Article

Antimicrobial resistance in generic *E. coli* isolated from western Canadian cow-calf herds

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Abstract

Objective
To examine antimicrobial resistance (AMR) in commensal fecal *Escherichia coli (E. coli)* from extensively managed beef calves and cows in western Canada and describe the differences among cows and calves in the spring and fall.

Animal
Beef cattle, cow-calf.

Procedure
Antimicrobial susceptibility testing was conducted on generic *E. coli* isolates collected from 388 calves and 387 cows from 39 herds following calving in 2021, 419 calves from 39 herds near weaning, and 357 cows from 36 herds at pregnancy testing. Minimum inhibitory concentrations were measured with the NARMS CMV5AGNF plate for Gram-negative bacteria and interpreted using Clinical and Laboratory Standards Institute standard breakpoints for humans.

Results
Only 16% (242/1551) of all isolates from 97% (38/39) of herds were resistant to ≥ 1 antimicrobial. Generic *E. coli* isolates were most commonly resistant to sulfisoxazole (11%, 175/1551), followed by tetracycline (9.3%, 145/1551) and chloramphenicol (3.5%, 55/1551). Isolates from calves in the spring were more likely to be resistant to sulfisoxazole, tetracycline, and chloramphenicol than those from cows in the spring or calves in the fall. Multiclass-resistant isolates were identified in 5% (39/807) of calves. Only 2 isolates recovered from cows were resistant to antimicrobials of very high importance for human health.

Conclusion and clinical relevance
Most generic *E. coli* isolates were pansusceptible. The observed resistance patterns were consistent with earlier studies of AMR from commensal *E. coli* in this region. Baseline AMR data for cow-calf herds are not currently collected as part of routine surveillance, but are essential to inform antimicrobial use policy and stewardship.

Résumé

Résistance aux antimicrobiens chez *E. coli* générique isolé dans des troupeaux vache-veau de l'Ouest canadien

Objectif
Examiner la résistance aux antimicrobiens (RAM) chez *Escherichia coli* de la flore fécale commensale (*E. coli*) provenant de veaux et de vaches de boucherie en élevage extensif dans l'ouest du Canada et décrire les différences entre les vaches et les veaux au printemps et à l'automne.

Animal
Bovins de boucherie, vache-veau.

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Procédure
Des tests de sensibilité aux antimicrobiens ont été effectués sur des isolats génériques d'*E. coli* collectés auprès de 388 veaux et 387 vaches de 39 troupeaux après le vêlage en 2021, de 419 veaux de 39 troupeaux à l'approche du sevrage et de 357 vaches de 36 troupeaux lors des tests de gestation. Les concentrations minimales inhibitrices ont été mesurées avec la plaque NARMS CMV5AGNF pour les bactéries à Gram négatif et interprétées à l'aide des seuils standard pour les humains du *Clinical and Laboratory Standards Institute*.

Résultats
Seulement 16 % (242/1 551) de tous les isolats provenant de 97 % (38/39) des troupeaux étaient résistants à ≥ 1 antimicrobien. Les isolats génériques d'*E. coli* étaient le plus souvent résistants au sulfisoxazole (11 %, 175/1 551), suivi de la tétracycline (9,3 %, 145/1 551) et du chloramphénicol (3,5 %, 55/1 551). Les isolats provenant des veaux au printemps étaient plus susceptibles d'être résistants au sulfisoxazole, à la tétracycline et au chloramphénicol que ceux provenant des vaches au printemps ou des veaux à l'automne. Des isolats résistants à plusieurs classes ont été identifiés chez 5 % (39/807) des veaux. Seuls deux isolats récupérés chez des vaches étaient résistants à des antimicrobiens de très haute importance pour la santé humaine.

Conclusion et pertinence clinique
La plupart des isolats génériques d'*E. coli* étaient sensibles à l'ensemble des antimicrobiens. Les profils de résistance observés concordaient avec les études antérieures sur la RAM provenant d'*E. coli* commensal dans cette région. Les données de base sur la RAM pour les troupeaux vache-veau ne sont pas actuellement recueillies dans le cadre de la surveillance de routine, mais elles sont essentielles pour éclairer la politique et la gestion de l'utilisation des antimicrobiens.

(Traduit par D^{r} Serge Messier)

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Introduction

Canadian cow-calf herds are typically more extensively managed than other production animal commodities such as swine, poultry, dairy, and feedlot cattle. Whereas the literature regarding antimicrobial resistance (AMR), the role of antimicrobial use (AMU), and drivers of AMR in livestock is growing for other sectors (1–3), very few reports describe AMR in cow-calf herds, including those in western Canada. Antimicrobial resistance in commensal enteric bacteria is of interest because there is potential for transmission of mobile genetic elements between bacteria and for spread of resistant bacteria between animals and to humans through direct contact, food, or the environment (4). Generic *Escherichia coli (E. coli)* is commonly used as a sentinel organism in surveillance programs because it is easy to culture from both mammals and birds, facilitating comparisons among species and environments. In addition, *E. coli* can carry a relatively large number of resistance markers and is a sensitive indicator for AMR (4).

