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Abstract Proceedings

Abstract 1: Kat Smith

Efficacy of bacteriophage-mediated colibacillosis control in laying hens

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Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, a disease of laying hens that results in significant losses to the Canadian egg-industry. Chicken Farmers of Canada aims to eliminate the use of category II and III antibiotics for APEC control, necessitating development of novel APEC control strategies. Phage therapies applied via environmental sprays have been successful in reducing APEC infection in broilers and we hypothesize that phages with strong lytic activity against clinical APEC serogroups will reduce colibacillosis in laying hens. This study's objectives are to isolate and characterize broad host range anti-APEC phages and to utilize these as an APEC control strategy in a hen-challenge model. Fecal samples from hen farms and sewage water were collected and processed to isolate phages targeting APEC. 7 phages were chosen for further characterization and their host range and lytic activity was determined via microplate phage virulence assays. This assay determines the lowest phage: APEC ratio resulting in complete lysis and determines a phage's infectivity against multiple tested bacterial strains. Phages were also subjected to transmission electron microscopy to determine their virion structure, and whole genome sequencing was used to determine the content of their genomes. Results suggest that four phages have strong lytic activity against multiple APEC strains, especially serogroup O78 APEC which are predominant in Canada, showing promise for use in bird challenge studies. Morphologically, 3 phages belong to the *Myoviridae* and *Siphoviridae* families. Future efforts will focus on the determination of phage colibacillosis control efficacy in laying hen models.

Abstract 2: Julia Ramirez

Investigating Diverse Perceptions of Antibiotic Resistance in San Carlos, Alajuela, Costa Rica

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INTRODUCTION:

Antibiotic resistance is a human, animal, and environmental health problem of growing concern worldwide. To address the rising threat of antibiotic resistance, interprofessional research into the actions contributing to and preventing the spread of antibiotic resistance is needed. Using a One Health approach, this pilot study aimed to investigate various perceptions of antibiotic resistance through the lens of human and animal healthcare professionals as well as feed store and farm workers around Florencia, San Carlos, Alajuela province, Costa Rica.

MATERIAL & METHODS:

We conducted structured, in-person surveys with a convenience sample of physicians, pharmacists, veterinarians, feed store workers and farm workers located within a 30-kilometer radius of Florencia. All private health care entities in the study area were eligible for study participation and included: medical clinics, urgent care centers, pharmacies, veterinary clinics, and animal feed stores. We also interviewed farm workers on dairy farms located in our study region. Public-sponsored healthcare facilities, private entities which were closed, or those that refused participation for any reason were not included in the study. Eligible participants were approached at their place of work and invited to participate in a structured face-to-face survey. All health professionals were given survey questions specific to their field. Survey questions for feed-store workers and farmers focused on livestock care. All surveys were conducted in Spanish by researchers proficient in the language. Qualitative and quantitative data were analyzed descriptively and common themes in participant responses were aggregated.

RESULTS:

In total, 34 in-person surveys were conducted, including: 3 physicians; 16 pharmacists/pharmacy technicians; 8 veterinarians; 3 feed stores workers; and 4 farmer workers. The most common themes that emerged were as follows: first, most bacterial diseases in humans and animals are diagnosed through patient history and exam only and without laboratory confirmation. Second, most participants (in both human and veterinary health fields) are aware of antibiotic resistance and believe that many patients and clients misuse antibiotics. Lastly, the participants generally agreed that increased governmental regulation of antibiotics would help with the growing threat of antibiotic resistance in both human and animal health. Improvements to the current regulatory system proposed by study participants included: implementation of a mandatory prescription system for purchase of all human and animal use antibiotics, better education of both patients/clients and providers on proper antibiotic management of bacterial diseases, and improved availability of diagnostic testing to ensure proper identification and treatment of bacterial infections.

CONCLUSIONS:
Antibiotic resistance in Costa Rica is perceived to be an increasing threat to human and veterinary health and may be driven by widespread availability of antibiotics and their general overuse and misuse. Human and veterinary health care providers recognize the need to address this growing threat through increased regulation in both fields.

Abstract 3: Sofiya Manji

Community-based Antibiotic Prescribing Patterns in Alberta: A Population-based Study

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INTRODUCTION:

Antibiotic resistance has been acknowledged as one of the world's most pressing public health problems. Despite the ever-rising concerns, antibiotic over use and inappropriate selection remains common. Antibiotic dispensing in the community accounts for the majority of antimicrobial use compared to those dispensed in hospitals in Canada. Surveillance of population-based antibiotic prescribing in the community can aid in interpreting patterns and trends of antibiotic resistance.

The objective of this study is to determine the pattern of overall, antimicrobial class and agent-specific antibiotic prescription over time at the province, zone, and prescriber group level for calendar years 2010-2017. The specific research questions are:

1. What is the prescribing rate of overall, class and agent-specific antibiotic prescription grouped by indication and stratified by patient demographics, seasonality, prescriber group, and material deprivation at the provincial and zone level in Alberta?
2. Is there any change in those prescribing rates?
3. Within and across the prescriber groups, which group has significantly higher or lower overall and class-specific antibiotic prescribing rates than the group, zone, and provincial average by age, sex, and season?
4. Are there specific local geographic areas in Alberta that have significantly higher or lower antibiotic prescribing rates (overall and class-specific) than the provincial average over time?

MATERIALS AND METHODS:

The study will use data from Pharmaceutical Information Network and Practitioner Claims databases. Retrospective analysis of community-level antibiotic prescribing from Jan 2010 to Dec 2017 will be conducted. The estimates will be aggregated at the local geographic area, zone and province level. Overall, class and agent-specific antibiotic use (grouped by indication) will be quantified in terms of defined-daily dose (DDD)/1000 persons/year. The distributional assumptions of variables will be assessed, and appropriate measures of central tendency will be identified. Means, medians, percentiles and standard deviations will be calculated for continuous variables whereas frequencies and proportions will be calculated for categorical variables. In all instances, P-value<0.05 will be considered as statistically significant.

RESULTS:

The data for the study are currently in the process of being cleaned and analyzed. Preliminary results from Jan 2010 to Dec 2010 will be presented. Overall, 1,325,004 antibiotic prescriptions were dispensed for oral and parenteral systemic use in 2010. Beta-lactams including penicillins were most frequently prescribed (44%) followed by macrolides, lincosamides and streptogramins (26%), and quinolones (16%) (Table 1). Additional results will be available by March 2021.

Table 1. Proportion of antibiotic-class specific prescriptions in Alberta, 2010

Antibiotic class	**Number of prescriptions (%)**
Penicillin beta-lactam antibacterials	388,771 (29.3)
Macrolides, lincosamides and streptogramins	337,323 (25.5)
Quinolones	210,710 (15.9)
Other beta-lactam antibacterials*	200,237 (15.1)
Sulfonamides and trimethoprim	69,632 (5.3)
Tetracyclines	66,741 (5.0)
Other antibiotics**	50,917 (3.8)
Aminoglycosides	672 (0.1)
Amphenicols	1 (<0.01)
Total	**1,325,004**

*Beta-lactam antibacterials, other than penicillins, e.g., cephalosporins, monobactams, carbapenems

**Antibacterials with various modes of action not classified elsewhere, e.g., glycopeptide, polymyxins, steroid, imidazole and nitrofuran derivatives

CONCLUSION:

Beta-lactams were most commonly prescribed in the community setting in 2010.

The results of this study will provide baseline data to inform antimicrobial stewardship efforts in in Alberta.

Abstract 4: Diksha Gourkhede

Antibiofilm efficacy of **Cecropin A (1-7)-melittin and Lactoferricin (17-30) against multi-drug resistant field isolates of *Salmonella* Typhimurium and *Salmonella* Enteritidis.**

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INTRODUCTION:
Non-typhoidal Salmonellae (NTS) constitute an important class of foodborne pathogens forming a biofilm, which contributes to their virulence and antimicrobial resistance. Multi-drug resistance among NTS serotypes, especially in *S*. Typhimurium and *S*. Enteritidis, compounded with their biofilm-forming ability, has emerged as a nagging public health threat. Antimicrobial peptides (AMP) comprise novel anti-biofilm therapeutic candidates owing to their potent bactericidal activity. In the present study, we evaluated the antibiofilm efficacy of two cationic AMPs namely, Cecropin A (1-7)- melittin (CAMA), and lactoferricin (17-30) against MDR- field strains of *S*. Typhimurium and *S*. Enteritidis.

MATERIALS AND METHODS:
Initially, biofilm-forming ability of the characterized MDR- *S*. Typhimurium (n=3) and *S*. Enteritidis (n=3) isolates were assessed by 96- well microtiter plate assay at different time intervals (24 and 48 h). Further, employing the same biofilm assay, *in vitro* anti-biofilm activity of the peptides at their minimum inhibitory concentration were individually explored against biofilm-forming MDR- strains of *S*. Typhimurium and *S*. Enteritidis at 24 and 48 h post-treatment (p.t.).

RESULTS:
All three strains of MDR- *S*. Typhimurium and *S*. Enteritidis were found to be moderate biofilm producers at 24 h, however, the same strains revealed higher biofilm producers at 48 h of incubation as compared to their respective controls. At both time intervals CAMA revealed a highly significant ($p< 0.001$) anti-biofilm activity, whereas, lactoferricin (17-30) treatment resulted in a significant ($p<0.05$) antibiofilm activity.

CONCLUSION:
The present study explores newer insights into the anti-biofilm efficacy of AMPs against MDR- strains, particularly that of *S*. Typhimurium and *S*. Enteritidis. Further, to explore its utility from a public health perspective, in-depth anti-biofilm efficacy studies of AMPs against NTS strains are warranted in appropriate *in vivo* models.

Abstract 5: Kathleen Pick

The role(s) of temperate bacteriophage in the mammalian gastrointestinal tract.

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INTRODUCTION:

The mammalian gastrointestinal tract is home to a rich and diverse community of microorganisms, termed the microbiome. The human microbiome has been the spotlight of recent research and has been shown to be a vital part of human health; however, the viral aspect of our microbiome remains largely unexplored. Bacteriophage (or simply phage) are viruses that infect bacteria, and we are only beginning to understand the abundance, diversity, and role(s) of phages in the human microbiome. Most enteric bacteria carry temperate phages; these phages integrate into the chromosome of their host where they lie dormant. When the host cell encounters stress, the phage excises from the chromosome, begins replicating, and lyses and kills the host cell to release progeny phage. Bacteria that carry these dormant phage are called lysogens and have been shown to have different physiological properties than their non-lysogenic equivalents, including more robust biofilm formation, enhanced antimicrobial resistance, and altered lipopolysaccharide (LPS) structures. The simple fact that most enteric bacteria carry these dormant phage suggests a role for temperate phage in the host environment. We aim to explore the interactions between temperate phage and their bacterial hosts, to understand what role(s) temperate phage play in the mammalian gastrointestinal tract. Increasing our fundamental understanding of the biology of bacteria in the gastrointestinal tract may lead to the development of novel antimicrobial therapies against gastrointestinal pathogens or improve the efficacy of existing treatments and probiotics.

METHODS:

To study phage-host interactions, we are utilizing a recently isolated commensal strain of *Escherichia coli*, MP1, that harbors a novel temperate phage, Kapi1. This “wild” strain differs drastically from our domesticated *E. coli* lab strains, and we hope that because it is more adapted to the host environment, we will be able to uncover novel aspects of *E. coli* biology that are relevant to its life in the gastrointestinal tract. Using a variety of genetic and microbiological techniques, we will study the differences between MP1 carrying or lacking Kapi1 in physiologically relevant conditions, and *in vivo* using mouse models.

RESULTS:

Preliminary data shows that incubation in simulated intestinal fluid selects for maintenance of Kapi1 in its lysogenic (dormant) form, compared to incubation in standard lab media. This suggests that carriage of Kapi1 is beneficial for the host cell in the intestinal environment and may be dispensable under lab conditions. We have also shown that MP1 lysogenized with Kapi1 has an altered O-antigen structure compared to its non-lysogenic counterpart. O-antigen is a component of the LPS which has a direct interaction with the host immune system and has been implicated in a variety of phenotypes including increased resistance to serum complement and antimicrobial peptides.

CONCLUSION:

Future work will aim to identify which traits Kapi1 confers to its host that are beneficial in the intestine and to uncover the molecular mechanisms behind these traits. This work will increase our understanding of the fundamental biology of *E. coli* in the gastrointestinal tract and thus may contribute to the development of future antimicrobial strategies.

Abstract 6: Lindsay Rogers

Antimicrobial resistance in Enterococci isolated from cattle, poultry, and retail meat in Alberta, Canada

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INTRODUCTION:
Antimicrobial resistance (AMR) is a significant threat to global public health where antimicrobial use in humans and animals is an important risk factor. Antimicrobial use in food producing animals can lead to the transmission of AMR from bacteria and/or their genetic elements to humans through a variety of pathways including meat products. The genetic relationships between isolates from different sources can be studied using bioinformatics to infer if there is transmission of AMR genes through the food chain. Bioinformatics is a means of analyzing bacterial genomes and characterizing resistant bacteria isolated from food producing animals. The objective of this study is to describe and analyze the molecular epidemiology and comparative genomics in *Enterococcus faecium* and *Enterococcus faecalis* isolated from feedlot cattle, broiler chickens, and retail poultry and beef products with respect to their chromosomal genes and mobile genetic elements.

