0.40 (0.0 to 3.8)	0.40	**0.26 (0.0 to 1.8)**	**0.19**

Table IV. Unconditional associations between herd management and antimicrobial use practices and antimicrobial resistance in at least 1 *E. coli* isolate from a composite fecal sample collected from 20 cows per herd at fall pregnancy testing (n = 96 herds) *(continued)*.

	Resistant to at least 1 antimicrobial		Resistant to at least 2 antimicrobials		Resistant to sulfisoxazole and streptomycin		Intermediate or resistant to tetracycline	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Category-II drugs used in cow herd[c]	1.0 (0.21 to 4.7)	0.99	0.58 (0.08 to 3.2)	0.72	0.74 (0.10 to 4.6)	0.99	1.6 (0.34 to 8.1)	0.74
Highest % of preweaned calf herd in which antimicrobials were used for any reason	**1.0 (1.0 to 1.0)**	**0.10**	1.0 (0.99 to 1.0)	0.22	1.0 (0.98 to 1.0)	0.54	1.0 (0.98 to 1.0)	0.41

[a] Samples collected in November compared to the samples collected in October (reference category).

[b] Contained in intra-mammary preparation (Special Formula 17900-Forte Suspension).

[c] Importance to human health as categorized by Health Canada (15). Category I includes drugs of very high importance to human health and Category II is of high importance.

OR — odds ratio; CI — confidence interval.

Values in bold signify factors where $P < 0.20$.

similar to that in the present study (99%), recovery of *Campylobacter* was lower (45%).

The most common types of resistance identified for *E. coli* isolates in the present study were tetracycline, sulfisoxazole, and streptomycin. This agrees with both the 2002 study from western Canada (8) and the 2008 study from the US (10). Multiple resistance in *E. coli* was uncommon in the current study with only 3% of isolates resistant to ≥ 2 antimicrobials in 2014. This compares to 7% from western Canada in 2002 (8), 3% in 2003, and 10% from 2008 in the United States (10). The most common multiple resistance patterns in the present study included both streptomycin and sulfisoxazole, which is consistent with previous reports of multiple resistance in studies of beef cattle and the suggestion of genetic linkage (17,18).

While resistance to ceftiofur, ceftriaxone, and ciprofloxacin was not detected in the present study, 1 *E. coli* isolate was resistant to amoxicillin/clavulanic acid. Similarly, resistance to category-I antimicrobials in the previous study from western Canada (8) was limited to 1 isolate that was not susceptible to ceftiofur. Only 2 ceftiofur-resistant *E. coli* isolates were reported in 2008 from the United States (10). The current results were substantially lower, however, than those reported for *E. coli* isolates from poultry in the 2015 CIPARS report of farm surveillance, which showed that 24% of placement and 12% of preharvest isolates were resistant to ceftriaxone (6). The results for beef cows were also lower than the 2% of *E. coli* farm isolates from swine that were resistant to ceftriaxone (6). The 2015 CIPARS report did not include feedlot surveillance.

Similar to what has been described in previous studies, *C. jejuni* was more common than *C. coli* and, while the numbers of *C. coli* were small, the prevalence of resistance was higher than for *C. jejuni* (19). No multiple resistance was identified in *Campylobacter* spp. in the present study, although resistance to ≥ 2 antimicrobials was detected in 8% of isolates from the US herds in 2008 (10). The most common type of resistance for *Campylobacter* spp. in both studies was to tetracycline. While resistance to ciprofloxacin was the second most common in the 2007–2008 beef study in the United States (10), it was not detected in the 2014 Canadian cow isolates.

The present study differed from previous cow-calf studies in that pooled samples were used and just 3 *E. coli* isolates per herd were tested for AMR and only 1 for *Campylobacter* spp. This approach is similar to that used by CIPARS for ongoing on-farm surveillance in swine and poultry (6). The description of isolates from pooled samples was also justified in part by the observation in a previous study of beef herds that more variability in AMR was observed among isolates and herds than among animals within herds (7). Despite the use of pooled samples and smaller number of isolates per herd, the AMR results were comparable to those from previous studies (8,10). However, additional work is needed under current management conditions to examine the proportion of variation in AMR among isolates within cows and among cows within herds in order to formally validate this approach for surveillance in cow-calf herds.

Other limitations of this study included the potential for type-I error with the large number of risk factors examined. There was also the potential for type-II error due to the limited sample size. Despite the limited sample size when compared to the 2008 US study (10), there was sufficient power to identify risk factors for AMR in cow-calf herds. Herds that treated cows with florfenicol were

Table V. Unconditional associations between herd management and antimicrobial use practices and the presence of antimicrobial resistance in *Campylobacter* spp. isolated from a composite fecal sample collected from 20 cows per herd at fall pregnancy testing (*n* = 80 herds).