Although pooled fecal samples were collected for AMR testing from western Canadian beef cows in 2014 (5) and beef herds in eastern Canada in 2001 and 2002 (6), the relative frequencies of AMR in enteric organisms based on individual-animal data have not been measured in Canadian cow-calf herds in almost 2 decades. Individual-animal data are necessary to determine if the frequency of AMR is changing within herds. In western Canada, AMR was evaluated in commensal *E. coli* isolates obtained from individual fecal pats of beef calves and cows in the spring and calves in the fall of 2002 (7,8). In addition, *E. coli* isolates from individual fecal samples from cow-calf pairs were also examined in 2003 (8), and individual samples were tested from beef cows from the United States in 2008 (9).

Antimicrobial resistance could be more seasonal in cow-calf herds than in other commodities as disease and resulting AMU are linked to the cow-calf production cycle, which is more seasonal than for most other livestock species (7,8,10,11). Canadian cow-calf herds also differ in that beef cows are typically managed outdoors on pasture for much of the year, potentially affecting the risk for environmental transmission of AMR from cow-calf herds through soil and water. The 54 000 cow-calf herds in Canada (12) substantially outnumber operations for more intensively managed commodities such as dairy, pork, and poultry, making surveillance of AMR crucial in this commodity. Furthermore, cow-calf herds are a potential direct source of resistant enteric organisms in the human and pet food chains through the sale of cull cows and bulls to processors. Finally, cow-calf herds are the primary source of cattle for backgrounding and feedlot operations, which provide most beef to consumers.

Although the Canadian Public Health Agency leads a surveillance program in feedlot cattle monitoring resistance of *E. coli* to antimicrobials of interest to human health, there is no similar initiative in cow-calf herds (13). Comparable, current, and more complete AMR data are needed from cow-calf herds to provide a baseline for measuring the effects of stewardship initiatives, as well as to target the most appropriate age groups and seasonal timing for future on-farm sampling programs. Previous work has documented higher relative frequencies of AMR in generic *E. coli* isolated from calves in the spring as compared to cows in the spring and from calves in the spring as compared to calves in the fall (7,8). The objectives of this study were to describe the relative frequency of AMR in fecal generic *E. coli* isolated from beef cows and calves from western Canada and to compare the relative frequencies of *E. coli* AMR between cows and calves in the spring and the fall.

Materials and methods

This project was approved by the University of Saskatchewan's Animal Research Ethics Board (AREB) under the Animal Use Protocol # 2014003.

Producer recruitment

The Canadian Cow-Calf Surveillance Network (C3SN) is a national program funded by the industry through a federal support program. Its goals are to monitor herd productivity, production-limiting diseases (14), AMU (11), and AMR in Canadian cow-calf herds. Participants were recruited to the network during 2018 and 2019 through consultation with veterinarians, producer organizations, and word of mouth. Recruitment targeted herds with $>$ 40 breeding animals for which pregnancy checking, basic records for calving and production, and access to email were reported (15).

For this study, 50 participants were recruited from 98 producers from western Canada who completed an AMU survey for C3SN in 2020 (11). The survey questions asked about record collection, whether individual- or herd-level records were available, and if producers would be willing to share records. Producers who agreed to share animal health records in 2021 were contacted in December of 2020, *via* email, to determine their interest in participating in collection of fecal samples and analysis for AMR.

The 50 herds recruited to participate in the fecal AMR project included 25 from Alberta, 12 from Saskatchewan, 8 from Manitoba, and 5 from British Columbia. Veterinarians were asked to collect fecal samples on-farm in 2021, using sample kits and instructions supplied by the study team. Convenience samples were collected from 10 cows and 10 calves per operation following calving (spring) and near the time of weaning and pregnancy testing (fall). The age of calves at sampling was recorded in the spring.

Random sampling of cattle in the spring during busy calving season was not considered feasible, nor was repeatedly sampling the same animals in the fall. Samples were collected either directly from the rectum or from fresh fecal pats observed to have dropped from the animal. Samples were packaged in a cooler with ice packs and shipped *via* overnight courier to Prairie Diagnostic Services (Saskatoon, Saskatchewan), for bacterial culture and susceptibility testing.

Laboratory methods

The fecal samples were weighed and 4.0 g were transferred into 50-milliliter centrifuge tubes containing 36 mL 1% buffered peptone water. The mixture was vortexed thoroughly and placed in a mixer for 1 h. The pre-enrichment mixtures were incubated at 35°C for 18 to 24 h under normal atmospheric conditions.

MacConkey agar (Difco Laboratories, Detroit, Michigan, USA), a selective medium for *Enterobacteriaceae,* was inoculated with 10 μL of the pre-enrichment mixture. The plates were streaked to obtain isolated colonies and incubated at 35°C for 18 to 24 h under ambient atmospheric conditions.