METHODS:
A bank of *E. faecium* (n=107) and *E. faecalis* (n=132) isolates was developed during AMR surveillance and research activities on Alberta poultry, cattle, and retail meat. These were speciated using high throughput pyrosequencing. Antimicrobial susceptibility testing was completed following CLSI guidelines. Whole genome sequencing of isolated DNA was completed using Illumina MiSeq technology with bioinformatic analysis of the resulting sequences. The isolates' genetic relatedness and AMR genes were compared through the creation of phylogenetic trees. AMR genotypes were compared to their phenotypes.

RESULTS:
In multivariable analysis, the odds of multiclass resistance (phenotypic resistance to three or more antimicrobial classes) did not differ ($p<0.05$) between feedlot and poultry fecal samples, however *E. faecium* was 3.09 (95% CI: 1.5, 6.5) times more likely to be multiclass resistant than *E. faecalis*. Resistance to antimicrobials commonly used in food animal production (i.e., tetracyclines, macrolides) was prevalent in fecal samples. The AMR genes found generally corresponded to the AMR phenotype of the isolate, although a sensitivity and specificity analysis of the use of genotype to predict phenotype had inconsistent results with wide confidence intervals. Phylogenetic analysis of *E. faecalis* identified no distinct clustering by sample source, whereas *E. faecium* clustered into two distinct clades (cattle feces and poultry feces). Virulence genes identified were associated with enterococcal survival in a broad range of environments. The *esp* virulence gene typical of pathogenic enterococci was not found in these isolates.

CONCLUSION:

Multiclass resistant bacteria are present in both food producing animals and their meat products. The majority of resistance in enterococci isolated from food producing animals is consistent with the antimicrobials used in those species. Multiclass resistant bacteria in retail meats may be influenced by bacteria from the food producing animals or during processing. Genomic analysis allows for assessment of the relatedness of these bacteria between animals and their meat products intended for human consumption. These results will help better understand the transmission of antimicrobial resistant *Enterococcus* spp. and their genes via retail meat products. This information will be used to inform mitigation strategies and policies to decrease antimicrobial resistant bacteria in animal production and the environment.

Abstract 8: Dana Tschritter

A risk assessment of ciprofloxacin-resistant *Campylobacter* infection from Canadian broiler meat

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Presenting and corresponding author: Dana Tschritter, Masters student

INTRODUCTION:

Health Canada and the World Health Organization have recognized fluoroquinolones as one of the most critically important families of antimicrobials, indicating them as essential for human medicine and a priority for antimicrobial resistance (AMR) research. Despite restrictions on the use of fluoroquinolones in broiler chicken production in Canada, the prevalence of fluoroquinolone-resistant *Campylobacter* found within retail chicken continues to increase. Campylobacteriosis is the third-most prevalent foodborne illness in Canada and infections with resistant strains of *Campylobacter* may increase the risk of serious health conditions and death. Additional investigation is required into the spread of FLQR *Campylobacter* through broiler chicken production. Quantitative microbial risk assessment (QMRA) is an advantageous tool for understanding the transmission of pathogens through selected exposure routes, estimating the likelihood of consequent infection, and identifying mitigation strategies that may be most effective. Here, we conduct a QMRA of ciprofloxacin-resistant (CIPR) *Campylobacter* in broiler chicken using a farm-to-fork framework to evaluate the likelihood of resistant campylobacteriosis in Canadians from broiler chickens and explore opportunities of reducing this risk.

METHODS:

A QMRA of *Campylobacter* in broiler meat published by the FAO/WHO in 2009 was used as a framework and updated with surveillance data to reflect the behaviour of CIPR *Campylobacter* in Canada. A stochastic Monte Carlo simulation model was used to track the prevalence and concentration of CIPR *Campylobacter* along the farm-to-fork pathway, culminating in a probability distribution estimating the quantity ingested from one serving of cooked Canadian broiler meat. Using previously published data and newly proposed methods that employ stochastic simple death processes, a novel dose-response model is under evaluation for CIPR *Campylobacter*. These new models allow for the estimation of a probability of infection due to the magnitude of exposure, similar to a traditional dose-response model, as well as the probability that any resulting illness will be resistant to treatment with ciprofloxacin. Lastly, a sensitivity analysis is being utilized to detect key model inputs for which data gaps exist and, lastly, various intervention scenarios will be explored to identify effective risk management options.

ANTICIPATED RESULTS:

This study will provide a comprehensive description of the probability of exposure to CIPR *Campylobacter* by estimating its occurrence throughout the broiler chicken supply chain. Additionally, a novel dose-response model for CIPR *Campylobacter* will be developed using recently published mathematical adjustments. Influential data gaps in surveillance and research will be identified, and our understanding of the characteristics and transmission dynamics of resistant *Campylobacter* via retail chicken exposure will be improved.

CONCLUSION:

This work will contribute significantly to the mathematical modeling of antimicrobial-resistant bacterial species throughout the farm-to-fork food chain and the relationship between AMR exposure and treatment-resistant illness. Additionally, this work will identify risk mitigation strategies to limit the spread of a specific antimicrobial-resistant bacteria through broiler chicken products in Canada.

Abstract 9: Maria Anto Dani Nishanth

Antibacterial efficacy of Cecropin A (1-7)-Melittin in-house designed cell-penetrating peptide against multi-drug resistant *Salmonella* Enteritidis and *S.* Typhimurium strains

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INTRODUCTION:

Non-typhoidal *Salmonellae* (NTS) has emerged as one of the leading causes of food-borne illnesses around the world. The emergence of antimicrobial resistance among NTS serotypes has made the treatment difficult, which could further increase mortality rates and unaffordable medical costs. The cell-penetrating peptides (CPPs) would be an ideal therapeutic approach to combat the intracellular drug-resistant pathogens, as they have an inherent ability to enter inside the cell without damaging the infected cell. The present study was undertaken to explore the *in vitro* antibacterial efficacy of in-house designed CPP from a cationic antimicrobial peptide i.e. Cecropin A (1-7)-Melittin (CAMA) against MDR field strains of *S.* Typhimurium and *S.* Enteritidis.

MATERIAL AND METHODS:

The cell-penetrating peptide (CPP) modification of a cationic antimicrobial peptide CAMA was performed using online CellPPD software. Further, its antibacterial efficacy against the characterized field isolates of MDR- *S.* Enteritidis (n=3) and MDR- *S.* Typhimurium (n=3) was investigated by determining its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by using micro broth dilution method. The modified CAMA- CPP was then evaluated for its stability at high- end temperatures (70 ºC and 90 ºC), proteases (Trypsin and Proteinase-K), cationic salts (150 mM NaCl and 2 mM $MgCl_2$), pH (2.0 to 8.0), and sheep serum. Additionally, the safety of modified CPP was evaluated against erythrocytes (sheep and human), its cytotoxicity against HEp-2 and RAW 264.7 cells, and its adverse effect against beneficial gut lactobacilli (*L. acidophilus, L. plantarum,* and *L. rhamnosus*). Finally, to evaluate the intracellular antibacterial activity of modified CPP, *in vitro* time-kill assay of CAMA- CPP against the selected MDR- strains of NTS was carried out in HEp-2 and RAW 264.7 cell lines.

RESULTS:

The MIC and MBC values of the in-house designed CPP (CAMA-CPP) against the characterized MDR-NTS strains were found to be 3.906 µM and 7.8725 µM, respectively. Besides, CAMA- CPP was found to be stable on exposure to different treatments *viz*., high-end temperatures, proteinase-K, cationic salts, pH (4.0 to 8.0), and serum; however, a two-fold increase in the MIC and MBC levels was noticed after trypsin treatment. CAMA- CPP exhibited a non-haemolytic pattern in human erythrocytes; however, moderate hemolysis (30.50%) was observed at higher concentrations (10X) in the sheep erythrocytes. CAMA- CPP exhibited minimal cytotoxicity at lower peptide concentrations (1X and 2X MIC) in both the secondary cell lines tested. Irrespective of the CPP- treatment, a similar growth pattern was observed by the commensal gut lactobacilli. Finally, *in vitro* time-kill kinetic assay revealed complete clearance of MDR-NTS strains was observed with CAMA- CPP treated HEp-2 and RAW 264.7 cells at 6 h post-treatment (p.t.) and 12 h p.t., respectively.

CONCLUSION:

The promising results of CAMA-CPP to eliminate the MDR- NTS strains tested provides ample scope for further translation study in various *in vivo* laboratory (invertebrate and/or mammalian) models. Additionally, encapsulation of peptides could be explored for its targeted delivery to overcome its degradation by protease enzymes

Abstract 10: Angela Ma

Development of a public-facing and interactive cumulative antibiogram for the province of Alberta

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INTRODUCTION:
Increasing rates of antimicrobial resistance is of significant global concern. Antibiograms that report antimicrobial susceptibility patterns of clinically important microorganisms are used for guiding decisions for empiric antimicrobial therapy and antimicrobial stewardship programs. Across the province of Alberta, local antibiograms are published per calendar year; however, an antibiogram representing the antimicrobial susceptibility rates across the entire province does not exist. The objective of this project is to create and publish a public-facing, cumulative provincial antibiogram. This antibiogram will be used to analyze trends in antimicrobial susceptibility rates and to target research to the most concerning areas of antimicrobial resistance in Alberta.

MATERIALS AND METHODS:
Antimicrobial susceptibility test results from clinically relevant bacterial pathogens in Alberta from 2020 were retrieved from 3 laboratory information systems and merged into a single database for analysis. Only the first isolate of a species per patient during the analysis period was included. Other exclusion parameters included species with susceptibility data for less than 30 isolates and antimicrobial agents with testing data for less than 70% of the highest number of isolates tested per species. Percent susceptibilities were calculated for all clinically relevant antimicrobial agents routinely reported for each species and uploaded onto Tableau to serve as the dashboard for an interactive provincial antibiogram.

RESULTS:
Twenty four Gram positive species and 29 Gram negative species were included in the Tableau antibiogram dashboard (Figure 1A and 1B). Percent susceptibilities are presented and the degree of shading represents increasing percent susceptibility. The online version of the dashboard is interactive and provides numbers of isolates tested for each microorganism/antibiotic combination. As the cumulative provincial antibiogram is updated with antimicrobial susceptibility test results going forward, this data will be reflected on the dashboard. Once 2021 data is available, trend analysis charts will be included on the dashboard. Future incorporation of patient demographics such as gender, age, and infection source into the antibiogram will also provide a more comprehensive overview of the province's antimicrobial susceptibility trends.

CONCLUSIONS:
A cumulative provincial antibiogram will be highly beneficial for identifying areas of antimicrobial resistance research worth investigating. The antibiogram provides extensive data on antimicrobial susceptibility trends that may not be captured in local antibiograms and testing results for rare microorganisms. If similar endeavours by other provinces to publish cumulative provincial antibiograms across the country are made, a national antimicrobial resistance surveillance network can be developed, which will undoubtedly be a valuable resource.

Abstract 11: Claudia Cobo-Angel #1

Producer-reported reasons for antimicrobial usage on Canadian dairy farms

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INTRODUCTION:

Antimicrobial resistance is an important threat to human and animal health. Antimicrobial use (AMU) in food-producing animals is a modifiable factor driving its occurrence. Consequently, authorities in many countries have recommended the implementation of antimicrobial stewardship programs to reduce AMU in agriculture, particularly antimicrobials of high importance to human. However, those programs may not be effective without understanding current AMU practices on farms. The objective of this analysis was to describe the most frequent reasons for AMU on Canadian dairy farms.

MATERIALS AND METHODS:

These data are from the Canadian National Dairy Study (https://www.nationaldairystudy.ca/). A questionnaire was administered in person on 371 dairy farms in 2015 in the 10 provinces of Canada, with the majority of respondents located in Ontario (36%) and Quebec (32%). There were 52 questions about herd management practices, but here we report descriptive statistics on questions about AMU.

RESULTS:

Although the majority of dairy farmers (93%) had regular visits to the herd by veterinarians, 65% of farmers regularly made decisions about animal health and antimicrobial treatments without veterinarian consultation. Eight farms (2%) were organic, therefore antimicrobials were not used or used very rarely. Almost one third of the farmers (28%) who used antimicrobials indicated not having a written protocol for AMU. On farms with protocols, these were mainly for mastitis (93%) and drying off (91%). Most farmers ranked mastitis as the most common reason for AMU in lactating dairy cows (72%), followed by uterine infections (10%), lameness (8%), respiratory infections (5%), post-surgical wound management (2%) and digestive tract infections (1%). In pre-weaned calves, digestive tract infections were the leading reason for AMU (52%), followed by respiratory infections (35%), navel ill (3%), lameness (2%) and post-surgical wound management (1%). Antimicrobials were not used, or used very rarely, in pre-weaned calves by 4% of respondents. In weaned calves, 5% of producers indicated cero or very rare use of antimicrobials. Of those who used antimicrobials in weaned calves, respiratory infections were the most frequent reason (65%), followed by lameness (10%) and digestive tract infections (10%).

