National surveillance of antimicrobial use and antimicrobial resistance in Canadian feedlots

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Abstract

There is public demand for the demonstration of responsible antimicrobial stewardship, especially in the livestock sector. Surveillance can detect temporal trends in antimicrobial use (AMU) and antimicrobial resistance (AMR) that identify emerging issues and research priorities, support stewardship goals, and meet the growing demand for reliable data. In this project, inclusion/exclusion criteria were used to define and randomly enrol eligible feedlots in proportion to feedlot capacity and the number of fed cattle in target provinces. Data was abstracted from both veterinary dispensing and AMU records from randomly sampled production lots closed in the previous calendar year. Composite fecal samples were collected yearly from randomly selected pens of cattle within 30 days of slaughter. Fecal culture identified *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp. and *Enterococcus* spp.; recovered isolates were subject to antimicrobial susceptibility testing (AST) via broth microdilution. Deep-guarded nasopharyngeal (NP) samples were also collected yearly from individual animals at feedlot entry and subsequent re-handling. Sample culture was performed to identify Mannheimia haemolytica, Pasteurella multocida and Histophilus somni, and recovered isolates were subject to AST as described above. Integration of longitudinal surveillance data provides a more comprehensive picture of AMU and AMR in the finishing feedlot sector over time.

Key words: antimicrobial use, antimicrobial resistance, feedlot cattle, surveillance

Introduction

Antimicrobial resistance (AMR) is a worldwide health concern that requires the focused and ongoing assessment of antimicrobial use (AMU) practices as well as the monitoring of AMR development in both human and veterinary medicine. There is a growing expectation among trading partners, foodservice, and food retailers that countries quantify AMU in food animal production, including in the feedlot cattle sector.

With continuing calls from national and international groups to describe AMU and AMR in the livestock sectors, the Canadian feedlot industry aims to provide meaningful and accurate data to demonstrate transparency and to protect global trade markets. In the fall of 2018, an expert group consisting of industry, feedlot veterinarians and government, was convened to develop a sampling framework for conducting surveillance of AMU and AMR in the fed cattle sector. From this collaborative effort, the current surveillance system was launched in 2019.

The feedlot AMU/AMR surveillance program is part of the Public Health Agency of Canada's Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). CIPARS monitors trends in AMU and AMR in select bacterial organisms from human, animal and food sources across Canada.[7,8,9] The program is similar to the National Antimicrobial Resistance Monitoring System (NARMS) in the United States, and is based on several representative and methodologically unified surveillance components which can be linked to examine the relationship between AMU and health impacts in food animals and humans.[7,8,9] AMR data are obtained through the isolation and antimicrobial susceptibility testing (AST) of specific enteric organisms detected via the sampling of core commodities along the food chain (farm, abattoir, and retail). Additionally, diagnostic laboratories submit isolates obtained from human and animal cases of Salmonellosis to CIPARS for AST and serotyping. Enrolled sentinel farms provide AMU data, and pharmaceutical companies provide antimicrobial drug sales data through the veterinary antimicrobial sales reporting (VASR) system. Information on AMU in humans is obtained from pharmacy sales, hospital purchases and physicians' diagnosis diaries. The integration of this information through CIPARS supports: (a) the creation of evidence-based AMU stewardship policies in hospital, community, and agricultural settings to help prolong the effectiveness of antimicrobials, and (b) the identification of appropriate measures to contain the emergence and spread of resistant bacteria between animals, food, and people in Canada.[8,9]

The goal of the proposed surveillance system is to capture how feedlot cattle are raised in Canada with respect to AMU, and to detect emerging trends in AMU and AMR over time. The system incorporates a One Health approach by including both bovine respiratory disease (BRD) pathogens of importance to animal health and enteric organisms of potential concern to human health. By monitoring AMR in BRD pathogens, feedlot veterinarians and their clients can use this information to support decisions as they strive to reduce disease risk, improve treatment efficacy (reduce morbidity and mortality), improve feedlot production sustainability, and address antimicrobial stewardship. By assessing the presence of AMR in fecal bacteria, which may pose a risk to human safety through fecal contamination of beef products or environmental pathways, the surveillance system can provide information for source attribution studies.

The surveillance system works closely with feedlot veterinarians to acquire data and samples. Individual animal health tracking/data management systems and specific induction and treatment protocols in commercial feedlots, which are under the direction and supervision of feedlot veterinarians with valid veterinarian-client-patient relationships, are a valuable resource for surveillance purposes. Reporting on these practices through the surveillance system will help to provide retailers and consumers with an impartial, third-party source of information regarding antimicrobial stewardship activities in this sector and support on-going stewardship practices in feedlots.