After incubation, the plates were examined for lactose-fermenting colonies consistent with *E. coli* grown on MacConkey agar. Individual colonies were then subcultured onto 5% sheep blood agar to obtain pure colonies. The subcultured plates were incubated at 35°C for 18 to 24 h under 5% CO_2. The colonies grown on blood agar were identified using MALDI-TOF MS (Bruker, Vancouver, British Columbia). A MALDI-TOF score $>$ 2 indicated secure species-level identification. Only isolates that had secure species identification were selected for further analysis. Three isolates per sample of the target organism were saved in Tryptic Soy Broth (TSB; Difco Laboratories) containing 15% glycerol and stored at -80°C.

Minimum inhibitory concentrations (MICs) were measured for an *E. coli* isolate from the most prominent colony in each sample according to the Clinical and Laboratory Standards Institute standard breakpoints for humans (Table 1) (16) using NARMS CMV5AGNF plate for Gram-negative bacteria (TREK Diagnostic Systems, East Grinstead, UK).

A bacterial broth (0.5 McFarland turbidity equivalent) was prepared from a pure bacterial isolate using the Sensititre Nephelometer (ThermoFisher Scientific, Nepean, Ontario). The dosing broths were prepared by transferring 10 μL of the suspension to 11 mL of a Sensititre Cation Adjusted AutoRead Muller-Hinton Broth w/TES (ThermoFisher Scientific). The dosing broth was inoculated onto a Sensititre plate using a Sensititre AIM Automated Inoculation System (ThermoFisher Scientific). The inoculated plates were tightly sealed and incubated at 35°C for 18 to 24 h under normal atmospheric conditions. A BioMic V3 system (Giles Scientific, Santa Barbara, California, USA) was used to report MICs. A manual mirror box was used to confirm shallow bacterial growth. Bacteria were classified as either susceptible or resistant, with intermediate MIC values classed as susceptible.

Data management and analyses

Data were managed using commercial spreadsheet and database programs (Microsoft Access and Excel; Microsoft, Redmond, Washington, USA) and analyzed using a commercial software program (STATA Version 16.1; StataCorp, College Station, Texas, USA).

Random effects logistic regression was used to estimate differences in the relative frequencies of resistance between seasons for cows and calves and between cows and calves within each season. Outcomes considered included resistance to each antimicrobial where the relative frequency of resistance $\geq$ 5%, multiclass resistance, and pansusceptibility. Pansusceptibility was defined as isolates that were susceptible to all tested antimicrobials, whereas multiclass resistance was defined as isolates resistant to $\geq$ 3 classes of antimicrobials (17). Models included a random effect for herd and fixed effects for season, cow *versus* calf, and the interaction between animal type and season. Odds ratios (OR) described the relative differences in outcomes among groups, and $P < 0.05$ was reported as statistically significant. In addition, intracluster correlation coefficients (ICCs) were reported as a measure of clustering of resistance outcomes within the herd after accounting for differences between cows and calves and spring and fall.

Random effects logistic regression was similarly used to evaluate the association between calf age and the occurrence of AMR

Table 1. Minimum inhibitory concentration (MIC) breakpoints (16).

Antimicrobial	Breakpoints (µg/mL)		
	Susceptible	Intermediate	Resistant
Category I: Very high importance[a]			
Amoxicillin/clavulanic acid	≤ 8	16	≥ 32
Ceftriaxone	≤ 1	2	≥ 4
Ciprofloxacin	≤ 0.06	0.12 to 0.5	≥ 1
Colistin	N/A	≤ 2	≥ 4
Meropenem	≤ 1	2	≥ 4
Category II: High importance[a]			
Ampicillin	≤ 8	16	≥ 32
Azithromycin	≤ 16	N/A	≥ 32
Cefoxitin	≤ 8	16	≥ 32
Gentamicin	≤ 4	8	≥ 16
Nalidixic acid	≤ 16	N/A	≥ 32
Trimethoprim/sulfamethoxazole	≤ 2	N/A	≥ 4
Category III: Medium importance[a]			≥ 32
Chloramphenicol	≤ 8	16	≥ 32
Sulfisoxazole	≤ 256	N/A	≥ 512
Tetracycline	≤ 4	8	≥ 16

N/A — Not applicable.

[a] Categories of antimicrobial drugs based on importance in human medicine — antimicrobial resistance (18).

outcomes in the spring, as previously described, after accounting for clustering by herd.

Antimicrobials were summarized based on Health Canada's categorization of importance to human health (18): Category I: very high importance, Category II: high importance, Category III: medium importance, and Category IV: low importance (not currently used in human medicine) (18).

To simplify the description of AMR relative frequency throughout the paper, terms used in the European Union and European Economic Area (EU/EEA) for reporting AMR data were followed as a guide (19): rare: < 0.1%, very low: 0.1 to 1.0%, low: > 1.0 to 10%, moderate: > 10 to 20%, high: > 20 to 50%, very high: > 50 to 70%, and extremely high: > 70%.

Results

Study population

Of the 50 herds enrolled, 39 herds provided fecal samples from calves in both the spring and fall: 20 (51%) herds from Alberta, 9 (23%) herds from Saskatchewan, 5 (13%) herds from British Columbia, and 5 (13%) herds from Manitoba.