CONCLUSION:

Most of the farms enrolled were frequent users of antimicrobial treatments. There is an opportunity to improve communication with veterinarians at the moment of deciding the use of antimicrobial treatments on dairy farms. Reasons for AMU differ between age cohorts of animals on dairy farms, indicating that specific preventive measures for these diseases could decrease AMU. There is a need to gather more detailed information about AMU and farmers' motivations and understanding of antimicrobial resistance to improve and potentially reduce AMU on dairy farms

Abstract 12: Claudia Cobo-Angel #2

Qualitative study of Canadian dairy farmers' knowledge and perception of antimicrobial resistance

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INTRODUCTION:
The increasing prevalence of antimicrobial resistance (AMR) in pathogens is a global health challenge for human and veterinary medicine. Antimicrobial use (AMU) in agriculture is a contributing and modifiable factor for the AMR emergence. It is important to understand farmers' knowledge and perceptions of AMR, and their perceived role in the development of resistant pathogens when aiming to promote prudent use of antimicrobials on farms. Our objective was to explore knowledge and perception of AMR among Canadian dairy farmers.

MATERIALS AND METHODS:
We conducted seven focus groups with 42 farmers, in two provinces of Canada: four in Ontario, and three in New Brunswick. A semi-structured interview guide was developed to elicit information about farmers' perceptions of AMR and their decision-making process for AMU. We used thematic analysis to identify, analyze, and report patterns within the transcribed focus group discussions. We developed and refined a codebook using an iterative process through of discussions with all the authors. Afterwards, all transcriptions were coded by the same researcher (CC-A) using NVivo 12 software.

RESULTS:
The majority of the farmers were familiar with the concept of AMR and could explain it to the researchers. Farmers believed that currently AMR is not a big problem in dairy production, although it was commonly mentioned that mastitis was a difficult disease to treat. Most considered themselves to be low users of antimicrobials, yet participants frequently stated that the main cause of AMR on dairy farms was overuse of antimicrobials. Farmers mentioned other causes of AMR such as incomplete treatment and the use of the same antimicrobial for long periods of time. Whereas participants were less concerned about feeding calves waste milk containing antimicrobial residues from cow's treatments, they considered that ingestion of antimicrobials through medicated feed was an important cause of AMR.

Participants discussed that agriculture was scrutinized more closely than human medicine regarding AMR and commented that AMR was more problematic for human health than for animal health. Many expressed doubt that they could reduce AMU without affecting animal health and welfare. However, some participants expressed concern about the impact of farming on AMR in humans and affirmed that they try to avoid certain antimicrobials due to their importance for human health.

CONCLUSION:
The majority of participants in this study were familiar with the concept of AMR and some of its causes. However, AMR in dairy cattle was not seen to be a current problem. Participants were aware of the impacts of AMR on human health, but most were skeptical about the magnitude of the role of AMU in agriculture and specifically of dairy farming on AMR in human pathogens.

Abstract 13: Youba Ndiaye

Evaluating economic performance and antimicrobial consumption in French broiler production: improved healthcare management as a win-win strategy.

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INTRODUCTION:

Supervising antimicrobial consumption is of paramount importance in both animal and human health sectors because of the public health and economic burden due to antimicrobial resistance for various reasons. Yet, antimicrobials are used in farms as they enable to curb economic damages generated by infectious diseases, and they have proved beneficial for farmers. To better orient towards sustainable antibiotic prescribing practices, it is important to assess the impact of the consumption of antimicrobials on the profitability of farmers. The main objective of this paper is to evaluate the relationships between the economic performance of farms, and the management of animal health.

MATERIALS AND METHODS:

We collected an original dataset covering 1,086 lots of broilers raised and harvested in France between 2017 and 2019. The dataset consists of technical and economic information, as well as drug consumption at the flock level. We perform different estimation strategies in order to (i) analyze the determinants of economic performance of farms, (ii) quantify the impact of veterinary practices on farmer's income.

RESULTS:

Estimation results show that the profitability of farms is highly correlated with the intrinsic characteristics of the farms, such as the density of the broilers, the average daily gain, and the feed consumption index. This suggests that the possibilities of increasing the income of farmers are very limited. However, when looking at the consumption of antimicrobials and vaccines, we observe that (i) the farmers not using antimicrobials have fairly similar gross revenue than the farmers using antimicrobials at low levels; and (ii) the more farmers use antimicrobials, the more their marginal revenue decrease. Specifically, the point estimates for antibiotic uses variables exhibit both positive and negative signs, indicating a non-monotonic relationship between antibiotic consumption and economic performance. The increasing phase may be related to an insurance effect provided by antimicrobials. Indeed, antimicrobials may buffer potential damages due to fair management practices. Afterwards, the economic performance of farms decreases due to the various reasons e.g., severity of diseases, negative side effects of antimicrobials on digestive commensal.

Additionally, we also find that the use of high priority critically important antimicrobials, as defined by the World Health Organization, have negative and significant effects on the profitability of farms. Finally, we show that farmers having a higher consumption of medical prevention, such as vaccine, also use less antimicrobials.

CONCLUSION:

Overall, our results highlight the importance of a judicious veterinary management for improving the profitability of farms. Globally, this study suggests that encouraging farmers to better prevent diseases, by using medical prevention and veterinary counseling, is a win-win strategy as this enables curbing antimicrobial use while improving revenue. Hence, moving towards rationed and reasoned uses of antibiotics is crucial both for animal welfare and farmer's portfolio.

Abstract 14: Daniel Major

Identification, production, purification, and characterization of putative leaderless bacteriocins

Leah Lussier[1], Daniel Major[1], Jeella Acedo[2]

Mount Royal University

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Bacteriocins are a highly heterogeneous group of antimicrobial genome-encoded peptides produced by a wide array of bacteria. These peptides have commercial applications in food preservation and livestock industries; and also have enormous potential as next-generation antibiotics. Routine use of antibiotics has contributed to widespread antimicrobial resistance, hence the need for further research on the discovery, application, and mechanisms of bacteriocins. The focus of this project is on leaderless bacteriocins, a class of bacteriocins which are understudied and not well understood. They are defined by the absence of an N-terminal leader peptide and the typical post-translational modifications which are required for bacteriocin activation. In other classes of bacteriocins, the leader peptide is integral for processing, transport and regulation of transcription of the peptide, however, the ability of leaderless bacteriocins to function in its absence calls for further research. Through genome mining, we identified and clustered 277 putative leaderless bacteriocins based on homology using the precursor peptide sequences of experimentally validated peptides. We selected two of these peptides, which we named VigA and MitA, and used a heterologous *Escherichia coli* BL21 expression system to produce His_6-SUMO tagged peptides. VigA was chosen because it shares sequence homology among the largest sequence similarity cluster where no experimentally validated peptides were included (Fig 1). MitA is of interest because it demonstrates no significant sequence homology with any other putative or experimentally validated bacteriocin, which indicates novelty (Fig 1). We successfully performed crude purification of these two peptides using affinity chromatography and verified through SDS-PAGE. Our next steps will be to further purify these peptides using high performance liquid chromatography prior to characterization using MALDI-TOF mass spectrometry and bioactivity assays against a consortium of selected bacteria. We anticipate being able to isolate the peptides and generate data on their minimum inhibitory concentrations. We are aiming to prove this approach is effective in identifying, producing, and testing putative bacteriocins. The identification and characterization of alternative antibiotics are vital. These peptides may be essential interventions in the rising incidence of antimicrobial resistance worldwide.

Abstract 15: Kayley McCubbin

Knowledge gaps in the understanding of antimicrobial resistance in Canada

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INTRODUCTION:

With worldwide rise in antimicrobial use (AMU), antimicrobial resistance (AMR) has become increasingly pervasive and may vastly reduce antimicrobial efficacy in the coming years. Countering both reduced efficacy and other projected impacts on global health and wellbeing requires a true 'One Health' approach. This paper identifies priorities for AMR research and policy across human, animal, and environmental sectors. These priorities describe the change necessary for effective AMR countermeasures within a Canadian context.

MATERIALS AND METHODS:

In a comprehensive narrative literature review, limitations in current understandings of AMR in Canada are summarized.

RESUTS:

Three priority areas for AMR research and policy action in Canada were identified for review: 1) treatment optimization, 2) AMU/AMR surveillance, and 3) prevention of AMR transmission. Within these areas, we identified specific knowledge gaps as described in the following table:

Table 1. Most important knowledge gaps hampering AMR prevention and control in Canada.

Area	Knowledge gap
AMR Development	- Relationship between AMU and AMR - Impact of heavy metals, cleaning agents, biocides and other xenobiotic compounds on AMR - How AMU in one health sector directly impacts AMR in another sector (i.e. human AMU and animal AMR, and the reverse) - Relative importance of AMU routes in AMR - How to employ policy to effective limit AMR development
Treatment Optimization	- Extent of antimicrobial misuse in Canada - Best management practices (BMPs) regarding antimicrobial prescribing in human/animal medicine - Economics of various efforts in lieu of AMU - Socio-economic/behavioural drivers of AMU (prescriber and patient perspectives) - Efficacy of widespread adoption of alternative therapies to AMU - Understand and shift perspectives that identify AMU is a harmless 'cure all' therapy - Identifying barriers and enablers of optimal human/animal AMU
Surveillance	- Up-to-date AMR prevalence estimates in the community, domestic animals, wildlife, production animals, not currently included in CIPARS, and the environment - AMR bacteria trends and emergence in Canada - BMPs to integrate AMU/AMR data collection and reporting
Prevention of AMR Transmission	- Long-term efficacy of AMR mitigation efforts - BMPs to prevent hospital-acquired AMR infectons - BMPs to reduce wastewater AMR and subsequent impacts on human/animal health - How to reduce AMR prevalence in various resistance reservoirs - How to limit the AMR risk in food systems - How to prevent cross-species AMR transmission - Quantitative risks in various AMR transmission pathways
Role of Environment	- Impact of human AMU/AMR on the environment - Impact of AMU/AMR in livestock on the environment - Relative importance of various environmental transmission routes (ground water and livestock derived manure spreading, etc.) - Impact of antimicrobial residues in soil, water and on pasture - Benefits/costs of reducing environmental AMR reservoirs and antimicrobial residues
Role of wildlife	- Impact of AMR on wildlife health - Wildlife role in AMR transmission - Benefits/costs of reducing AMR transmission from wildlife to livestock or humans

IMPORTANT CONCLUSIONS:

The knowledge gaps identified by this review underscore the necessity of using a One Health approach to identify and understand priorities in Canada's response to AMR.

Abstract 16: Alyssa Butters

Comparative genomics and molecular epidemiology of antimicrobial resistance in *Escherichia coli* isolated from poultry, beef cattle, retail meat, and the environment

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Presenting Author: Alyssa Butters, Corresponding Author: Sylvia Checkley

INTRODUCTION:

Antimicrobial use in food animals is hypothesized to yield a reservoir of antimicrobial-resistant bacteria that represents a human health risk. However, there is a need for a better understanding of the routes of transmission of antimicrobial-resistant bacteria and antimicrobial resistance genes (ARG) between food animals and humans. The role environmental bacteria could play in this transfer is not well understood, and antimicrobial resistance (AMR) mitigation strategies addressing environmental transmission are lacking.

Escherichia coli, an enteric bacterium shed in the feces of common food animal species, is a well-established human pathogen. Originally thought incapable of replication outside of animal hosts, it is now recognized that *E. coli* can survive and reproduce in environmental habitats. It is proposed these "naturalized" *E. coli* can act as reservoirs for AMR genes acquired from fecal bacteria contaminating water or soil. However, environmental strains of *Escherichia* are perhaps distantly related to enteric strains, and ecological barriers may impede the transfer of genes between enteric and naturalized *E.coli*. It is unclear if this affects the ability of naturalized bacteria to acquire and maintain AMR genes originating from enteric strains.

E.coli can carry mobile genetic elements (MGEs) identified as significant contributors to the evolution of AMR. In addition to direct contact with animals, exposures to *E. coli* through consumption or handling of retail meats or ingestion of contaminated water have received attention as potential routes of antimicrobial resistance transmission to humans. Yet, AMR surveillance in *E. coli* has primarily relied on phenotypic AMR profiles, creating difficulty detecting if AMR genes detected in retail meats or water isolates arise from food animals.

This study will employ whole-genome sequencing to investigate the relatedness of *E. coli* from food animal production, retail meats, and environmental sources. The genetic basis of AMR carried by the isolates will be determined and compared between isolates from the different sources.

MATERIAL AND METHODS:

E. coli isolates have been collected in Alberta from poultry barns, feedlots, geographically relevant well water and wastewater, and locally sourced retail beef and poultry meats. Whole genome sequencing will employ short-read and long-read methods, and the sequencing data combined using bioinformatic techniques.

ANTICIPATED RESULTS:

The study will produce high quality assemblies of *E. coli* genomes including resolution of MGEs. Phylogenetic trees will be created, and associations in the pattern and frequency of resistance determinants will be assessed using regression analysis with adjustment for clustering. Epidemiological analysis of resistance determinant associations among the isolates will identify associated factors.

ANTICIPATED IMPORTANT CONCLUSIONS:

The assembly of MGEs, not feasible in many AMR surveillance studies, will significantly contribute to the existing body of knowledge. Phylogenetic trees will allow a comprehensive comparison of isolates' genetic relatedness from different sources, and factors identified will be used to shape AMR transmission reduction strategies.

Combatting AMR requires considering the roles animal production practices and the environment play in the global context of transmission. This project will provide insight into the interplay of these components in a unique and robust way to inform vital mitigation strategies.

Abstract 17: Olufunto Adewusi

Title: Application of genomic technology for food animal diagnostics to inform antimicrobial stewardship: a scoping review.