The objectives of the project are to (a) provide representative estimates of AMU and AMR in the Canadian finishing feedlot sector; (b) provide a unified approach to monitor trends in AMU and AMR over time; (c) investigate associations between AMU and AMR on a targeted basis related to emerging AMR trends; and (d) provide collated industry data for the assessment of the potential public and animal health risk of AMU in the Canadian finishing feedlot sector.

Materials and methods

Population of interest and sample size calculations
Sampling is designed to be representative of the number of fed cattle produced in the provinces of Alberta, Saskatchewan and Ontario. Almost 90% of Canadian feedlot cattle are located in these provinces.[15] To be included in this program, feedlots must: be engaged in the finishing phase of cattle production (cattle not in the finishing phase may be on-site and included in data collection, but at least some of the cattle must be sent directly from the feedlot to slaughter); have a valid veterinarian-client-patient relationship with the veterinarian enrolling the feedlot; and have a one-time capacity of > 1,000 animals.

A sample size calculation for the number of fed cattle required for a representative estimate of AMU was performed with the Epitools (Ausvet) sample size calculator, using an estimate based on the expected treatment incidence as described in Timmerman et al. (2006).[5,13] The expected treatment incidence used for this calculation came from Canadian research and feedlots.[2] The total number of fed cattle (151,000) to be sampled were then distributed proportionally among the provinces, the varying feedlot capacities, and the participating veterinary clinics. A random sample was selected from a list of eligible feedlots identified by each participating veterinary practice to ensure that the correct number of cattle were represented within each feedlot capacity stratum. The feedlots were deidentified and coded to protect their confidentiality.

Sample size calculations for the BRD pathogens were based on a 13% recovery rate for *Mannheimia haemolytica* from deep-guarded nasopharyngeal swabs (NP), and a 33% prevalence of resistance to tetracyclines in these isolates.[12] Using a precision of 0.05 and confidence level of 95%, 832 NP were required to provide 107 *M. haemolytica* isolates. The number of composite fecal samples required was based on *Enterococcus* spp. detection because enterococci provide useful AMR information for macrolides, which are commonly used antimicrobials in feedlots. Data were obtained from Beukers et al. (2015) and concerned the prevalence of macrolide resistance among *Enterococcus* spp. detected in fecal samples at the end of the feeding period.[3] The estimated prevalence of tylosin resistance in *Enterococcus* spp. isolated from cattle manure in this study was 30%; using a precision of 0.05 and a confidence level of 95%, the required sample size was 421 samples, based on a 98% recovery rate.[3]

Sample collection
Ten composite fecal samples per feedlot were collected each year following a prescribed protocol. Each composite sample was composed of 20 fresh and pooled fecal pats from unique pens containing cattle within 30 days of slaughter. The target bacteria to be cultured from the composite fecal samples included *Escherichia coli*, *Salmonella* spp. *Campylobacter* spp., and *Enterococcus* spp. Nasopharyngeal (NP) samples were collected twice yearly from 16 individual animals at entry processing and again at implant re-handling (20 to 105 days on feed (DOF)) following a standard protocol. The same group of cattle, but not necessarily the same individual animals, were sampled at both time points. The target organisms to be isolated from the NP swabs included *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*. Where possible, the collection of samples was distributed across the year in enrolled feedlots to capture any variation by season. For each group of sampled cattle, data were collected on breed type (beef, dairy, mix), BRD risk-category (low, medium, high) and the age of animals sampled (calf, yearling, adult).

Primary isolation/bacterial identification
Primary isolation and susceptibility testing of *Salmonella* spp., *E. coli* and *Campylobacter* spp. were performed at the National Microbiology Lab in St. Hyacinth, Quebec, using the standard CIPARS protocols and methodology.[7] Isolation and susceptibility testing of organisms not routinely surveyed by CIPARS (*Enterococcus* spp. and the respiratory pathogens) was conducted at Prairie Diagnostic Services (PDS) in Saskatoon, Saskatchewan. For each sample, only 1 isolate per identified organism was saved for AST. AST was performed using Sensititre Complete Automated broth microdilution (SensititreTM Trek Diagnostic Systems Ltd, West Sussex, England). Plates used included the Sensititre™ NARMS Gram Negative CMV4AGNF AST plate, Sensititre™ NARMS Gram Positive CMV3AGPF AST plate, Sensititre™ CAMPY[2] plate and the Sensititre™ bovine/porcine AST plate (BOPO7F). Clinical Laboratory Standards Institute (CLSI) guidelines were followed for primary isolation and AST. Minimum inhibitory concentration (MIC) values were interpreted according to CLSI standards when available.