The median number of cows calving in the sampled herds was 244 (range: 46 to 1044, mean: 212). Of the enrolled herds, all were identified as having some commercial cattle (range: 10 to 90%); 15 were identified as having some seedstock, ranging from 2 to 90% of the herd consisting of purebred cattle.

In the spring of 2021, *E. coli* was recovered from 99.5% (388/390) of fecal samples from calves and 100% (387/387) of samples from cows. Participating herds started calving between December 2020 and May 2021; most calved in March (33%, 13/39) and April (28%, 11/39). Samples following calving (spring) were collected in the months of March through August, with 59% (23/39) in June. The average age at sampling for calves in the spring was 8 wk (SD: 5 wk, range: 1 to 23 wk).

Similarly in the fall of 2021, *E. coli* was recovered from 100% (419/419) of sampled calves and 99.4% (357/359) of cows. Three herds did not submit cow samples in the fall, but rather submitted 20 samples from calves. The fall samples were collected in the months of September through January, with most in October (33%, 13/39) and November (44%, 17/39). Age at sampling was not available for calves in the fall. Four herds collected cow and calf samples on different days: 3 herds sampled within 1 wk and 1 herd sampled cows in October and calves in January.

Antimicrobial resistance

Sixteen percent (242/1551) of all isolates from 97% (38/39) of the herds enrolled in the study were resistant to ≥ 1 antimicrobial. Overall, resistance was low or moderate across all animal classes and seasons, with no single animal class in either season having more than 20% of isolates resistant to a single antimicrobial (Table 2).

The highest resistance relative frequency across all seasons and animal classes was to sulfisoxazole (11.3%, 175/1551), followed by tetracycline (9.3%, 145/1551) and chloramphenicol (3.5%, 55/1551). *Escherichia coli* isolates were also resistant to ampicillin (1.4%, 21/1551) in both cows and calves in the spring and fall (Table 2).

Isolates resistant to trimethoprim/sulfamethoxazole were recovered from 2 calves in the spring and 1 cow in the fall. Two isolates resistant to gentamicin were recovered from calves in the spring (Table 2). One calf isolate from the spring and 2 cow isolates from the fall were resistant to cefoxitin. Only 2 isolates were resistant to any of the Category I antimicrobials tested: 1 isolate from a cow in the spring and 1 isolate from a cow in the fall were resistant to amoxicillin/clavulanic acid (Table 3).

Overall, 8.5% (132/1551) of isolates were resistant to just 1 antimicrobial class and 7.1% (110/1551) of isolates were resistant to > 1 antimicrobial class (Table 3). An extremely high proportion of isolates (84.4%, 1309/1551) were pansusceptible. Resistance to ≥ 3 antimicrobial classes was detected in 3.0% (46/1551) of the isolates (Table 3).

The most common resistance pattern from calf isolates recovered during the spring that were resistant to > 1 antimicrobial

Table 2. Relative frequencies of antimicrobial resistance [% (observed frequency)] for *Escherichia coli* isolates recovered from 39 cow-calf herds in spring and fall of 2021.

	Spring 2021		Fall 2021	
Antimicrobial	Calves (n = 388) Resistant	Cows (n = 387) Resistant	Calves (n = 419) Resistant	Cows (n = 357)[b] Resistant
Category I: Very high importance[a]				
Amoxicillin/clavulanic acid	0% (0)	0.3% (1)	0% (0)	0.3% (1)
Ceftriaxone	0% (0)	0% (0)	0% (0)	0% (0)
Ciprofloxacin	0% (0)	0% (0)	0% (0)	0% (0)
Colistin	0% (0)	0% (0)	0% (0)	0% (0)
Meropenem	0% (0)	0% (0)	0% (0)	0% (0)
Category II: High importance[a]				
Ampicillin	2.3% (9)	0.8% (3)	1% (4)	1.4% (5)
Azithromycin	0% (0)	0% (0)	0% (0)	0% (0)
Cefoxitin	0% (0)	0.3% (1)	0% (0)	0.6% (2)
Gentamicin	0.5% (2)	0% (0)	0% (0)	0% (0)
Nalidixic acid	0% (0)	0% (0)	0% (0)	0% (0)
Trimethoprim/sulfamethoxazole	0.5% (2)	0% (0)	0% (0)	0.3% (1)
Category III: Medium importance[a]				
Chloramphenicol	9% (35)	0.3% (1)	3.1% (13)	1.7% (6)
Sulfisoxazole	17.8% (69)	2.3% (10)	11.9% (50)	12.9% (46)
Tetracycline	17.8% (69)	4.7% (18)	9.8% (41)	4.8% (17)

[a] Categories of antimicrobial drugs based on importance in human medicine — antimicrobial resistance (18).
[b] Samples submitted from 36 herds.

Table 3. Relative frequencies of resistance patterns based on antimicrobial class [% (observed frequency)] for *Escherichia coli* isolates recovered in 2021 from 39 cow-calf herds.