Olufunto O. Adewusi[1] (presenting author), Cheryl L. Waldner[2], Janet E. Hill[3], Barbara J. Wilhelm[4], Simon J. G. Otto[1,5]

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INTRODUCTION:

Veterinarians are an important part of antimicrobial stewardship in food animal production. They work to preserve the effectiveness of antimicrobial medications through diligent and accountable medical decision-making while preventing health and other associated economic losses to the animal, public, and environment. In feedlot settings, Bovine Respiratory Disease (BRD) complex is the most common cause of substantial financial losses and parenteral antimicrobial use in Western Canada. Current diagnostic tests for antimicrobial susceptibility take 5 – 7 days to provide results to veterinarians, forcing them to make decisions based on clinical judgement without diagnostic information.

Genomic ASSETS (Antimicrobial Stewardship Systems from Evidence-based Treatment Strategies) for Livestock is a revolutionary application of rapid genomic technology to inform antimicrobial use (AMU) in livestock. Genomic ASSETS aims to develop a timely, metagenomics-based diagnostic system to provide BRD results to feedlot veterinarians to inform antimicrobial decision-making and support antimicrobial stewardship.

The objective of this scoping review is to identify and summarize published literature for the direct application of metagenomic tools to identify known respiratory pathogens and antimicrobial resistance (AMR) genes from samples in livestock.

METHODS:

This review will follow procedures endorsed by the Joanna Briggs Institute and PRISMA Scoping Review reporting guidelines. The search strategy was developed in consultation with a librarian. Inclusion criteria comprise studies of metagenomics applied to respiratory disease diagnosis, without restriction on year, the language of publication or study location. MEDLINE®, AGRICOLA™, *BIOSIS Previews*®, CABI and EMBASE® will be queried and de-duplicated citations uploaded to DistillerSR™ for screening (Evidence Partners, Ottawa, ON). First stage screening of titles and abstracts will appraise relevance for inclusion. Second level screening on the full text will confirm inclusion and begin categorization of studies (e.g., based on genomic methods used) for data extraction. The inclusion criteria will be the application of metagenomics tools in respiratory infections. The exclusion criteria will be other types of infections (not respiratory). The populations sampled will not be limited to animals. We will conduct a descriptive synthesis of specific indications populations sampled, techniques used, and current challenges identified, in tables, figures and narrative form.

PRELIMINARY DATA:

The development of the initial search strategy yielded the following results; MEDLINE® (2,167), AGRICOLA™ (162), *BIOSIS Previews*® (790), CABI (303) and EMBASE® (3,621). After de-duplication, the total number of unique citations is 4,465. Scoping review results will be presented.

CONCLUSION AND IMPLICATIONS:

This review intends to inform further development of genomic tools and methods for Genomic ASSETS, which will change the way veterinarians make AMU decisions for livestock by providing rapid, robust diagnostic information to end-users.

Abstract 18: Nele Caekebeke

Efficacy of coaching on Belgian and Dutch broiler farms aimed at optimizing management and reducing antimicrobial use

Nele Caekebeke[1,*], Moniek Ringenier[1], Franca J. Jonquiere[2], Tijs J. Tobias[2], Merel Postma[1], Angelique van den Hoogen[2], Manon A.M. Houben[3], Francisca C. Velkers[2], Nathalie Sleeckx[4], Arjan Stegeman[2] and Jeroen Dewulf[1]

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INTRODUCTION:

Reduction of antimicrobial use (AMU) is needed to curb the increase of antimicrobial resistance in broiler production. Improvements in biosecurity can contribute to a lower incidence of disease and thereby lower the need for AMU. However, veterinary advice related to AMU reduction and biosecurity is not often complied with, which has been linked to the attitudes of farmers. A behavior change promoted by coaching may facilitate uptake and compliance with veterinary advice.

MATERIALS AND METHODS:

A longitudinal study was performed on 30 conventional broiler farms with high AMU in Belgium and the Netherlands. Farms were followed-up for around 13 months. The design of the study is displayed in Figure 1.

For quantification of behavioral change, the ADKAR® model was used. ADKAR® is an acronym for the five building blocks necessary for successful change; Awareness, Desire, Knowledge, Ability, and Reinforcement. All five elements have to be addressed sufficiently in the right sequence to achieve change. The model was adapted for use in veterinary medicine, where the attitude of a farmer towards AMU was scored for each element. Each element was scored 1 to 5, with 5 being the best score.

Biosecurity level was scored using the Biocheck.UGent™ survey. Scores range from zero to 100, with zero corresponding to the absence of any biosecurity measure and a score of 100 corresponding to the ideal biosecurity situation.

Production parameters were collected from the farmer through the farm management programs and antimicrobial use was quantified using the treatment incidence per 100 days, which represents the number of days an animal was treated with antimicrobials per 100 days.

Associations between all parameters were investigated using a linear mixed model.

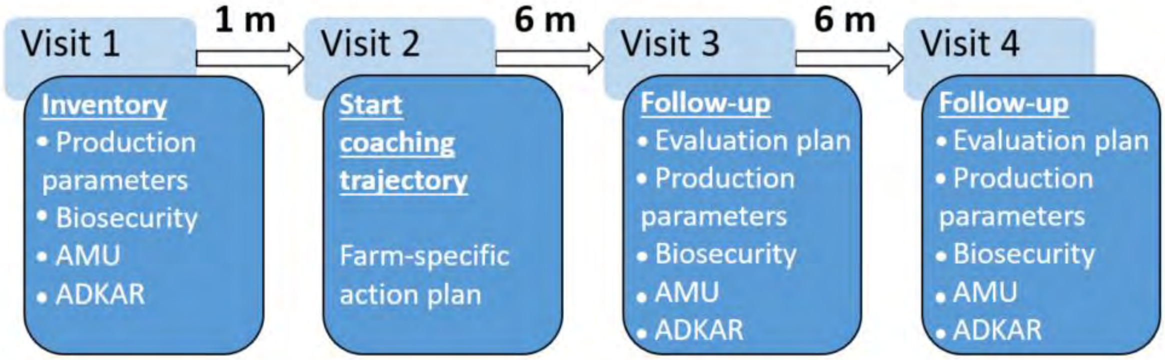

Figure 1 Study design

RESULTS:

In both countries, biosecurity levels increased, as well as ADKAR®-scores over time, meaning that a behavior change was more likely to occur. Antimicrobial use on average was lower in the Netherlands, however, a reduction was achieved in both countries. This reduction in AMU did not harm production parameters (mortality, feed conversion ratio). A negative trend between the individual ADKAR®-scores and AMU was found (not significant).

CONCLUSIONS:

After coaching of farmers, there was a change in attitude and behavior regarding AMU, reflected by an increase in ADKAR®-scores. This indicates that coaching can help in behavioral changes towards better antimicrobial stewardship. By coaching, biosecurity could be improved, while reducing AMU. We found a negative association between ADKAR and AMU, indicating the ADKAR® model can be a useful tool in achieving sustainable AMU reduction in veterinary medicine, however further research is needed. As ADKAR®-profiles were very different between Belgian and Dutch farmers, the approach to achieve behavioral change has to be adapted to the specific audience.

Abstract 19: Christine Neustaedter

Avoiding a superbug: A scoping review of risk factors for infection with antimicrobial-resistant *Campylobacter*

Christine M. Neustaedter[a,b], Richard J. Reid-Smith[b,c,d], Melissa C. MacKinnon[c,d], Carolee A. Carson[b,c], Colleen P. Murphy[b,c], Brennan Chapman[c,d], Simon J. G. Otto[a,b,e]

[a]HEAT-AMR Research Group, University of Alberta School of Public Health; [b]AMR-One Health Consortium, [c]Public Health Agency of Canada; [d]University of Guelph, Ontario Veterinary College, Department of Population Medicine, [e]Thematic Area Lead, Healthy Environments, Centre for Health Communities, School of Public Health, University of Alberta

INTRODUCTION:

Campylobacter is a leading cause of acute diarrheic illness around the world. To make matters worse it has developed resistance to antimicrobials important for human medicine. Infection with antimicrobial-resistant (AMR) *Campylobacter* is an important public health concern, as it may be linked with increased severity of illness and risk of death. Arising from this, there is a need to better understand potential risk factors for these infections. The objective of this study was to perform a scoping review of factors associated with human infection with AMR *Campylobacter*.

MATERIALS AND METHODS:

The scoping review followed the methods described in the Joanna Briggs Institute Reviewer's Manual and PRISMA Scoping Review reporting guidelines. Inclusion criteria were English-language publications reporting factors (e.g., food sources, prior antimicrobial use) that were associated with human infections with AMR-Campy (resistant to macrolides, tetracyclines, and/or quinolones). Databases searched were: ProQuest® AGRICOLA, CAB Abstracts® and Global Health®, Ovid EMBASE®, Scopus®, and Ovid MEDLINE®. Grey literature sources were: World Health Organization's Global Index Medicus, the first 250 relevance-sorted Google Scholar results, and Bielefield Academic Search Engine. Using Distiller SR®, two independent reviewers completed primary screening (title and abstract) and secondary (full text) screening based on inclusion criteria. Extracted data will include reported factors, their description and associated results, in addition to characteristics of the studies and study population and participants.

ANTICIPATED RESULTS:

The search returned 7910 articles after de-duplication. 37 articles made it through primary and secondary screening and will be data extracted. Results of the article screening and data extraction will be available for presentation in May. The anticipated results of this search will identify factors specific to an AMR-*Campylobacter* infection. Some tentative themes appearing in the articles to be extracted include: men who have sex with men, HIV status, travel, proximity to animals, and age.

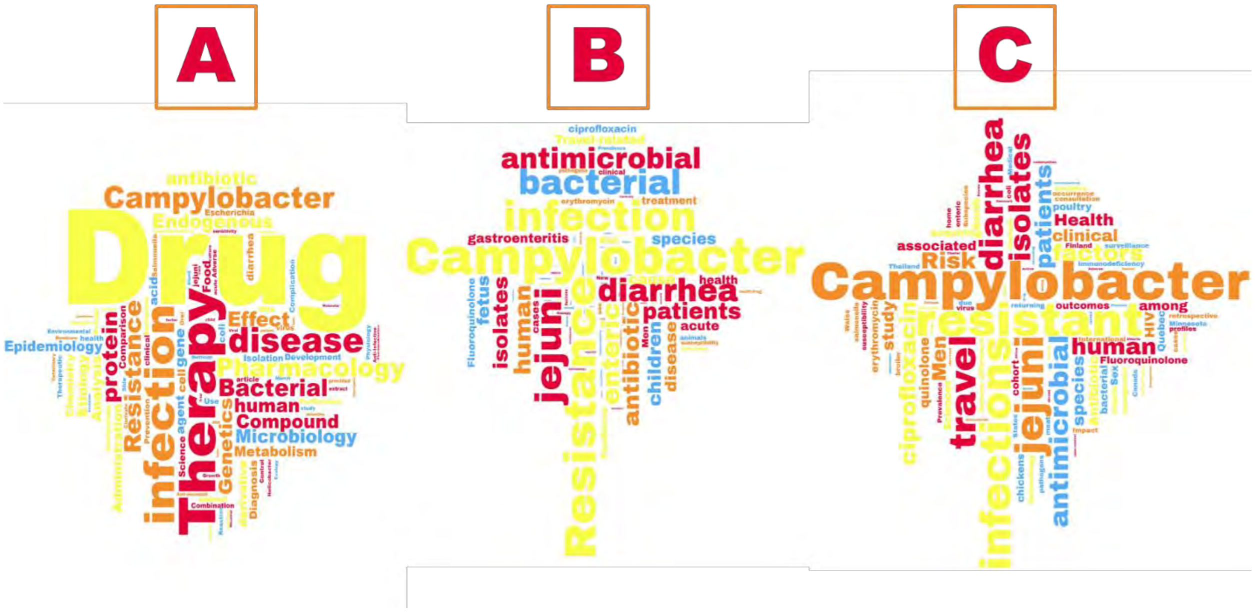

Figure 2. Word clouds comparing top 100 keywords, by frequency, from: A) all returned article titles and B) article titles that passed primary screening C) article titles that will be data extracted.

ANTICIPATED IMPORTANT CONCLUSIONS:

The impacts of this scoping review are two-fold. First, identification of data gaps in the literature helps to focus future research, and refine our approaches to solving the puzzle of human infection with AMR *Campylobacter*. Second, existing data can be used to inform quantitative modeling; more specifically, results are anticipated to provide insight into if there are separate risk factors for a resistant *Campylobacter* infection, compared to a susceptible infection. These data will be incorporated into an integrated assessment model for antimicrobial resistance for human exposure to resistant *Campylobacter*.

Abstract 20: Nikky Millar

Evidence of a drastic decrease in the use of antimicrobials of very high importance for humans in dairy farms after a new regulation restricting their use in Quebec, Canada

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Since February 2019, a new regulation restricted the use of antimicrobials of very high importance in human medicine (3rd and 4th generation cephalosporins, fluoroquinolones, and polymyxins; also defined as category I antimicrobials by Health Canada), in food producing animals in Quebec, Canada.

OBJECTIVES:
The objectives were (1) to estimate the changes in antimicrobial use (AMU) in dairies at the farm-level after the implementation of the regulation and (2) to quantify the effect of two factors (the quantity of AMU on a herd before the regulation and the associated veterinary facility) on the magnitude of these changes.