Antimicrobial classification
Consistent with CIPARS methodology, AMR results were interpreted with reference to the classification of antimicrobial drugs based on their importance to human medicine; these classifications reflect the categorizations created and used by

Health Canada's Veterinary Drugs Directorate.[7,8,9] Antimicrobials are considered to be of Very High Importance in Human Medicine (Category I) when they are essential for the treatment of serious bacterial infections and there is no or limited availability of alternative antimicrobials for effective treatment.[7,8,9] Antimicrobials of High Importance in Human Medicine (Category II) consist of those that can be used to treat a variety of infections, including serious infections, but for which alternatives are generally available; Antimicrobials of Medium Importance in Human Medicine (Category III) are not the preferred treatment for serious infections; Antimicrobials of Low Importance in Human Medicine (Category IV) are currently not used to treat bacterial infections and rarely used in human medicine. [7,8,9]

Antimicrobial use data

Each year, participating veterinary practices randomly sampled closed production lots from enrolled feedlots until the required number of cattle to be sampled for that feedlot was reached. As was described previously, the total number of cattle required for each feedlot was proportional to the feedlot's size. AMU information and veterinary dispensing records for each of the closed and randomly selected production lots were assembled and submitted by the participating veterinary practice. Data were summarized in a standardized format and identifying information was removed prior to submission to protect the confidentiality of the feedlot. The AMU data collected represented the minimum information identified by the Canadian Animal Health Surveillance System (CAHSS)/CIPARS committee to quantify AMU and was consistent with Canadian and international standards.[7] Sufficient detail was requested to be able to describe what and how antimicrobials were administered (in-feed/parenteral), the quantity of antimicrobials used, the reason(s) for AMU, and the type of cattle receiving the antimicrobials with respect to days on feed, source, and BRD risk category.

Statistical analyses

Antimicrobial resistance analysis

Statistical analyses accounted for the potential clustering of resistance patterns within individual feedlots using generalized estimating equations (GEE). All statistical models included a logit-link function and exchangeable correlation structure. Null binomial response models were used to estimate the population-averaged prevalence of resistance to each antimicrobial. A separate model was fit for each antimicrobial tested for each pathogen with isolate-level resistance as a binary outcome (i.e., resistant vs. non-resistant, as determined by the MIC breakpoints for the relevant antimicrobial and pathogen). If no CLSI breakpoint was available for a particular antimicrobial/ organism combination, the antimicrobial was omitted except in the presentation of MIC distribution data. Isolates with intermediate susceptibility were classified as susceptible. When the prevalence of resistance was 0% or 100%, an exact binomial test was performed to estimate an exact upper or lower confidence interval, respectively.

This method was repeated to stratify susceptibility testing results by province. Analyses for *Enterococcus* spp. and *Campylobacter* spp. were additionally stratified by species, and analyses for the BRD pathogens were stratified by the sample collection time point (i.e., arrival or re-handling).

All statistical analyses were conducted in RStudio v1.4.1106 using R v4.0.4. Most data handling and cleaning were done using the "tidyverse" packages, primarily "dplyr" (**https://tidyverse. tidyverse.org**/); models were fit using the "geepack" package.

Antimicrobial use analysis

Analysis of the 2019 AMU data is not complete as data are still being validated. Validation of AMU data includes but is not limited to ensuring that the submitted data make biological sense, that outliers are identified, that calculations are correct, and that the datasets are complete. Practitioners providing the data are contacted with any questions arising from the validation process. Once the AMU validation is complete, the analysis will proceed in accordance with current CIPARS methodology.[7] Indicators used by CIPARS include a) milligram (mg) of active ingredient (AI)/population correction unit (PCU). The PCU is based on the population size and average weight at treatment. Average weight at treatment for fed cattle is to be confirmed; consideration is being given to using a mean weight of 741lbs (336 kg) based on a prior Canadian project;4 b) mg AI/kilogram (kg) animal biomass. The kg animal biomass pertains to live weights documented immediately prior to the expected slaughter date; c) number of animal defined daily doses using Canadian standards (nDDDvetCA)/1000 animal days at risk or Treatment Incidence 1000 (TI1000). The TI1000 is a species and herd-specific indicator that expresses the number of doses a thousand animals would receive per day over the observation period. The DDDvetCA values have been developed by CIPARS for each livestock species; d) nDDDvetCA/PCU. This indicator is the total number of DDDvetCAs adjusted for PCU; e) nDDDvetCA/kg animal biomass. This indicator is interpreted as the total number of DDDvetCA for every kg of live pre-slaughter weight.