	Resistant to at least 1 antimicrobial		Resistant to tetracycline	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Sample collection date		0.22		**0.20**
November: October[a]	1.4 (0.26 to 9.4)	0.99	4.1 (0.45 to 200)	0.35
December: October	0.61 (0.09 to 5.0)	0.86	2.1 (0.18 to 116)	0.90
January and February: October	0.30 (0.00 to 3.0)	0.32	1.3 (0.00 to 51)	0.99
Samples collected after weaning	0.39 (0.10 to 1.6)	0.21	0.60 (0.14 to 3.1)	0.47
Producer age (years)	0.99 (0.95 to 1.0)	0.83	0.98 (0.93 to 1.0)	0.42
Produces purebred cattle for sale	0.40 (0.04 to 2.0)	0.40	0.54 (0.05 to 2.9)	0.71
Total cows calved 2014 > 300	0.81 (0.20 to 2.9)	0.97	1.2 (0.27 to 4.7)	0.99
Number of cows to calf in 2014	0.999 (0.995 to 1.001)	0.49	1.000 (0.997 to 1.001)	0.99
Calves retained after weaning	0.88 (0.23 to 3.1)	0.99	1.4 (0.33 to 5.3)	0.84
Feedlot operation	1.7 (0.15 to 12)	0.86	2.2 (0.19 to 16)	0.64
Calving started before March 2014	1.1 (0.30 to 3.7)	0.99	1.2 (0.30 to 4.7)	0.99
Calf mortality > 5%	2.9 (0.49 to 13)	0.30	2.4 (0.34 to 13)	0.45
Antimicrobials provided to cows in feed	0.29 (0.01 to 2.3)	0.42	0.39 (0.01 to 3.1)	0.66
Injectable antimicrobials used on cows	0.98 (0.10 to ∞)	0.99	0.75 (0.08 to ∞)	0.99
Highest % of cow herd in which antimicrobials were used for any reason	**0.94 (0.74 to 1.0)**	**0.11**	**0.95 (0.71 to 1.0)**	**0.20**
Antimicrobials used in cows				
Aminoglycosides (not topical)	4.0 (0.10 to ∞)	0.40	5.2 (0.13 to ∞)	0.33
1st generation cephalosporins	1.0 (0.00 to 9.9)	0.99	1.3 (0.00 to 13)	0.99
Ceftiofur	3.4 (0.44 to 23)	0.28	2.2 (0.19 to 16)	0.64
Florfenicol	**2.5 (0.67 to 9.1)**	**0.19**	1.8 (0.41 to 7.4)	0.52
Lasalocid	1.6 (0.00 to 22)	0.99	2.1 (0.00 to 28)	0.99
Macrolides	0.22 (0.004 to 1.7)	0.23	0.29 (0.01 to 2.3)	0.42
Monensin	0.33 (0.00 to 2.3)	0.31	0.43 (0.00 to 3.1)	0.45
Penicillin	0.71 (0.17 to 2.6)	0.78	1.1 (0.24 to 4.1)	0.99
Polymyxins[b]	0.87 (0.08 to 5.0)	0.99	1.2 (0.11 to 6.9)	0.99
Sulfa boluses (including intra-uterine)	0.57 (0.00 to 4.5)	0.63	0.74 (0.00 to 5.8)	0.80
Tetracyclines	0.63 (0.15 to 3.2)	0.69	1.3 (0.24 to 14)	0.99
Trimethoprim-sulfadoxine	0.39 (0.00 to 2.8)	0.39	0.50 (0.00 to 3.7)	0.55
Category-I drugs used in cow herd[c]	1.6 (0.38 to 6.2)	0.63	1.5 (0.30 to 6.5)	0.74
Category-II drugs used in cow herd[c]	0.47 (0.12 to 1.6)	0.29	0.74 (0.18 to 2.9)	0.84
Highest % of preweaned calves in which antimicrobials used for any reason	0.98 (0.94 to 1.0)	0.21	0.98 (0.95 to 1.0)	0.50

[a] Samples collected in November compared to the samples collected in October (reference category).

[b] Contained in intra-mammary preparation (Special Formula 17900-Forte Suspension).

[c] Importance to human health as categorized by Health Canada (15). Category I includes drugs of very high importance to human health and Category II is of high importance.

OR — odds ratio; CI — confidence interval.

Values in bold signify factors where $P < 0.20$.

more likely to be multi-resistant to *E. coli.* Herds with calf mortality of > 5% were more likely to have at least 1 *E. coli* isolate with resistance to streptomycin and sulfisoxazole. As calf mortality data were collected before the fecal samples were taken, it is reasonable that more calves could have been treated in herds with greater calf mortality, which would lead to an increase in resistance prevalence. That information may not have been completely captured in the herd AMU records used in this analysis. Calf mortality had not been examined as a risk factor in the previous analysis of risk factors for AMR in beef cows in western Canada (8). There were no significant risk factors identified for AMR in *Campylobacter.*

Comparing the evidence in the present study to historical work, there is no indication that the frequency of AMR has increased in generic *E. coli* from mature beef cows. This study provided the first information on the prevalence of AMR in fecal *Campylobacter* spp. in western Canada. Future studies of AMR in beef cows should

evaluate the potential for seasonal variability in shedding, given the differences previously demonstrated in beef calves (7). Work is also necessary to optimize the number of samples collected per herd and determine the number of isolates per herd needed to accurately reflect changes in response to antimicrobial use and infection control practices. Studies should also be considered to compare the relative benefits of phenotypic description of isolate AMR, genomic analysis of isolates, and metagenomics approaches to evaluate best management practices in cow-calf herds.

Acknowledgments

This study was partially funded by the Beef Cattle Research Council and the Public Health Agency of Canada.

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