MATERIALS AND METHODS:
Changes in AMU were estimated using electronic, centralized drug sales data from June 2016 to May 2020, extracted from a veterinary billing software, Vet-Expert ©, used by most food animal veterinary facilities in Quebec. The database was extensively cleaned up to identify dairy clients and antimicrobial drugs, since it is also used by veterinarians to keep tracks of general sales to their clients. Antimicrobial sales data were used to compute defined course doses (DCDbovCA) for each antimicrobials' categories used at the farm-level. For descriptive purposes (part 1), DCDbovCA were summed up for all farms and monthly total DCDbovCA from June 2016 to June 2020 were estimated. To evaluate the average change in AMU per herd and the impact of the two predictors (part 2), number of DCDbovCA by herd from June 2017 to June 2018 (pre-regulation period) were compared to those of June 2019 to June 2020 (post-regulation period).

RESULTS:
Data from 3339 (70%; part 1) and 3569 (75%; part 2) of the 4766 dairy herds present in the province at the end of June 2020 were retrieved. The monthly category 1 AMU went from an average of 18,241 DCDbovCA in the province before the implementation of the regulation to a monthly average usage of 4280 DCDbovCA after the implementation of the regulation. Moreover, during the pre-regulation period, the herd-level average category 1 AMU was 26 DCDbovCA and we observed an average reduction of 19 DCDbovCA (95%CI: -24.2; -14.8) after the implementation of the regulation without a significant increase in category 2 AMU, in category 3 AMU, nor in the total AMU. The pre-regulation AMU on a farm was significantly associated with the change in AMU during the post-regulation period for category 1 antimicrobials ($p<0.01$). The more category 1 antimicrobials were used on a farm prior to regulation, the greater the farm reduced category 1 AMU after the implementation of the regulation. Finally, the

reduction in category 1 antimicrobials on a farm was influenced by the veterinary facility associated with the farm. For 46 of the 52 (88%) veterinary facilities included in the study, we observed, on average, a reduction of usage of category 1 antimicrobials of the dairy farms they deserved.

CONCLUSION:
There was a significant reduction in category 1 AMU following implementation of the new regulation in dairy herds in Quebec, Canada. The veterinary facility and the historical AMU impacted the level of reduction on a given herd.

Abstract 21: Sydney Pearce

Using knowledge translation to support dairy veterinarian antimicrobial stewardship and antibiotic decision-making

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With growing focus on antibiotic usage among health and agricultural sectors, the dairy industry experiences increasing pressure to implement novel, evidence-based antibiotic use (AMU) practices as they develop. Four recent systematic reviews (SRs), which provide best available evidence for health interventions, synthesized AMU best practices for two diseases contributing a significant portion of industry AMU and with significant economic and welfare impacts. They identified antibiotic efficacies for preventing intramammary infections (IMI) and treating bovine respiratory disease (BRD), blanket vs. selective treatment for IMI, and teat sealant efficacy. The SRs have potential to improve AMU decision-making by increasing utilization of effective antimicrobials and practices, effective antimicrobial alternatives, and reducing use of antimicrobials important to humans when combined with Canada's categorization of importance to human medicine; all important components of antimicrobial stewardship and reducing antimicrobial resistance (AMR) occurrence. To access this potential, best methods of translating SR results to AMU decision-makers must be determined. Ontario dairy veterinarians will be enrolled and assessed via questionnaire for antimicrobial prescription decision-making before and after a knowledge translation (KT) tool intervention. Four remotely accessible (i.e., not in person) KT tools will be randomly distributed among participants to determine how best to reach this population when intensive, in-person learning is not an option due to limiting schedules or resources. Tools include an online copy of the published SRs, website, podcast series, and an educational, mobile gaming app. The SR copies will be the control intervention representing the current standard of KT among academic groups. The entrance questionnaire will address i) factors in veterinary decision-making regarding antimicrobial prescription, ii) veterinary antibiotic prescription and practice preferences in described scenarios, iii) how they prefer to receive new information, iv) veterinarian and clinic-level demographic characteristics, and v) barriers to obtaining new health information. The follow-up (4 weeks after tool provision) and exit (2 months after follow-up) questionnaires will re-address topics i) and ii) to assess changes with the additions of vi) quantity and quality of time spent with tool, vii) perception of tool, viii) barriers to using the tools, ix) suggestions for improvement of tools. The objectives of this randomized controlled trial are to identify the efficacy of novel, remotely accessible KT tools (i.e., website, podcast, app) for changing IMI and BRD antibiotic practice decision-making among Ontario dairy veterinarians in comparison to traditional academic KT (i.e., published research papers). It is hypothesized that the novel tools will affect behavior change more than the control tool (SR copies) and provide a more positive user experience. We also hope to better understand common barriers to accessing information and information mediums of choice within this population through the questionnaires. By incorporating animal and human health evidence in our tools, as well as assessing social environment factors influencing access to information, we are attempting to take a One Health approach to supporting antimicrobial stewardship efforts in this industry.

Abstract 22: Hélène Lardé

Comparison of farm-level quantification methods to estimate the usage of antimicrobial agents from products other than medicated feed in Quebec dairy farms, Canada.

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INTRODUCTION:

A monitoring system to assess antimicrobial use (AMU) is expected to be sustainable, easy to implement, representative of the actual consumption, and applicable to a large population. The objective of the study was to compare three quantification methods for antimicrobials from products other than medicated feed to a "garbage can audit" (reference method, GCA) in Quebec dairy farms.

MATERIALS AND METHODS:

Data were collected over one year in 101 Quebec dairy farms from 3 sources: electronic veterinary invoices obtained from the billing software used by Quebec's dairy practitioners (method VET), the "Amélioration de la santé animale au Québec" provincial reimbursement program (method GOV), and livestock treatment records completed on the farm as part of the "Canadian Quality Milk" program (method CQM). The AMU rate was reported for each method (GCA, VET, GOV, and CQM) in number of DCDbovCA per 100 cow-years for total AMU, AMU by route of administration, and AMU by antimicrobial category according to the World Health Organization and to Health Canada. Comparisons with the reference method were performed using concordance correlation coefficients, Bland-Altman plots, and negative binomial regressions (with the alternative method as the outcome variable in the model, and the reference method as the independent variable).

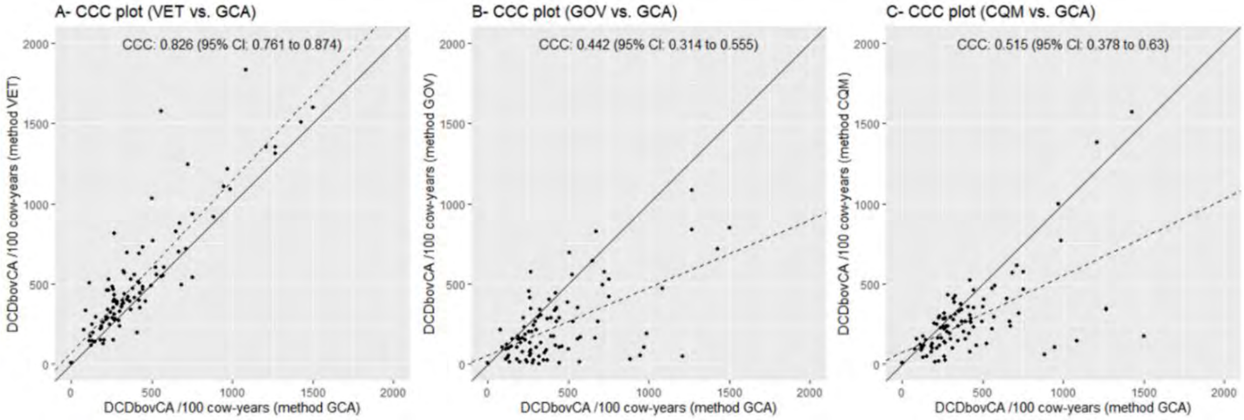
RESULTS:

Most invoices were extracted from the same software (Vet-Expert, 87 farms; Sysvet, 14 farms). Overall, an almost perfect agreement (Figure 1-A) was identified between methods VET and GCA, except for the oral and intrauterine routes, possibly due to the inability of the method GCA to collect efficiently oral and intrauterine formulations. Method VET identified an average of 105 DCDbovCA /100 cow-years (95% confidence interval: -237, 446) more than the method GCA, but the difference was not statistically significant. Only moderate agreement was observed between methods GOV and GCA (figure 1-B), and between methods CQM and GCA (Figure 1-C).

CONCLUSIONS:

Method VET showed the highest correlation with GCA, while methods GOV and CQM were deemed insufficient to measure reliably the AMU. The billing software used by Quebec's dairy practitioners seemed to be promising in terms of surveillance and benchmarking of AMU, including critically important antimicrobial agents. In 2018, 86% of Quebec's dairy practitioners used the same software (Vet-Expert), and therefore, the database is expected to include most of Quebec's dairy farms (provincial reports).

Figure 1. Concordance plots showing strength of agreement for quantification of total AMU rate in products other than medicated feed (in DCDbovCA /100 cow-years) between reference method GCA and methods VET (A), GOV (B), and CQM (C). Each black point represents one farm of the project. The solid and dashed lines represent the line of perfect concordance and the reduced major axis, respectively. Concordance correlation coefficient (CCC) is presented with its 95% confidence interval (95% CI).



Abstract 23: Michelle Cheng

Application of sociotechnical system analysis to the complex problem of antimicrobial resistance

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INTRODUCTION:

Antimicrobial resistance (AMR) is a complex problem and one of the greatest threats to public health in the 21st century. There are multiple approaches to addressing this threat, including infection prevention and control, antimicrobial stewardship, and research/innovation. Complex systems such as AMR can be better understood utilizing the sociotechnical framework[1] through analyzing the system elements including goals, people, processes, culture, buildings/infrastructure, and technology. This model reflects mixed-methods research to describe the barriers and facilitators to optimizing system performance across individual, organizational, geographical, and cultural boundaries.

Our study will generate a comprehensive collection of system information to subsequently inform technology development opportunities and knowledge translation interventions that apply to AMR. This research is part of the AMR – One Health Consortium, funded by the Major Innovation Fund program of the Ministry of Jobs, Economy and Innovation, Government of Alberta. Our initial phase of this work will be presented, identifying some of the sociotechnical system (STS) elements that influence the use of antimicrobials in humans and animals.

MATERIALS AND METHODS:

Scoping interviews were performed with the goal of allowing subject matter experts to steer the research team to promising systems to perform in-depth STS analysis. The study received approval from our Conjoint Health Research Ethics Board (REB20-0156). Participants were interviewed using a semi-structured interview guide. Participants were influential subject matter experts with seniority, at least 10 years in their specific field. Recruitment was performed though existing networks and referral/snowball networking, aiming for a total of 20-25 participants. Data from the interviews were collected in the form of audio recordings, notes, and a demographics questionnaire.

All data was analyzed using rapid analysis, with the goal of identifying the system ontology and different sub-systems in place. From the system ontology and analysis, scenarios/themes were extrapolated for further analysis for the impact on AMR and potential for interventions in the scenarios.

RESULTS:

We completed 21 scoping interviews. The participants were from various sectors including: animal health (n=11), pharmacy (n=6), dentistry (n=2), and market economics/research (n=2). They varied from academics (n=7) to frontline workers (n=14). Rapid analysis was performed by two researchers independently to ensure inter-rater reliability, and subsequently compiled to identify areas with high saturation of engaged experts and major themes. Data analysis identified most engaged experts were from the animal health and pharmacy sectors. Three common themes considered to have the greatest impact on AMR included: 1) surveillance, 2) technology improvements, and 3) knowledge transfer and breaking "old habits". A limitation is that under-representation from other sectors may have skewed the themes found.

CONCLUSIONS:

AMR is a complex problem. The initial phase of our STS analysis employed scoping interviews and identified that awareness of AMR is present, but more action must be taken to combat this issue. Three common overarching themes: surveillance, technology improvements, and knowledge transfer were identified as next steps for the continuation of this stage of our study.

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Abstract 24: Britteny Kyle

Understanding American Foulbrood in Ontario: prevalence, perception and antimicrobial usage protocols.

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INTRODUCTION:

Honey bees play a vital role in food security through crop pollination services. Currently the beekeeping industry is experiencing unsustainable gross colony losses across North America. Antimicrobial use in apiculture is under veterinary stewardship in Canada as of December 1st 2018. American Foulbrood (AFB) is the most devastating bacterial disease of honey bee brood. Antibiotics, and oxytetracycline in particular, have been administered to honey bees in Ontario for decades in order to prevent and control AFB. Concerns about prophylactic and metaphylactic practice include antimicrobial residues in a food of animal origin, the development of antimicrobial resistance in the etiological agent as well as commensal microbes, and detrimental effects on honey bee health. The goal of this study is to gain an understanding of AFB management protocols that beekeepers are employing and if certain counties may be at higher risk. The specific objectives are to (i) study antimicrobial usage protocols at the county level (ii) determine the observed risk distribution of AFB among Ontario's honey bee populations, and to (iii) investigate the potential association between antimicrobial usage and prevalence of AFB.

MATERIALS AND METHODS:

An online survey will be anonymously administered to beekeepers in Ontario. The sampling plan makes use of a snowball technique. A member of each of the 32 local beekeepers' association in Ontario will be contacted via email to request participation. Each respondent will be asked to share the survey by forwarding the email to 3 additional beekeepers. A disease map of AFB will be created using prevalence data obtained during the annual provincial apiary inspection program.