Results

The results presented were identified by the authors as key data obtained from the first year of the surveillance project, and do not comprehensively cover the breadth of information available.

General information

The goal was to recruit 26 feedlots to from which to obtain AMU data, fecal and NP samples. In order to meet the needs of another collaborative project, an additional 14 feedlots in Alberta were enrolled for fecal sample collection only. The target number of feedlots for Alberta was 30 (16 for AMU, fecal and NP sampling and 14 for fecal samples only), 2 for Saskatchewan and 8 for Ontario, totaling 40 feedlots. Only 5 of 8 feedlots were recruited in Ontario for 2019, and thus 37 feedlots were enrolled in the first year of sampling.

Enteric sample information

In year 1 of the sentinel feedlot surveillance project, 366 fecal samples were collected from participating feedlots across Alberta, Saskatchewan and Ontario. Sixty-six samples were collected from 7 feedlots with a capacity of 1000 to 5000, 120 samples from 12 feedlots with a capacity of 5001 to 10,000, 100 samples from 10 feedlots with a capacity of 10,001 to 20,000, and 80 samples from 8 feedlots with a capacity of >20,000. The median pen capacity for cattle sampled was 100 to 200 animals (range <100 to 600).

Table 1: Recovery rates from fecal samples by province, 2019.

Province	Percentage (%) of isolates recovered and number of isolates recovered/number of samples submitted							
	Escherichia coli		*Salmonella* spp.		*Campylobacter* spp.		*Enterococcus* spp.	
Alberta	99.0%	293/296	4.4%	13/296	37.2%	110/296	100.0%	290/290
Ontario	100.0%	50/50	26.0%	13/50	82.0%	41/50	100.0%	50/50
Saskatchewan	100.0%	20/20	0.0%	0/20	55.0%	11/20	100.0%	15/15
National	99.2%	363/366	7.1%	26/366	44.3%	162/366	100.0%	355/355

The CIPARS laboratory processed a total of 366 samples for primary isolation of *Salmonella* spp., *Campylobacter* spp. and *E. coli*. The PDS lab processed 355 samples for isolation of Enterococcus spp.; 11 fecal samples were lost in transportation or otherwise not received by PDS. Organism recovery varied between the provinces across Canada (Table 1).

Susceptibility of enteric organisms

E. coli

Except for a single ceftriaxone-resistant isolate, no resistance to antimicrobials of Very High Importance in Human Medicine (i.e., Category I antimicrobials) was observed among the *E. coli* isolates (n = 363). Nationally, resistance was highest to tetracycline (48.5%, 176/363, Category III), followed by streptomycin (19.3%, 70/363, Category III). No isolates were resistant to more than 6 classes of antimicrobials; however, 20 (5.5%) isolates were resistant to 4-5 classes of antimicrobials. In isolates with resistance to 4-5 antimicrobial classes, the identified classes included aminoglycosides, beta-lactams, folate pathways inhibitors, phenicols and tetracyclines.

Salmonella spp.

Of the 26 *Salmonella* spp. isolates recovered in 2019, the 2 most common Salmonella serovars were *S. heidelberg* (26.9%, 7/26), and *S. orion* (19.2%, 5/26). In total, 18 (69.2%) of 26 *Salmonella* spp. isolates were either pan-susceptible to all tested antimicrobials or resistant to 1 antimicrobial class. All recovered *S. heidelberg* isolates were resistant to 5 or more antimicrobial classes, including Category I and II beta-lactams (ampicillin, amoxicillin-clavulanic acid, and ceftriaxone), Category II aminoglycosides (streptomycin) and folate pathway inhibitors (trimethoprim sulfamethoxazole), and Category III phenicols (chloramphenicol), folate pathway inhibitors (sulfisoxazole) and tetracyclines (tetracycline). Six of the 7 isolates were also resistant to cefoxitin (Category II beta-lactam) and nalidixic acid (Category II quinolone). A single isolate was additionally resistant to Category I quinolone ciprofloxacin.

Campylobacter spp.