Resistant to	Spring calf (n = 388)	Spring cow (n = 387)	Fall calf (n = 419)	Fall cow (n = 357)[a]
Pansusceptible	76% (294)	94% (365)	83% (348)	85% (302)
1 class	7.7% (30)	3.4% (13)	11% (46)	12% (43)
> 1 class	17% (64)	2.3% (9)	6.0% (25)	3.4% (12)
2 classes	9.3% (36)	2.1% (8)	3.3% (14)	1.7% (6)
3 classes	7.0% (27)	0% (0)	2.4% (10)	0.8% (3)
4 classes	0.3% (1)	0.3% (1)	0.2% (1)	0.8% (3)
Multiclass-resistant ≥ 3 classes	7.2% (28)	0.3% (1)	2.6% (11)	1.7% (6)
Resistance to a Category I antimicrobial	0% (0)	0.26% (1)	0% (0)	0.28% (1)

[a] Cow samples submitted from 36 herds.

class was to tetracycline and sulfonamide (42%, 27/64). The most common resistance patterns detected to ≥ 3 antimicrobial classes included tetracyclines, phenicols, and sulfonamides (86%, 24/28), followed by tetracyclines, phenicols, and penicillins (7%, 2/28). Nine isolates from cows sampled in the spring were resistant to > 1 antimicrobial class, and 6 of these isolates (67%) were resistant to tetracycline and sulfonamide.

As with calves in the spring, the combination of tetracyclines and sulfonamides accounted for most (40%, 10/25) resistance to > 1 antimicrobial class from isolates from calves in the fall. In addition, 9 (36%) isolates from this group were resistant to tetracyclines, sulfonamides, and phenicols. The most common resistance pattern to > 1 antimicrobial class for cow isolates in the fall was to tetracyclines and sulfonamides (42%, 5/12).

One calf isolate from the spring, 1 cow isolate from the spring, 1 calf isolate from the fall, and 3 cow isolates from the fall were each resistant to 4 classes of antimicrobials (tetracyclines, phenicols, sulfonamides, and aminoglycosides).

The relatively frequency of herds with AMR in ≥ 1 sample varied from 36% of herds for cows in the spring to 82% of herds for calves in the spring (Table 4). Within herd, sample frequency was most variable for sulfisoxazole resistance, followed by tetracycline (Table 4).

Differences among susceptibility of isolates from cows and calves from the spring and fall

Three antimicrobials were identified for additional analysis: sulfisoxazole, tetracycline, and chloramphenicol. For calves sampled in the spring, the odds of being resistant to sulfisoxazole (OR: 0.90, 95% CI: 0.82 to 0.99; $P = 0.03$) and tetracycline (OR: 0.89, 95% CI: 0.82 to 0.96; $P = 0.005$) decreased with increasing calf age in wk, but the decrease in odds of resistance to chloramphenicol (OR: 0.90, 95% CI: 0.80 to 1.01, $P = 0.08$) was not significant.

Isolates from calves in the fall were less likely to be resistant to sulfisoxazole, tetracycline, and chloramphenicol compared to isolates from calves in the spring (Table 5). Isolates from calves in the fall were also less likely to be multiclass-resistant as well as be resistant to ≥ 1 antimicrobial compared to isolates from calves in the spring (Table 5). Furthermore, isolates from calves in the fall were more likely to be pansusceptible than isolates recovered from calves in the spring (Table 5).

Table 4. Comparison of the proportions of resistance-positive herds as well as the average within-herd relative frequencies for resistance-positive herds.

	Spring calf (n = 39 herds)			Spring cow (n = 39 herds)			Fall calf (n = 39 herds)			Fall cow (n = 36 herds)		
		Within-herd percentage for resistant herds			Within-herd percentage for resistant herds			Within-herd percentage for positive herds			Within-herd percentage for positive herds	
Antimicrobial	% (#) of resistance-positive herds	Mean	Min and Max	% (#) of resistance-positive herds	Mean	Min and Max	% (#) of resistance-positive herds	Mean	Min and Max	% (#) of resistance-positive herds	Mean	Min and Max
Category I: Very high importance[a]												
Amoxicillin/clavulanic acid	0% (0)	0%	0%, 0%	2.6% (1)	10%	10%, 10%	0% (0)	0%	0%, 0%	2.8% (1)	10%	10%, 10%
Ceftriaxone	0% (0)	0%	0%, 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%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%
Colistin	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%
Meropenem	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%
Category II: High importance[a]												
Ampicillin	13% (5)	18%	10%, 30%	7.7% (3)	10%	10%, 10%	10% (4)	10%	10%, 11%	11% (4)	13%	10%, 20%
Azithromycin	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%
Cefoxitin	0% (0)	0%	0%, 0%	2.6% (1)	10%	10%, 10%	0% (0)	0%	0%, 0%	5.6% (2)	10%	10%, 10%
Gentamicin	2.6% (1)	17%	17%, 17%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%
Nalidixic acid	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%
Trimethoprim/sulfamethoxazole	5.1% (2)	14%	8%, 20%	0% (0)	0%	0%, 0%	0% (0)	0%	0%, 0%	2.8% (1)	10%	10%, 10%
Category III: Medium importance[a]												
Chloramphenicol	51% (20)	18%	10%, 63%	2.6% (1)	10%	10%, 10%	23% (9)	13%	10%, 30%	14% (5)	12%	10%, 20%
Sulfisoxazole	67% (26)	27%	8%, 80%	26% (10)	10%	10%, 10%	62% (24)	19%	5%, 70%	53% (19)	24%	10%, 80%
Tetracycline	72% (28)	25%	10%, 75%	31% (12)	15%	10%, 20%	56% (22)	17%	10%, 40%	36% (13)	13%	10%, 30%
Pansusceptible	100% (39)	76%	20%, 100%	100% (39)	94%	70%, 100%	100% (39)	83%	30%, 100%	100% (36)	85%	20%, 100%
Herd with ≥ 1 resistant isolate	82% (32)	30%	10%, 80%	36% (14)	16%	10%, 30%	79% (31)	10%	10%, 11%	64% (23)	24%	10%, 80%
Multiclass-resistant	41% (16)	18%	10%, 30%	2.6% (1)	10%	10%, 10%	21% (8)	13%	10%, 20%	14% (5)	12%	10%, 20%
Resistant to ≥ 1 Category I antimicrobial	0% (0)	0%	0%, 0%	2.6% (1)	10%	10%, 10%	0% (0)	0%	0%, 0%	2.8% (1)	0%	0%, 0%