ANTICIPATED RESULTS:

It is anticipated that there will be a difference in the perception and approach to AFB management between commercial and small-scale beekeepers, with commercial beekeepers monitoring more frequently and being more likely to administer oxytetracycline. Additionally, management practices of AFB may vary geographically with the level of urbanization and disease prevalence. It is unknown if there will be any correlations to spatial patterns of perception and preventative practices when compared to the risk distribution of AFB.

ANTICIPATED IMPORTANT CONCLUSIONS:

The most important conclusion will be a furthering of our understanding of current levels of beekeeper knowledge, attitudes, awareness and preventative practices as well as highlighting counties that are at greater risk of AFB infections. This will allow for the development of more precise and efficient prevention and control strategies. Furthermore, this understanding will aid veterinarians tasked with decision making regarding the prescription of medically-important antimicrobials in apiculture.

Abstract 25: Nelima Ibrahim

Antimicrobial use and biosecurity in broilers & sonali (local bird) in Bangladesh in a One Health perspective

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INTRODUCTION:

Antimicrobial use (AMU) is a major contributing factor in the rising threat of antimicrobial resistance (AMR) in food animal production worldwide. Therefore, to be able to control the AMR problems, AMU has to be addressed. Therefore the aim of this study was to quantify and compare AMU and biosecurity status of broiler and sonali farms in the northern region of Bangladesh. Sonali is a cross-breed of Rhode Island Red (RIR) cocks with Fayoumi hens and has a similar phenotypic appearance & meat tastes to that of local chickens. It's rearing type is similar to broiler production system and has a production length between 60 to 70 days.

METHODS:

The data of antimicrobial use of each flock and total antimicrobials purchased over one year of that particular flock were collected from 53 broiler and 52 sonali farms in northern part of Bangladesh. Five individual fecal samples and two individual environmental samples were collected from each farm to determine the level of AMR in *E. coli*. AMU at flock level was quantified as treatment incidences, based on Defined Daily Dose (TI DDDvet). Biosecurity status of the farms was determined using Biocheck.ugent: a scoring system to measure and quantify the level of biosecurity on broiler farms.

RESULTS:

The median of TIDDDvetFl* which express the number of days per 100 animal days that the flock received a dose of antimicrobials in broiler flocks was 18 and minimum to maximum value range is 0.30-160.84. It means that the median broiler was treated with antimicrobials 18% of their live time. . The median of TIDDDvetFl* in sonali flocks was 10 and also range of minimum to maximum value is 0.38-31.5. Sonali bird is treated with antimicrobials for 10% of its live time. In broiler, birds were treated with antimicrobials in 37% of the cases for curative purpose and in 63% for preventive purpose. In sonali 49% of the antimicrobial treatments were curative and 51% preventive. Ninety-two percent of farms started treatment on the first day of production. All farms started using antimicrobials in the first week. The most frequently used antimicrobials in broilers are Aminopenicillins, Fluoroquinolone, Sulfonamides, Macrolides and Tetracyclines with respectively 22%, 18%, 19%, 18% and 16% of the total number of treatments. In sonali, Aminopenicillins, Fluoroquinolone, Cephalosporin, Sulfonamides, Macrolides, Tetracyclines and Quinolone antimicrobial classes are used in respectively 10%, 10%, 12%, 15%, 12%, 13% and 15%.

The standard deviation of external biosecurity in both broiler and sonali farms are different in 2 categories among 7 categories: depopulation of birds (6.28, 9.96) and visitors and farmworkers (7.40, 14.32) respectively broiler and sonali. Nevertheless, standard deviation of internal biosecurity in both farms are unlike in one categories among 3 categories: materials and measures between compartments (23, 21.75) broiler and sonali respectively.

CONCLUSION:
These preliminary results suggest that a difference in AMU has been observed in terms of treatment type and antimicrobial classes as well as the comparison between broilers and sonali. Further on these results will be completed with AMR results.

Abstract 26: Mariana Fonseca

Surveillance of antimicrobial use and antimicrobial resistance on dairy farms across Canada

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[*]Presenting author: Mariana Fonseca (Ph.D. Student)

INTRODUCTION:

In Canada, few studies have evaluated agricultural antimicrobial stewardship practices, including in the dairy sector. A farm-based surveillance program of antimicrobial use (AMU) and antimicrobial resistance (AMR) was developed and implemented on dairy farms in five Canadian provinces (CaDNet-ASR).

MATERIALS AND METHODS:

Data on AMU and farm-specific demographics are being collected using garbage can audits (GCA), farm records, and questionnaires. The minimum inhibitory concentrations (MIC) of a panel of antimicrobials against *Escherichia coli*, *Campylobacter* spp., and *Salmonella* spp. were determined using fecal samples collected from the bulk manure storage and composite samples of each of the following groups of animals: lactating cows, breeding age heifers, and pre-weaned calves.

RESULTS:

A total of 144 dairy farms were recruited and will be followed for four years. In year 1 of data collection, each farm was sampled once, and 560 composite fecal samples from 140 farms yielded a high prevalence of *Campylobacter* spp. among lactating cows (84%) and heifers (83%). The prevalence of *Salmonella* spp. was higher in bulk manure storage samples (11%) compared to calves (4%), lactating cows (3%), and heifers (2%). The highest prevalence of *Salmonella spp.* was observed in Ontario (Table 1). The MIC profiles for the first 667 isolates tested (476 isolates of *E. coli*, 193 isolates of *Campylobacter* spp., and 28 isolates of *Salmonella* spp.) indicated that resistance to tetracycline was most common among the isolates (55, 77, and 79% for *Campylobacter* spp., *E. coli, and Salmonella* spp., respectively). The susceptibility of isolates to category I antimicrobials is summarized in Table 2.
The AMU data were collected through GCA in four provinces and showed a mean of 619.5 DDD/100 animals/year. The summary is presented in Table 3.

CONCLUSIONS:

In the first year of data collection, the prevalence of *Campylobacter* spp. was high. Of all antimicrobials tested, tetracycline displayed the smallest proportion of susceptible isolates, and some isolates were resistant to category I antimicrobials. Only two farms did not use category I antimicrobials, and the category I use represented ~20% of the total DDD/100 animals/year.

Table 1. Overall prevalence (%) of enteric pathogens over provinces.

Province	*Escherichia coli*	*Campylobacter* spp.	*Salmonella* spp.
British Columbia	94	56	5
Alberta	99	76	0.8
Ontario	99	68	13
Quebec	98	55	5
Nova Scotia	94	63	0

Table 2. Susceptibility of isolates for category I antimicrobials (very high importance in human medicine).

Antimicrobial	Species	N	% Susceptible
Amoxicillin/clavulanic acid	*E. coli*	476	96.4
	Salmonella spp.	28	96.4
Ceftriaxone	*E. coli*	476	97.0
	Salmonella spp.	28	100
Meropenem	*E. coli*	476	99.8
	Salmonella spp.	28	100
Ciprofloxacin	*Campylobacter spp.*	193	86.5

Table 3. Summary of total antimicrobial use (DDDadur[a]/100 animals/year) in dairy farms across Canada

Variable	No. farms	Mean	Std. Dev.	Min	Max
All antimicrobials	96*	619.5	1207.7	21.8	12019.1
Category I antimicrobial [b]	96*	123.8	144.0	0.0	673.2

a: Defined daily dose according to Lardé et al. (2020)

b: Category I antimicrobials reported: 3rd-generation cephalosporins, fluoroquinolone, and polymyxin.

*Quebec data not included

Abstract 27: Abishad P.M.

Antimicrobial efficacy of green synthesized Silver nanoparticles against multi- drug resistant enteroaggregative *Escherichia coli*

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[#]Presenting author (Ph.D. Scholar); *Corresponding author

INTRODUCTION:

Enteroaggregative *Escherichia coli* (EAEC) is an emerging food- borne pathogen responsible to produce chronic as well as persistent diarrhoea in humans and animals. An elevated pattern of drug resistance towards EAEC along with comparatively reduced discovery of antimicrobial agents would worsen the antimicrobial resistance (AMR). In order to combat the menace of AMR among pathogens of public health significance, alternative antimicrobial therapeutics like, biogenic nanostructures have attracted wide attention in the recent past. The present study was envisaged to explore the antimicrobial efficacy of green synthesized silver nanoparticles (Ag NPs) against three multi- drug resistant (MDR)- field strains of EAEC.

MATERIALS AND METHODS:

Initially, the Ag NPs were green synthesized by using the culture- free supernatant of decline phase culture of the probiotic strain, *Lactobacillus acidophilus* MTCC 10307. The morphological characterization and phase transformation of the synthesised Ag NPs were investigated using UV-Vis spectrophotometry, DLS, FTIR, XRD, TGA-DTA, SEM and TEM. The *in vitro* antimicrobial efficacy of the characterised Ag NPs against the MDR-EAEC strains was performed by resazurin- based microbroth dilution assay. Additionally, the *in vitro* antioxidant and free radical scavenging activity of Ag NPs were determined by using ABTS and ferric reducing antioxidant power (FRAP) assays.

RESULTS:

In this study, we successfully synthesized Ag NPs through an eco-friendly green synthesis protocol, which was then confirmed by its XRD pattern. A weight loss of 15 % up to 200°C with an intense exothermic peak between 150°C and 200°C was observed in TGA-DTA, while aggregated nanoclusters were observed in SEM. Moreover, the TEM imaging of Ag NPs revealed a spherical morphology and crystalline nature with an optimum size of 15.152 nm. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of green synthesised Ag NPs against MDR- EAEC strains were found to be 7.80 µM and 15.60 µM, respectively. Moreover, concentration- dependent antioxidant activity was observed in ABTS and FRAP assays.

CONCLUSIONS:

The promising results of this study to synthesis nanoparticles in an eco-friendly approach provides further avenues for its usage as appropriate alternatives in antimicrobial therapeutics and its translation in suitable *in vivo* models.

Abstract 28: Lee Wisener

Non-antibiotic approaches for disease prevention and control in beef and swine production: 2 scoping reviews

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INTRODUCTION:

Livestock producers are encouraged to practice antibiotic stewardship by replacing their use of antibiotics belonging to classes of medical importance to humans with non-antibiotic approaches. Our objective was to describe the volume and nature of research on non-antibiotic approaches for beef and veal (beef-veal) production and for nursery pig production through scoping reviews (ScRs). Scoping reviews systematically map the research for a broad topic and identify knowledge gaps and specific topics that could feasibly support systematic reviews that answer questions about summary effectiveness of specific interventions.

MATERIALS AND METHODS:

The beef-veal ScR search included available research from January 1990 to October 2016, whereas the nursery pig ScR included research from January 2000 to April 2018. The ScRs were limited to research conducted for the North American context. Multiple electronic databases were searched and for the nursery pig ScR, proceedings sourced from the American Association of Swine Veterinarians meetings were searched manually. The search was followed by relevance screening by two reviewers independently with disagreements resolved by consensus. Pre-specified data items were charted for each ScR separately. We charted the eligible clinical trials and observational studies for both ScRs and for the beef-veal ScR, we also charted challenge trials.

RESULTS:

For the beef-veal ScR we charted 722 eligible articles, (5%) of the 13,598 screened. For the nursery pig ScR we charted 441 articles and proceedings, (4%) of 11,316 screened. The interventions and outcomes reported were diverse for both ScRs. Replication of studies was less for the beef-veal ScR suggesting less depth of research compared to the nursery pig ScR.

Non-antibiotic feed additives and vaccines were the most common interventions studied. There were relatively few studies that evaluated management practices. The most common outcomes reported were indices of vaccine immunity, morbidity, and mortality. Among the vaccine clinical trials, 29% and 36% failed to report a clinically important outcome for the beef-veal and nursery pig ScR respectively. The beef-veal ScR identified seven specific topic areas evaluated in clinical trials that shared enough commonality which may support systematic reviews whereas the nursery pig ScR identified 13 specific topics. There was a dearth of studies in which non-antibiotic interventions were compared to a preventive antibiotic control group needed to enhance the evidence for antibiotic alternatives.

CONCLUSIONS:

Scoping reviews provided a guide for future research and research funding on non-antibiotic approaches to prevent and control diseases in beef and veal production and in nursery pig production. There was considerable volume and diversity of research for both broad topics but there was limited depth in the beef-veal research. Future primary research could replicate specific topic areas to generate additional studies to support systematic reviews. Other gaps in the research included management practices. Future primary research could also include preventive antibiotic use comparison groups where appropriate. Consistent reporting of clinically important outcomes in vaccine clinical trials is needed. If not already done, systematic reviews could be done for the specific topic areas identified as having enough commonality to combine.

Abstract 29: Rebecca Flancman

Enriched antimicrobial resistance gene sequencing to assess changes in the fecal resistome of dairy calves in Southern Ontario, following on-farm interventions to reduce antimicrobial usage

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INTRODUCTION:

Neonatal diarrhea accounts for 20-25% of morbidity among calves and antimicrobials are commonly administered. One approach for mitigating antimicrobial resistance is to systematically modify antimicrobial usage (AMU). Our study investigated one such strategy: the effects of a decision-tree in-farm based intervention to reduce AMU for diarrhea on the fecal resistome of dairy calves, across two farms.