Of the 162 *Campylobacter* spp. isolates, 121 were identified as *C. coli* (74.7%) and 41 were *C. jejuni* (25.3%). Nationally, resistance was highest to Category III tetracycline for both species; prevalence of resistance was 81.0% (98/121) and 75.6% (31/41) for *C. coli* and *C. jejuni* respectively. Resistance to ciprofloxacin (Category I) in combination with resistance to nalidixic acid (Category II) was the next most frequently detected pattern. A total of 31.4% (38/121) and 17.1% (7/41) of the *C. coli* and *C. jejuni* isolates, respectively, had this pattern of resistance. Thirty-six of 121 *C. coli* isolates (29.8%) were resistant to Category II macrolides (azithromycin and erythromycin) and lincosamides (clindamycin); no resistance to macrolides or lincosamides was observed among the *C. jejuni* isolates. No isolates were resistant to more than 4 antimicrobial classes.

Enterococcus spp.

AST was only performed on a subset of the 355 *Enterococcus* spp. isolates. Species selected for AST included the most common species in cattle, *E. hirae*, and two human pathogenic species, *E. faecalis* and *E. faecium*. As a result of an early miscommunication, only *E. faecalis* and *E. faecium* isolates were initially saved for testing; as a result, 42 *E. hirae* isolates were discarded and only 282 of 324 (87.0%) eligible isolates were tested for susceptibility to antimicrobials. In total, 206 (73.0%) isolates were *E. hirae*, 46 (16.3%) were *E. faecalis*, and 30 (10.6%) were *E. faecium*.

Resistance to macrolide (Category II) antimicrobials varied with the *Enterococcus* species identified. Nationally, resistance to macrolides was highest among *E. hirae* isolates, with 83.0% (171/206) and 72.3% (149/206) of *E. hirae* isolates resistant to tylosin and erythromycin, respectively. Twenty-one (45.7%) of the 46 *E. faecalis* isolates were resistant to both tylosin and erythromycin. In *E. faecium*, tylosin resistance was detected in 23.3% (7/30) of the isolates and erythromycin resistance was detected in 23.3% (7/30) of the isolates. Seven of 282 enterococci isolates (2.5%) were resistant to Category I ciprofloxacin. No Category I vancomycin-resistant enterococci isolates were detected. Many isolates (86.1%, 243/282) were resistant to 2 to 5 antimicrobial classes, including various combinations of aminoglycosides, lincosamides, lipopeptides, macrolides, nitrofurans, oxazolidinones, phenicols, quionolones, streptogramins and tetracyclines. Only 2.1% (6/282) of the isolates were susceptible to all antimicrobials tested. Overall, resistance to tetracyclines, macrolides, and lincosamides were the most common.

Respiratory pathogen sample information

In year 1 of the sentinel feedlot surveillance project, 624 NP samples were collected from participating feedlots across Alberta, Saskatchewan, and Ontario. This amounted to 75% (624/832) of the required samples estimated at the project outset. For *M. haemolytica, P. multocida* and *H. somni*, 608 samples were processed by PDS; 16 NP samples were lost in transportation or otherwise not received by PDS. The recovery of the BRD pathogens from NP samples varied between the provinces across the country (Table 2). Of the 608 samples, 52.6% (320/608) were collected at arrival and 47.4% (288/608) at re-handling. Forty-nine percent (298/608) of the samples came from yearlings

and 51.0% (310/608) from calves. Fifty-four percent (328/608) of the cattle sampled were classified as low risk for BRD and 46.0% (280/608) were classified as high risk.

M. haemolytica

A total of 46 *M. haemolytica* isolates were obtained from NP sampling; 23 from arrival sampling and 23 from re-handling sampling (Table 2). Nationally, macrolide resistance was highest for tilmicosin (19.6%, 9/46), followed by gamithromycin and tulathromycin (both 17.4%, 8/46) (Figure 1A). Most of the macrolide resistant isolates were collected at re-handling (34.8%, 8/23) (Figure 1A). Only 1 (4.3%) isolate had macrolide resistance at arrival. Two (8.7%) arrival isolates were resistant to danofloxacin and enrofloxacin (i.e., Category I quinolones) (Figure 1A). No other isolates were resistant to Category I antimicrobials. Most isolates (73.9%, 34/46) from either collection time, were pan-susceptible to the panel of antimicrobials tested. Six (13.0%) isolates were resistant to 2-5 antimicrobial classes, including quinolones and macrolides.

P. multocida

Nationally, 180 *P. multocida* isolates were recovered from NP samples; 91 from arrival and 89 from re-handling (Table 2). Resistance was highest to tetracycline (37.2%, 67/180), followed by macrolides (tildipirosin, 32.8%, 59/180; gamithromycin, 23.8%, 43/180; tulathromycin, 23.3%, 42/180; Figure 1B). Except for the phenicol class, the proportion of isolates resistant to each antimicrobial class was higher at re-handling than at arrival (Figure 1B). Resistance to Category I antimicrobials danofloxacin (11.7%, 21/180), ciprofloxacin (7.8%, 14/180), and ceftiofur (0.6%, 1/180) was detected (Figure 1B). Twelve of 180 (6.7%) isolates were resistant to 4 or 5 antimicrobial classes, including fluoroquinolones and macrolides.