Max — Maximum; Min — Minimum.

[a] Categories of antimicrobial drugs based on importance in human medicine — antimicrobial resistance (18).

Table 5. Summary of relative differences in antimicrobial resistance outcomes of interest for *Escherichia coli* isolates collected from cows and calves in the fall and spring of 2021, accounting for clustering within 39 herds using generalized estimation equations.

	Fall calf *versus* spring calf[a]				Spring cow *versus* spring calf				Fall cow *versus* fall calf				Fall cow *versus* spring cow				
		95% CI				95% CI				95% CI				95% CI			
Antimicrobial	Odds ratio	Lower	Upper	*P*-value	Odds ratio	Lower	Upper	*P*-value	Odds ratio	Lower	Upper	*P*-value	Odds ratio	Lower	Upper	*P*-value	ICC
Chloramphenicol[b]	0.31	0.16	0.6	0.001	0.02	0.00	0.19	< 0.001	0.51	0.19	1.38	0.18	6.49	0.77	54.5	0.09	0.19
Sulfisoxazole[b]	0.62	0.41	0.93	0.20	0.11	0.06	0.22	< 0.001	1.05	0.68	1.64	0.82	5.79	2.85	11.8	< 0.001	0.13
Tetracycline	0.47	0.31	0.73	0.001	0.22	0.12	0.37	< 0.001	0.46	0.26	0.84	0.01	1.02	0.51	2.03	0.95	0.10
Pansusceptible[b]	1.61	1.13	2.29	0.009	5.64	3.43	9.27	< 0.001	1.15	0.77	1.70	0.50	0.33	0.19	0.55	< 0.001	0.09
Multiclass-resistant[b]	0.33	0.16	0.68	0.003	0.03	0.00	0.23	0.001	0.61	0.22	1.68	0.34	6.51	0.78	54.7	0.08	0.21
≥ 1 resistant isolate[b]	0.63	0.44	0.89	0.009	0.18	0.11	0.29	< 0.001	0.87	0.59	1.29	0.5	3.06	1.81	5.17	< 0.001	0.09

CI — Confidence interval; ICC — Intracluster correlation coefficient.

[a] All odds ratios reflect the relative difference in the odds of the listed outcome between the first listed group and the second. For example, the odds of chloramphenicol resistance are 0.3× as high in calves in the fall as in calves in the spring. The odds of the isolate from a calf being pansusceptible are 1.6× higher in a calf from the fall as in a calf from the spring.

[b] Interaction between type of animal and antimicrobial ($P < 0.01$).

Isolates recovered from cows in the fall were more likely to be resistant to sulfisoxazole than those from cows in the spring (Table 5). Also, fall cow isolates were more likely to be resistant to ≥ 1 antimicrobial compared to isolates from cows in the spring (Table 5). In contrast to the observed pattern in calves, isolates from cows in the spring were more likely to be pansusceptible compared to isolates from cows in the fall (Table 5).

Isolates from calves in the spring were more likely to be resistant to chloramphenicol, sulfisoxazole, and tetracycline, as well as multiclass-resistant and resistant to ≥ 1 antimicrobial, compared to isolates from cows in the same season (Table 5). Isolates from cows in the spring were more likely to be pansusceptible than isolates recovered from calves in the spring (Table 5). The only difference between cows and calves in the fall was that isolates from calves were more likely to be resistant to tetracycline than isolates from cows (Table 5).

The ICC values determined from the herd random effect for the complete models ranged from 0.09 to 0.21, suggesting a moderate level of clustering of AMR by herd (Table 5). The highest levels of clustering were observed for chloramphenicol resistance and multiclass resistance.