MATERIALS AND METHODS:

Farm1 used intramuscular (IM) trimethoprim-sulfonamide (TMS), spectinomycin, and lincomycin in the Pre-intervention period (Pre), and only TMS in the Post-intervention period (Post). Farm2 used oral TMS and either ceftiofur or IM-TMS in the Pre period, and ceftiofur or IM-TMS in the Post period. A total of 31 fecal samples from dairy calves across two timepoints, on two different farms (N=7-8 per sampling point) were used in the study. Samples were obtained before the antimicrobial reduction intervention and 12 months afterwards. Metagenomic sequencing of the resistome with enrichment protocols was performed (Illumina HiSeq) using a commercial system that was customized for target-enrichment of sequencing for antimicrobial resistance genes (Agilent SureSelect XT-HS). Bioinformatics processing and statistical analysis were performed using the MEGARes antimicrobial resistance database, AmrPlusPlus pipeline, and custom R scripting.

RESULTS:

Inter-farm comparison in the Pre-intervention period shows there were distinct differences between farms, with increased relative abundances consistent with the antimicrobial classes used on each farm.

Intra-farm results show significant decreases over time in the relative abundances of sulfonamides and fluoroquinolones on Farm2. The relative abundance of beta-lactams also decreased to a lesser degree. On Farm1, there were increased relative abundances of resistance genes in the Post period versus the Pre period, with significant increases in tetracycline, fluoroquinolone, and aminoglycoside resistance classes, and the latter approached significance. The increases identified on Farm1 were unexpected since the AMU for the fluoroquinolone and aminoglycoside classes were decreased post-intervention and tetracyclines were never used in either period. It is hypothesized these increases were due to the presence of multiple resistance genes on the same plasmid (which has little to no fitness cost, ensuring their persistence among bacteria), and resulted in the overall increased resistance seen in the Farm1 Post period.

Many class-level changes were driven by specific resistance mechanisms, especially in relation to the tetracycline, MLS, and beta-lactam classes on both farms, and aminoglycosides on Farm1 and fluoroquinolones on Farm2. Additionally, many changes (including for beta-lactams and sulfonamides) were driven by only one or two animals at a given timepoint, and not necessarily changes across all samples.

Richness of antimicrobial resistance classes increased between timepoints on Farm1, but this did not translate to a statistically significant change in Inverse Simpson diversity, and there were no changes for either on Farm2. However, NMDS data indicate resistances showed significant clustering by farm and farm timepoint.

CONCLUSIONS:

These results suggest that reducing AMU decreases the relative abundance of resistance seen however, it is hard to overcome and undo decades of AMU quickly. In addition, this emphasizes the importance of follow up on interventions to discern their actual effect, both in the immediate and longer term.

Abstract 30: Sakib Rahman

The Antibiotic Resistance Paradox

Sakib Rahman* and Dr. Aidan Hollis

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This paper uses a panel setting to quantify the ecological associations between the variety of bacterial pathogens and the classes of antibiotics used to treat them, at an aggregate level of the member states of the European Union. Specifically, it estimates the elasticities of resistance with respect to usage in order to help identify the sensitive bug-drug combinations and focus public health strategies on those combinations.

Using antibiotic sales data from IQVIA MIDAS and resistance level data from European Centre for Disease Prevention and Control (ECDC), econometric tools are employed to quantify the relationships. Aggregate hospital and retail sales data are used, for 26 European countries for 11 years and resistance data for 26 bug-drug combination for the same countries and years. For each bug-drug combination the time dimension is used to control for unobserved time-invariant omitted variables, referred to as country fixed effects. The relationship is assumed to be given by a linear additive model where country fixed effects do not vary in time. In addition, the possibility where differences across countries have influence on the relationships of interest is considered and a random effects model is employed. The panel setting allows for this type of short-run contemporaneous estimation and it is the first usage-resistance estimate of its kind to our knowledge. Moreover, a simple cross-sectional long-run analysis is also done by fragmenting the data into early and late years.

In the short run we find a positive contemporaneous relationship between usage and resistance for some combinations of *Enterococcus Faecalis, Enterococcus Faecium, Escherichia coli, Klebsiella Pneumoniae and Pseudomonas Aeruginosa*. This relationship holds when different measures of antibiotic usage are used. Among the bug-drug combinations which showed a positive and significant relationship, the average elasticity is 1.78, i.e., a 1% increase in usage increases resistance by 1.78% on average. The elasticities differ for individual bug-drug combinations. The strongest elasticity is observed in the combinations of *Enterococcus Faecalis*, while for others the observed relationships are quite weak. The long-run analysis, however, presents a paradox. Even for the few observed short-run cross-sectional relationships that are positive, there is no observable effect of antibiotic usage in a country on future resistance levels.

Our findings imply that there are other factors along with and/or other than usage which determines the occurrence of resistance. While Antimicrobial Stewardship policies focusing on mitigating over and injudicious use of antibiotics are important, policy makers should also consider additional factors relevant for each context to tackle the problem of growing antimicrobial resistance. Lastly, the relationships estimated are relevant for broader population.

Abstract 31: Shane Renwick

Stewardship of Antimicrobials by Veterinarians Initiative (SAVI)

Dr. Shane Renwick DVM, Project Manager, Canadian Veterinary Medical Association (Presenting and Corresponding), Mr. James Dunlop, Deputy Project Manager, TDV Global Inc.

INTRODUCTION:

The Stewardship of Antimicrobials by Veterinarians Initiative (SAVI) is intended to both generate and share knowledge on prudent antimicrobial stewardship as well as pilot collection of prescription and dispensing-based antimicrobial use (AMU) data for the beef, poultry and swine sectors. The project was designed in keeping with One Health principles and is aligned with the Pan-Canadian Framework on Antimicrobial Resistance and associated Action Plan.

MATERIALS AND METHODS:

SAVI is intended to provide veterinarians with the most current and critical knowledge and intelligence they need to make effective decisions with regard to AMU.

With respect to the generation and sharing of knowledge and guidance, SAVI has upgraded its platform for the hosting of the CVMA Guidelines for Veterinary Antimicrobial Use and will be adding aquaculture and equine species groups to the existing guidelines that span six different species groups. SAVI will also be updating and revising the existing guidelines and exploring the use of alternate treatments, products and protocols to complement or optimize antimicrobial use.

From a data collection perspective, SAVI has worked with leaders within the veterinary community in generating initial data extracts to populate the test database and is in the process of developing automated electronic data transfer protocols to ease the burden on reporting by project participants as well as the crafting of customized reporting to deliver relevant information on an as-required basis.

As a pilot initiative, data collection efforts have been initiated with a select group of participants to test, refine and improve the system prior to wider adoption, with an initial focus on leading clinics that treat large numbers of animals. Standardization of AMU metrics from a One Health perspective is being pursued with support from the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) and other ongoing initiatives. As a key tenet of the project, SAVI is committed to the safeguarding of all collected data and to the confidentiality and privacy of participant information.

An Expert Advisory Group (EAG) was convened from representatives of key veterinary and sector stakeholder organizations to leverage their expertise in both design, planning and implementation activities as well as the development of a communications and engagement approach to encourage adoption of project guidance and participation in data collection.

ANTICIPATED RESULTS:

The ultimate goal of SAVI is the development and sustainment of a national decision-support system that arms veterinarians with the latest knowledge, guidance and data on AMU in service to their patients and clients.

Anonymized and aggregated data will be employed to analyze prescription and dispensing practices on a national basis and assist in the targeting of future antimicrobial stewardship efforts and investigation of the relationship between AMU and antimicrobial resistance.

ANTICIPATED IMPORTANT CONCLUSIONS:

Optimization of AMU requires the generation and uptake of best practices for both antimicrobial prescription and dispensing as well as the means and the evidence base to identify and evaluate change in behaviours over time. SAVI will address that existing gap in capability as part of a larger One Health collaboration on antimicrobial stewardship.

Abstract 32: Chloé Bâtie

Patterns of antibiotic usage among chicken farmers in North and South Vietnam

Chloé Bâtie [1,2,3,*], Le Thi Thu Ha [4], Etienne Loire [2], Truong Dinh Bao [4], Ha Minh Tuan [3], Ngo Thi Kim Cuc [3], Mathilde Paul [5], Flavie Goutard [6,2,5]

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In response to the threat of antibiotic resistance (AMR), it is urgent to implement effective and tailored strategies to reduce antibiotic consumption. In this context, the European project RoadMap aims to encourage the prudent use of antibiotics in livestock production systems in various contexts to contribute to the reduction of AMR. In Vietnam, there is a great diversity of chicken rearing practices, from backyard farming to intensive contract farming, resulting in a very heterogeneous use of antibiotics. In order to propose adapted solutions, it is necessary to find out whether socio-economic and technical management of the farms can be associated with antibiotic usage patterns.

In this preliminary study, we aim to analyze patterns of antibiotic usage among chicken farmers in Vietnam according to different farms type. A structured questionnaire was addressed to chicken farmers rearing broilers, layers or breeders between May and June 2020 in two districts of Hanoi province and three districts of Long An province, in North and South Vietnam. Farms were randomly selected, using four strata based on farms type: 29 backyard family farms (<100 chickens), 33 semi-intensive family farms (100 – 2000 chickens), 38 intensive family farms (>2000 chickens) and 25 contract farms (>2000 chickens).

Out of the 125 farms studied, 111 were using antibiotics. These farms were included in further analysis. A multiple correspondence analysis followed by a consolidated hierarchical clustering on the principal components (PC = 15 with 70% of explained variance) was performed on 18 variables describing antibiotic usage. The *FarmType* variable related to the four types of farming was introduced as a supplementary variable. A total of three groups based on the within-group inertia were retained, these groups were best characterized by the variables *ABUadvices*, *ABUwayofadvices, ABsource* and *FarmType..* The first group included farmers (n=24) who receive company advice, with a veterinarian or technician coming directly to the farms to treat their poultry and provide them with antibiotics. They record information related to antibiotic treatments, are trained in antibiotic use and comply with the withdrawal time. Farmers from the second group (n=59) buys antibiotics from the drugstores that provide them with advices; the group follows the prescribed dosage. Most farmers in this group use antibiotics for treatment purpose only. The last group (n=28) includes farmers who also buy their antibiotics from the drugstore but use them according to their own experience. They use antibiotics for treatment but also for prevention and generally use higher dosage than prescribed. Contract farms were overrepresented in the first group (91,7%, $p<0.001$), whereas backyard farms and intensive family farms were more frequently found in group 2 (90,5%, $p<0.001$) and 3 (43,3%, $p<0.01$) respectively.

Findings may help to improve antibiotic awareness campaigns by tailoring messages to the specific practices of these different farm types. In the framework of the RoadMap project, these results, together with other approaches, should facilitate the joint development of innovative AMR control strategies with stakeholders involved in the decision-making process.

Abstract 33: Ellen de Jong

Mastitis-related antimicrobial use: Current practices on Canadian dairy farms

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INTRODUCTION:

Prevention and treatment of mastitis accounts for the majority of antimicrobial use on dairy farms. Various stewardship programs aimed at reducing antimicrobial use in the dairy sector have emerged over the last decade. In Canada, collaborative efforts of various stakeholders have resulted in the 'Canadian Dairy Network of Antimicrobial Stewardship and Resistance' (CaDNetASR) initiative. This network encompasses a cohort of 150 dairy farms in five regions and monitors antimicrobial resistance and antimicrobial use patterns.

MATERIALS AND METHODS:

To help identify areas for reduction in mastitis related treatments, structured questionnaires captured current on-farm antimicrobial use practices on a subset of 146 farms in British Columbia, Alberta, Ontario, Quebec and Nova Scotia.

RESULTS:

Of surveyed farms, 35% routinely practiced selective dry cow therapy (DCT), and 59% practiced selective treatment of clinical mastitis (CM). Of the farmers practicing selective DCT, 86% used somatic cell counts (SCC) to select cows for treatment (timepoint of SCC measure ranging from the last 2 weeks, to last 12 months). Cut-offs for selecting cows to treat ranged from 15,000 to 500,000 cells/mL (median 150,000 cells/mL). Time point when the previous CM case occurred was used by 45% of the farmers as part of their selective DCT strategy; with most farmers taking the current lactation into account. 33% considered at the number of CM events (ranging from 1 case in her lifetime, to 4 in the same lactation) in their selective DCT protocol. Regarding selective treatment of CM, multiple factors played a role in the decision making of the farmers selectively treating CM. Severity of the symptoms and confirmed or suspected bacteria were the most important considerations, followed by SCC and CM history. 41% used SCC to select CM cases for treatment, based on either the last DHI report, or the last three reports. Cut-offs for selecting cases to treat ranged from 150,000 to 1,000,000 cells/mL (median 300,000 cells/mL). With regards to CM history, treatment considerations were most often based on the presence of 1 or 2 CM cases in the same lactation.

CONCLUSIONS:

These preliminary results suggest that there may be an opportunity for reducing antimicrobial use related to DCT and treatment of CM on dairy farms in Canada. Information on current practices will provide data for intervention studies in British Columbia and Alberta that will measure the effect of selective DCT and selective CM treatment on antimicrobial use and resistance through the CaDNetASR program.

Abstract 34: Adrienn Gréta Tóth

A One Health approach study on alimentary products, as sources in the process of animal-to-human antimicrobial resistance gene transfer

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INTRODUCTION:

Antimicrobial resistance genes (ARGs), that are responsible for the expression of antimicrobial resistance (AMR), can appear in the bacterial genome due to mutations or horizontal gene transfer (HGT) among bacteria. HGT can occur among non-pathogens and/or pathogens if the right triggers are present and if various bacterial populations are in each other's physical proximity. The fulfillment of such requirements may be provided in the human gut where foods including live bacteria enter. Incoming gene sets may either derive from probiotic cultures used in food industry or as dietary supplements, or from bacteria included in raw animal products.