H. somni

A total of 60 (20 arrival, 40 re-handling) *H. somni* isolates were obtained from NP sampling in 2019 (Table 2). No resistance to Category I antimicrobials was observed among the *H. somni*

isolates recovered (Figure 1C). Nationally, resistance was highest to tetracycline (35.0%, 21/60); less than 10% of isolates were resistant to any macrolide, aminocyclitol or penicillin antimicrobials (Figure 1C). The majority of isolates were pan-susceptible (63.3%, 38/60) or resistant to 1 antimicrobial class (21.7%, 13/60).

Dispensing and AMU

Dispensing and AMU data for 26 feedlots has been collected for 2019. Despite attempts to collect the required information in a standardized format, there were discrepancies between participating veterinary practices as to which data were received. Therefore, additional time was required to assemble these data into a format that supports robust data analysis. Lessons learned from the first year contributed to significant improvements in the data collection process and should facilitate the more streamlined analysis of these data in the future.

Discussion

The first year of a national feedlot AMU/AMR surveillance system produced several key findings. Except for a single ceftriaxone-resistant isolate, no resistance to antimicrobials of Very High Importance in Human Medicine (i.e., Category I antimicrobials) was detected in the recovered *E. coli* isolates. Given that in-feed tetracyclines are commonly used in Canadian feedlots, it is not surprising that the prevalence of resistance in the *E. coli* isolates was highest for tetracycline (Category III).

Historically, the prevalence of *Salmonella* spp. in samples obtained from healthy feedlot cattle in Canada has generally been between 1-2%.[14,16] At 7%, the recovery of *Salmonella* spp. from this surveillance project was unexpectedly high. Additionally, *Salmonella* spp. isolated from healthy Canadian feedlot cattle in the past have had low levels of AMR.[9] However, in 2019, resistance to 5 or more antimicrobial classes was detected among S. heidelberg isolates. After investigating the differences in historical recovery and AMR patterns of *Salmonella* spp. as compared to the current data, it became apparent that 6 of the 7 the pens with multidrug resistant (wMDR) *S. heidelberg* housed Holstein

Table 2: Recovery rates from nasopharyngeal samples by province and time of sample, 2019

Province	Time of sample	Percentage (%) of isolates recovered and number of isolates recovered/number of samples submitted					
		Mannheimia haemolytica		*Pasteurella multocida*		*Histophilus somni*	
Alberta	Arrival	5.9%	15/256	27.3%	70/256	5.9%	15/256
	Re-handling	8.3%	20/240	33.3%	80/240	15.0%	36/240
	Overall	7.1%	35/496	30.2%	150/496	10.3%	51/496
Ontario	Arrival	16.7%	8/48	29.2%	14/48	10.4%	5/48
	Re-handling	6.3%	1/16	12.5%	2/16	0.0%	0/16
	Overall	14.1%	9/64	25.0%	16/64	7.8%	5/64
Saskatchewan	Arrival	0.0%	0/16	43.8%	7/16	0.0%	0/16
	Re-handling	6.3%	2/32	21.9%	7/32	12.5%	4/32
	Overall	4.2%	2/48	29.2%	14/48	8.3%	4/48
National	Arrival	7.2%	23/320	28.4%	91/320	6.3%	20/320
	Re-handling	8.0%	23/288	30.9%	89/288	13.9%	40/288
	Overall	7.6%	46/608	29.6%	180/608	9.9%	60/608