Discussion

The intent of this study was to describe the relative frequency of AMR of interest for human health for generic *E. coli,* a commensal enteric organism isolated from cattle feces in western Canada. As an indicator organism, *E. coli* has some advantages over other enteric organisms such as *Campylobacter* spp., *Salmonella* spp., and *Enterococcus* spp., including the number of studies available for comparison for cattle and across other commodities, ease of culture and high expected isolation rates, and relatively well-established interpretation criteria for antimicrobial susceptibility (1,4–9). Overall, the relative frequency resistance in the current study of the cow-calf industry was moderate, with 16% of all isolates resistant to ≥ 1 antimicrobial class. This was notably lower than on-farm resistance reported for other commodities in 2019, such as feedlot cattle, pork, and chicken, for which resistance to ≥ 1 antimicrobial class was 52, 78, and 66%, respectively (20).

In contrast to the individual animal samples reported in the present study, 2 previous Canadian studies of cow-calf herds, including the only report within the last decade, examined pooled fecal samples. In the most recent Canadian study, pooled samples were collected from 20 cows per herd in the fall of 2014 (5). Three *E. coli* isolates from each pool were tested for AMR, compared to the single isolate per animal in the current study. Despite the differences in sampling methods, tetracycline resistance was similar for cows in the fall (5). However, both sulfisoxazole resistance (13%) and resistance to ≥ 1 antimicrobial (15%) were higher for cows in the fall of 2021, compared to 3 and 4% in 2014 (5). Only a single isolate from each of the 2014 and 2021 studies was resistant to any antimicrobial very important to human health: amoxicillin/clavulanic acid (5). Nevertheless, the percent of herds with AMR would very likely have been underestimated in 2014 as compared to 2021 because there were only 3 isolates per herd, compared to 10 in the present study.

Pooled fecal samples were also collected from cows and nursing calves in 13 Ontario beef herds from January through August in 2001 (6). As in the current study, tetracycline resistance was highest in cow-calf herds, although the overall crude prevalence was lower than in the current study, at 9.6% in pooled fecal samples (6). The relative frequency of tetracycline resistance in the Ontario study was, however, almost identical to that for the time-matched spring data, when current tetracycline resistance was adjusted to reflect the relative contribution of cows and calves from the Ontario sampling scheme (6). Also similar to the 2021 data, 89% of all cow and 81% of calf isolates from Ontario were pansusceptible (6). The disadvantage of these previous pooled studies was they did not provide a baseline by which to assess whether the frequency of AMR is changing within herds over time.

Like the current study, 2 other comparable reports from the United States and Canada described AMR based on individual samples. Tetracycline resistance (16%) was higher in the 2008 individual cow samples from the United States than for the 2021 samples from western Canada, which averaged 4.7% for both seasons (9). Sulfisoxazole resistance in cows was similar, with 6.7% in the 2008 isolates compared to 7.2% in 2021. The only previous Canadian data with individual cow sample results comparable to the American data and the cow data from the current study were from 69 herds sampled in the spring of 2002 (8). The 2002 Canadian data also mirrored the American data, in that sulfamethoxazole resistance for cows in the spring was nearly identical to that from 2021, at 7.1% (8). However, also as in the American data, tetracycline resistance was slightly higher in 2002, at 8.7%, as compared to 4.7% in 2021 (8).

Single isolates recovered from cows in the spring of 2002 and from the 2008 American study were resistant to ceftiofur (8,9). No 3rd-generation cephalosporin resistance was detected in the current study, despite reports of ceftiofur use in some participating herds (21,22). Ceftiofur was not included on the current MIC plate, which reflects drugs important in human medicine; rather, ceftriaxone alone represented the 3rd generation cephalosporins.

Also similar to findings in the current study, resistance to antimicrobials of high or very high importance to human health was also very low for individual calf samples from 91 herds in the spring and 45 herds in the fall of 2002 (7). In both 2021 and 2002, calves in spring were more likely to have ≥ 1 resistant isolate than calves sampled in the fall and cows sampled in the spring (7,8). In addition, cow-calf pairs were examined from 10 herds in 2003 with a similar higher prevalence in calves than cows (8). Resistant isolates were recovered from both the cow and calf from the same pair in only 5% of pairs sampled (8).

Selection pressure associated with AMU (5) could contribute to the observed differences between calves in the spring and fall and between calves and cows. The most common targets of AMR in the present study, tetracycline and sulfisoxazole, were also consistent with described AMU patterns. In western Canada in 2019 to 2020, 84% of herds reported using oxytetracycline on their operation at least once (11); 33% reported using injectable sulfadoxine/trimethoprim in any animal and 40% reported using sulfamethazine oral boluses in calves (11). Most treatments administered to calves for neonatal diarrhea and respiratory disease typically occur in the first 3 mo after birth (10). Calves are also more likely to be treated than cows in the spring, potentially explaining the difference in AMR between calves and cows (21). However, AMU differences do not explain the higher prevalences of AMR to both tetracycline (46%) and sulfamethoxazole (43%) reported in calves in the spring of 2002 (7).

Another potential factor contributing to differences in resistance levels between calves in the spring and the fall is physiological differences associated with calf age (7). In the present study, there was a decrease in the frequency of AMR with increasing age for calves within the spring sample period after accounting for clustering by herd. Gow *et al* had previously documented age differences in AMR in neonatal beef calves, with an increase immediately after birth followed by a decrease to weaning (7). Hoyle *et al* also reported that calves had fewer resistant *E. coli* relative to susceptible *E. coli* as they aged (23).