Figure 1: Percentage of *M. haemolytica* **(A)**, *P. multocida* **(B)** and *H. somni* **(C)** isolates resistant to select antimicrobials at arrival and re-handling. The percentage was adjusted to account for the clustering of samples by feedlot. When the prevalence of resistance was 0% or 100%, a confidence interval calculator was used to estimate an exact upper or lower confidence interval, respectively.
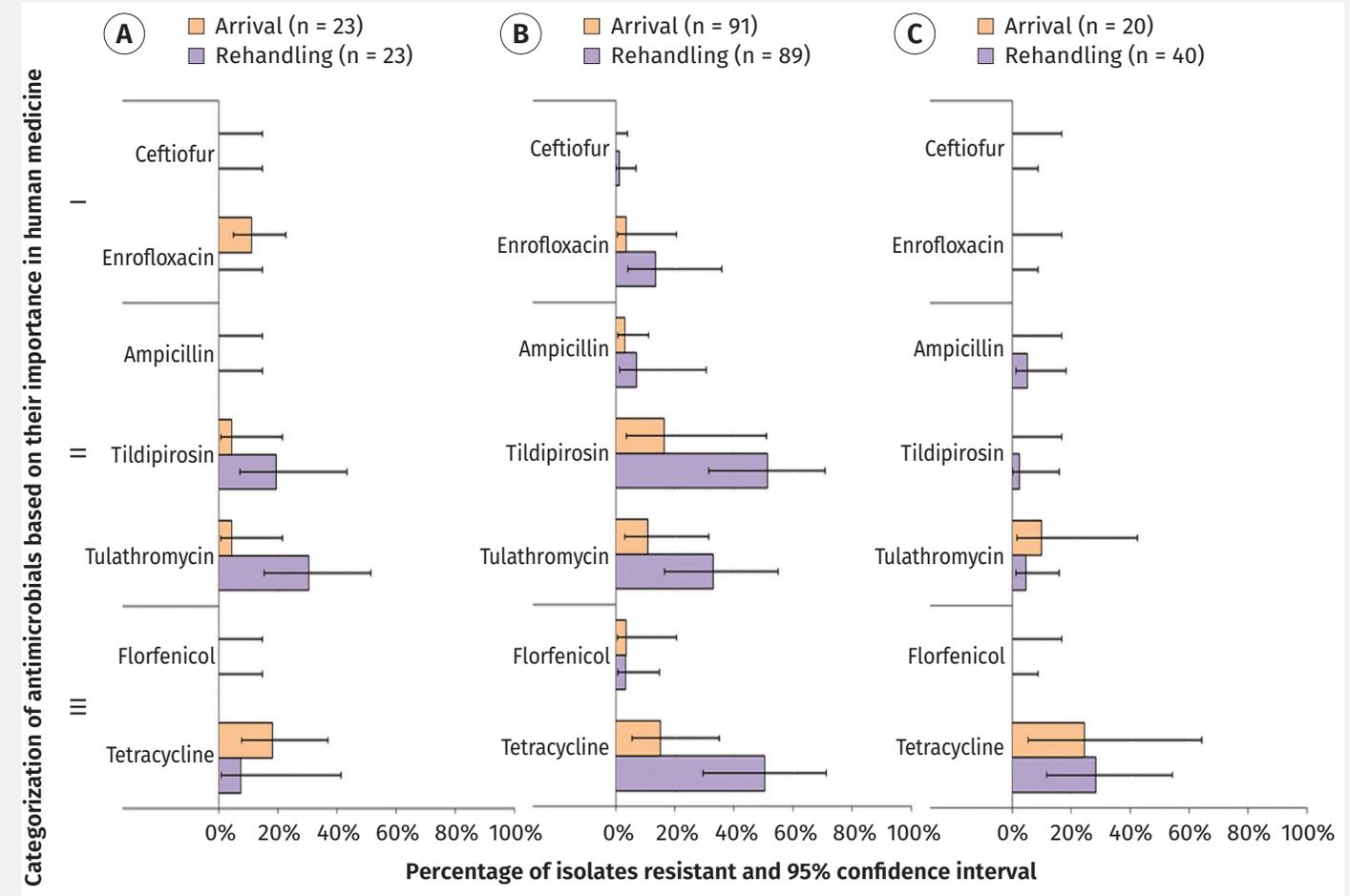
cattle sourced from the United States. The final pen contained beef cattle from Canada. The pattern of phenotypic resistance was indicative of clonal spread of *S. heidelberg* before and/or after arrival at the feedlot sites. Whole genome sequencing (WGS) on these MDR *S. heidelberg* isolates was performed by CIPARS. WGS revealed genetic relatedness between the feedlot isolates (differences of only 1-12 single nucleotide variants, [SNVs]). In contrast, the isolates were genetically distinct from 16 human *S. heidelberg* isolates available through CIPARS (differences of more than 150 SNVs). Based on this initial investigation, the practice of finishing Holstein cattle from the United States appears to have impacted the recovery of MDR *Salmonella* spp. in Canada. The finishing of Holstein cattle from the U.S. is a relatively recent phenomenon in western Canada, starting in earnest in 2017. It has since become an important part of the finished cattle supply in western Canada; consequently, it will be important to continue monitoring the future impact of importing American dairy feeders on the recovery of MDR *Salmonella* spp. from Canadian feedlot cattle.