The potential role of transmission in explaining the observed AMR patterns is not as obvious. Tetracycline and sulfamethoxazole resistance in cows was previously shown to be a predictor of resistance in calves in the same herd (7), suggesting the potential for transmission of AMR from cows — either directly from the dam or from other cows or calves through the environment. Whereas dam to calf transmission is very likely, one previous study reported no evidence that dam and calf status were strongly associated within 105 cow-calf pairs (8). Typically, cattle are managed under a relatively higher degree of confinement during and following calving season, providing opportunities for indirect environmental transmission of enteric organisms among all cows and calves in the same management group. Recent research has linked spring management activities and failure to disperse cattle following calving with increased risk of neonatal infectious disease (10).

Interestingly, chloramphenicol resistance was noted among calves and cows in different seasons, to varying degrees, with the highest frequency from calves in the spring. However, chloramphenicol is banned for use in food-producing animals in Canada due to the risk associated with aplastic anemia in humans (24). One potential reason for the observed resistance to chloramphenicol could be the use of florfenicol on cow-calf operations. Florfenicol has consistently been 1 of the 3 most reported antimicrobials used within this cohort of cow-calf operations, together with tetracyclines and all products containing sulfonamides, with 81 and 73% of herds reporting use at least once in both of 2014 and 2020 (11,21). Florfenicol is a structural analog of chloramphenicol approved for treating respiratory disease. The observed resistance might be attributed to a chromosomal *flo* gene that confers resistance to both florfenicol and chloramphenicol (25).

We inferred that, whereas the relative frequencies for resistance to individual antimicrobials have varied over time, the types of antimicrobials for which resistance is most common and the frequency of resistance to antimicrobials most important to human health have remained relatively unchanged across available reports. There are, however, some limitations in the available data. The choice of *E. coli* as an indicator organism limits the assessment of resistance to macrolides, an important class of drug in the beef industry and to human health. The only macrolide included on the MIC plate chosen for this study was azithromycin, to which *E. coli* was completely susceptible. However, *E. coli* is intrinsically resistant to many of the macrolides used in veterinary medicine due to the impermeability of the outer membrane (26,27). Because validated cut points for most veterinary macrolides of interest are not available for enteric commensal organisms, additional data were gathered for macrolides of interest to human health. Investigators examined various *Enterococcus* species from these same samples using Sensititre plates appropriate for Gram-positive bacteria that include macrolides; the results of this examination will be presented elsewhere.

Although several significant associations consistent with previous reports were identified, the power of the study was lower than originally intended, with only 39 herds providing samples in both the spring and fall sampling periods. The current protocol allowed for convenience sampling of 10 calves and 10 cows in the spring and fall; however, with only 10 calves and 10 cows, the samples would not fully represent the herd. Therefore, caution is needed in making generalizations, even though the number of isolates per herd was consistent with that reported for *E. coli* in a larger report from the United States in 2008 (9). Further as individual animals were not repeatedly sampled in both seasons, it was not possible to measure the change in status of individual animals from the spring to the fall.

Finally, because the herds enrolled in this study were part of a larger network, the sample population likely represented relatively progressive, intensively managed operations. The enrollment criteria included some basic record management and participation in industry best management practices such as pregnancy checking. Thus, whereas the data give some insight into *E. coli* resistance, this study reflects 1 segment of the western Canadian cow-calf industry and should be directly compared to data from herds of similar size and with comparable management practices. The herds were, however, recruited using very similar protocols to those reported for previous Canadian studies (5–8,28), facilitating direct comparisons to previous work. Herd-level AMU data from herds enrolled in this study are published elsewhere (11).

Additional studies are needed to better understand the now-repeated observations of higher relative frequency of AMR in calves than in cows and decreasing relative frequency in calves from the spring to the fall. If cow-calf herds were added to federal AMR surveillance initiatives, the existing information would be of value to inform on the potential effect of targeting sampling to cows or calves or to specific seasons. Although lower than for calves, the relative frequency of AMR for cows in the fall was higher than for cows in the spring. This was new information, as the previous comparable study (8) did not sample cows in the fall. Cows are typically culled in the fall, and these culls have the closest connection to the food chain and potential risk for human or pet foodborne exposure.

Evidence of moderate clustering of AMR outcomes should be considered in planning future studies, and the reported ICCs can be considered in sample size calculations. The observed clustering could result from a common management or AMU practice associated with AMR or be evidence of contagious transmission within a herd. Regardless, clustering suggests that the sample size per herd to detect common AMR outcomes might be lower than otherwise necessary without herd-level determinants. However, as clustering is only moderate, very small sample sizes per herd would still be likely to miss important information.

Other subsequent epidemiologic analyses using individual animal treatment data are also necessary to also examine the potential contribution of AMU to AMR. Future genomics studies could provide more insight into the observed AMR profiles (29). In addition, genomics studies could help to identify the role of contagious transmission in explaining observed patterns in AMR and any connections to environmental *E. coli.*

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