Increased azithromycin and ciprofloxacin resistance was observed for *Campylobacter* spp. isolates as compared to historical CIPARS/FoodNet Canada findings.[9] Azithromycin resistance in *Campylobacter* spp. isolates recovered from feedlot cattle feces increased from 0% in 2017 to 26% detected in the current study.[9]

Ciprofloxacin resistance in *Campylobacter* spp. isolates recovered from feedlot cattle feces has likewise increased from approximately 9% in 2016 to 28% detected in this study.[9] Isolates recovered from Canadian abattoirs demonstrate a similar upward trend in ciprofloxacin resistance over time.[9] The observed trend in ciprofloxacin resistance across *Campylobacter* spp. isolates from successive steps of the food chain help substantiate the findings reported here. In the future, targeted research may be required to confirm and investigate the drivers of these upward trends.

Resistance to tetracycline and erythromycin among *E. hirae* isolates has also increased compared to a recent (2014-2016) feedlot study in southern Alberta.[17] Ongoing surveillance will help determine if this increase is a verifiable trend. Interestingly, the current study detected lincomycin resistance at a very high level (91.1%), even though lincomycin is not labelled for use in beef cattle in Canada. This high level of resistance to lincomycin is not unique to Canadian cattle, as it has also been reported in American dairy cattle and Australian beef cattle.[1,10] Widespread resistance to lincomycin may stem from some measure of intrinsic resistance across enterococci species; alternatively, the high prevalence may be explained in part by cross-resistance arising from macrolide use, consistent with the macrolide-lincosomide-streptogramin B (MLSB) phenotype.[11]

The recovery of *P. multocida* and *H. somni* isolates via NP sampling was consistent with previous findings.[6] However, despite similar methodologies and cattle types, the recovery of *M. haemolytica* was considerably lower than recently published results and our projected recovery rate.[6,12] It is unclear why *M. haemolytica* recovery was lower than expected when the other BRD organisms were cultured at rates consistent with previous findings. If the low recovery of *M. haemolytica* continues in subsequent years, a sample size adjustment may be necessary.

Over half of the BRD pathogens were susceptible to the tested antimicrobials with established breakpoints. While not statistically significant, increases in resistance between arrival and re-handling time points were detected for several antimicrobials and BRD organisms. This finding is consistent with a 2015 Canadian study and is worth monitoring as increases in resistance over time may be due to pressure from AMU in the feedlot, spread of AMR within the feedlot, or both.[6] If these trends are verified over time, additional studies may be necessary to determine the drivers of resistance and the best strategies to mitigate further AMR development. In contrast to the findings reported by Erickson et al (2017), the current study detected higher levels of fluoroquinolone resistance in *M. haemolytica* (at arrival) and *P. multocida* (at both sampling times). Resistance to several macrolide/BRD organism combinations was also higher in the current study than in the Erickson et al (2017) study.[6]

At least 1 resistant *P. multocida* isolate was identified for every tested antimicrobial with an established breakpoint (ranging from 0.5% to 37.0%). Given that many of these antimicrobials are used to prevent and treat BRD, this finding may become a concern if the proportion of resistant *P. multocida* isolates continues to increase over time.

AMU and dispensing data were initially collected and submitted for 23 feedlots. This was a significant undertaking as feedlot operators and veterinarians determined the best approaches to obtain and summarize the information, especially for in-feed AMU. The validation of these data is currently being conducted. The 2020 AMU and dispensing data have been collected and will be integrated once the data have been verified. When the results are available, these data will contribute to our understanding of AMU and antimicrobial drug dispensing in Canadian feedlots and will be a significant resource for the industry.

In conclusion, the national feedlot AMU/AMR surveillance network has thus far provided extremely valuable data to the Canadian beef industry, veterinarians, federal and provincial governments, and other stakeholders. With significant trading partners collecting and reporting AMU and AMR data, Canada also needs to be able to report reliable AMU and AMR data to help ensure ongoing competitiveness and open market access. As more retailers begin to request AMU metrics from their supply chains, this information is critical to ensure that unrealistic and uneconomic production constraints are not imposed on industry, potentially with negative impacts on animal health and welfare. This surveillance network underscores the beef industry's social responsibility to the public while supporting economically sustainable beef production, trade, food security, and cattle health and welfare.

Ethics statement

The protocol for this project was reviewed and approved by the Feedlot Health Management Services Ltd. Animal Care Committee (a certified holder of a Certificate of Good Animal Practice), and in accordance with standards set by the Canadian Council of Animal Care.

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