MATERIALS AND METHODS:

New generation sequencing (NGS) datasets deriving from products that can be consumed containing live bacteria were bioinformatically analyzed. Two raw milk samples, one kefir and one yoghurt starter culture sample were shotgun sequenced by the authors. All other short read datasets (kefir, yoghurt, live microbial food ingredients) were obtained from the NCBI SRA repository. After the selection and taxonomic classification of bacterial reads, open reading frames (ORFs) of assembled contigs aligning to the reference genes of CARD (Comprehensive Antimicrobial Resistance Database) with high identity and coverage values were identified as ARGs. Each ARG was linked to the affected antibiotic groups. The mobility of ARGs (plasmid origin or sequential proximity to a mobile genetic element (MGE)) was examined. Results were visualized by each sample, along with the ARG abundance changes during fermentation by three kefir grain samples.

RESULTS:

By the two raw milk samples 47 ARGs were detected. Three of these ARGs harbored on plasmids and gene *blaZ,* was close to a phage-integrase (MGE) ORF.

By kefir, 22 ARGs were identified in products, 2 in grains and 1 in a strain. In yoghurt, 1 ARG was found in a product and 2 in grains. The highest ARG abundance was observed in the kefir strain samples. ORF including gene *lmrD* from a kefir grain contained a transposase (MGE) ORF close by. Datasets explaining the ARG abundance changes during kefir fermentation were also examined.

A further analysis focusing on live microbial ingredients showed that numerous bacterial species used by food and dietary supplement production have a wide ARG spectrum.

CONCLUSIONS:

Consumption of food including live bacteria may raise the chance of ARG transfer in the human gut. Raw animal products may contain bacteria with an elevated number of ARGs due to the use of antibiotics at farms. Without heat treatment the multiplication of these bacteria is not rendered, thus their possibly mobile ARG pool expands. By probiotic foods bacteria containing ARGs may also multiply before reaching the human gut that results in higher ARG counts in the products by the time of intake. Expanded bacterial ARG pools entering our gut may explain a way of the raw and probiotic food derived spread of AMR. ARG monitoring of food should be considered to control AMR passage by products of animal origin to humans. Further examinations would be reasonable to investigate whether the production of ARG-free dietary products is possible or not.

Abstract 35: Landon Warder #1

Comparison of dose-based metrics of antimicrobial use on Canadian dairy farms across provinces

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Antimicrobial use in dairy cattle is a potential source of antimicrobial-resistant enteric pathogens entering the food chain. This study looked to determine differences in dose based metrics of antimicrobial use between provinces in Canada from garbage can audits.

Garbage can audits were conducted over approximately six months in 2020. Thirty farms, each, were from Alberta and Ontario. Twenty were from Nova Scotia, and fourteen were from British Columbia. A custom Python code was used to determine the mass of each active ingredient. Conversion factors for defined daily doses (DDD) and defined course doses (DCD) were taken from Lardé et al. (2020). Antimicrobial drug usage rates (ADUR) were calculated per animal-year.

Across Canada, the mean DDD ADUR and DCD ADUR were 5.0 and 1.1, respectively. These results mean that, on average, each animal was treated with antimicrobials for five days and received 1.1 treatments over a year.

For DDD ADUR, British Columbia (3.44, 95% CI: 2.31, 4.58) significantly lower than Ontario (6.00, 95% CI: 4.67, 7.33), and marginally lower than Alberta (4.88, 95% CI: 4.01, 5.76) and Nova Scotia (4.73, 95% CI: 3.77, 5.70). For DCD ADUR, British Columbia (0.68, 95% CI: 0.46, 0.91) was significantly lower than Alberta (1.23, 95% CI: 0.95, 1.51), and also lower than Ontario (1.11, 95% CI: 0.79, 1.43), and not significantly different from Nova Scotia (1.03, 95% CI: 0.71, 1.36). Figure 1 shows the amount of antimicrobial used in each province.

To determine the effect of herd size and stocking density of lactating cows in the context of province on ADUR, a two-level model was built using MLWin. For DDD ADUR and DCD ADUR, stocking density was not a significant predictor. The number of cows was a significant predictor for DDD ADUR and, after transformation, DCD ADUR (square root of DDD ADUR vs herd size squared: $p = 0.007$; cube-root DCD ADUR vs herd size cubed: $p = 0.010$). In both cases, increasing herd size led to decreased ADUR. The interclass correlation was approximately 0.1 in both models, indicating that 90% of variation comes from variation between farms rather than between provinces.

Increased herd sized negatively correlates with antimicrobial usage by defined daily dose and defined course dose per animal-year. Though most of the variation in antimicrobial usage comes at the farm level, British Columbia had significantly lower usage than Ontario for DDD and Alberta for DCD. These data and analyses are vital for broad benchmarking metrics for antimicrobial stewardship in the Canadian dairy industry.

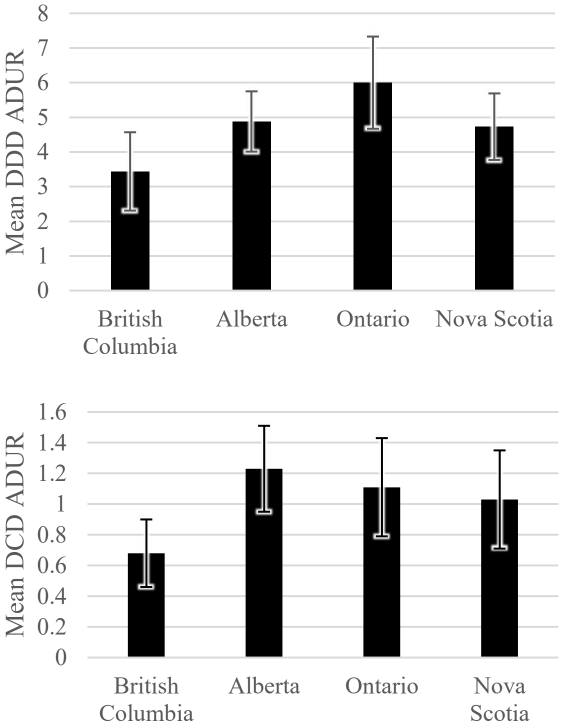

Figure 1. Mean DDD ADUR and DCD ADUR by province with 95% confidence intervals.

Abstract 36: Landon Warder #2

Determination of Defined Daily Dose Antimicrobial Drug Usage Rate from a garbage can audit of Canadian Dairy farms using Python

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Antimicrobial use in food-producing animals has been flagged as a potential source and reservoir of antimicrobial-resistant pathogenic bacteria. However, Canada does not have a system to determine the amount of antimicrobial drugs used on dairy farms. This project developed a system in Python for computing dose based metrics from garbage can audit data.

Garbage can audits were conducted over six to 12 months in 2020 on 94 farms from British Columbia, Alberta, Ontario, and Nova Scotia. Along with the garbage can audit, a pharmacy inventory was taken when the collection began and ended. An algorithm was built in Python to determine the mass of each active antimicrobial ingredient used on each farm during the study period.

To determine the dose based metrics, DDD_{bovCA} and DCD_{bovCA} factors from Lardé et al. (2020) were used to compute the defined daily dose (DDD) and defined course dose (DCD), respectively. This lookup table contains information for every drug Health Canada has approved for use in cattle. Producing DDD and DCD conversion factors is necessarily flawed. However, the values created by Lardé and colleagues is specific to Canadian bovine medicine and has taken care in assigning values for different production phases.

Antimicrobial drug usage rates (ADUR) were calculated automatically by using the computerized system per animal-year. Antimicrobial use in the four main production phases on a dairy farm (milk-fed calves, weaned heifers, lactating cows, and dry cows) is very different, with different ramifications for human public health. It is essential to ensure that producers are compared on an even playing field, even with different demographics. The current code uses the total number of cattle for the denominator, though limiting it to lactating cows has been investigated. Future directions may include determining the DDD and DCD per 10 000 kg of milk per year.

Using traditional methods, data entry typically takes a person with understanding of veterinary medications roughly two hours per herd. Then, conversion to dose based metrics typically takes two to four further hours. This computerized pipeline can produce DDD ADUR and DCD ADUR for 94 herds in 15-30 seconds. The code reliably produced summaries of antimicrobial use separated by route of administration, production phase, formulation, category of importance to human medicine, and class of active ingredient. Beyond running much quicker, the time to produce outputs is nearly independent of the number of farms in the data set, so it should scale to larger number of farms.

Future work will investigate extending this code to veterinary dispensing records, potentially increasing the number of and veterinary practices, and determine differences between dispensing records and garbage can audit results.

Abstract 37: Nikola May

Investigating the plasmid transfer dynamics of *Salmonella enterica* in a Simulated Human Intestinal Microbial Ecosystem (SHIME)

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INTRODUCTION:

The human gastrointestinal tract has been described as a “mixing pot” for dissemination of genetic elements due to the variety of organisms in the intrinsic gut microbiome and the occasional presence of foodborne pathogens. Antimicrobial resistance (AMR) genes are often transferred via conjugation although few studies to date have examined the frequency of plasmid transfer in different environmental conditions. *Salmonella enterica* have been shown to carry many different families of plasmids, which may carry a variety of AMR genes. The purpose of this research is to study how *S. enterica* plasmid transfer dynamics are affected by environmental factors (including pH, bile acids) consistent with transit through the gastrointestinal (GI) tract.

This research aims to better understand gene dissemination within the gut microbiota and the impact of transit through the intestinal tract on plasmid transfer by *Salmonella enterica*. By understanding plasmid dynamics in the gut microbiota, we hope to better control the spread of these plasmids.

MATERIALS AND METHODS:

Preliminary *in vitro* experiments will test how conjugation frequency changes after acid-shock conditions, in the presence of bile salts, and when both stressors are combined. Different *Salmonella enterica* serovars will act as both donors and recipients, and will carry plasmids from different replicon types. All the strains utilized in this study are wild type and are sourced from human, animal, food and environmental sources.

Strains selected after preliminary *in vitro* work will be introduced to a Simulated Human Intestinal Microbial Ecosystem (SHIME). The SHIME will be set with parameters to mimic the human digestive system, including different components having varying pH, established microbial communities, and bile salt concentrations that reflect the natural digestive process. Samples will be taken from various locations and time points related to the digestive process.

ANTICIPATED RESULTS:

We hypothesize that conjugation efficiency under stress conditions will vary between *S. enterica* subspecies and source. These differences may also extend to different plasmid families being transferred. The SHIME results will likely differ from the *in vitro* results because of the SHIME containing an established microbiota. The presence of microbial metabolites and competition will likely impact conjugation efficiency when compared to only having acid shock and/or bile shock stressors.

ANTICIPATED CONCLUSIONS:

This research aims to determine how environmental factors throughout the human digestive system influences conjugation efficiency in *S. enterica,* as well as how *in vivo* conjugation compares to traditional *in vitro* conjugation assays. This research will identify strains that alter their gene uptake or dissemination rates in response to environmental stressors of the GI tract. We will also determine whether this behavior is widespread or specific to strains of *S. enterica* or plasmid types.

Abstract 38: Danielle Julien

A Collaborative Co-Design Platform of an Application for Antimicrobial Prescribing and Stewardship in Veterinary Medicine: CVMA on Spectrum

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INTRODUCTION:

The continuing emergence of antimicrobial resistance (AMR) is well-recognized as a serious threat to global public health. The challenges presented by multi-drug resistant bacterial infections are numerous and negatively impact the One Health ecosystem involving humans, animals and our shared environment. Furthermore, many antimicrobials used in veterinary medicine are considered of high importance in human medicine. The complexity of AMR presents opportunities to operationalize the One Health approach promoting prudent use guidelines and best practices for optimal antimicrobial use in both humans and animals. Some antimicrobial prescribing guidelines exist, but within separate and static platforms. Algorithmic applications ("apps") present a novel resource for integrating existing antimicrobial guidelines, informing optimal use of antimicrobials, and encouraging antimicrobial stewardship in human and veterinary medicine. Therefore, our research question was: *Can an antibacterial algorithmic "app" be developed by and for the veterinary community using selected species-specific syndrome guidelines*?

The aim of our initiative is to co-design an "app" with the veterinary community using a transdisciplinary, multisectoral One Health approach.
Three goals of the research are to:

1. Educate users on antimicrobial stewardship principles and optimal prescribing through the "app" experience;
2. Improve the appropriate antimicrobial utilization for common bacterial infections across commonly treated animal species; and
3. Expand the product in scope, location, and media and improve existing content through incorporation of user feedback in an iterative manner.

MATERIALS AND METHODS:

Our multidisciplinary team includes the Canadian Veterinary Medical Association, academic investigators in Alberta, and Ontario, and software consultants in British Columbia. We have employed a mixed method approach to the co-design of the CVMA on Spectrum (application) with the veterinary community. The design of the study reflects a constructivist approach where knowledge is constructed through collective approaches. We have used the term 'co-design' to describe the process of developing the application collaboratively with the veterinary community. We will use the Participatory Action Research (PAR) methodology that focuses on social change and, through systematic inquiry, fosters collaboration among participants and researchers. This study was approved by the Research Ethics Board at the University of Calgary (REB19-1997). In the winter and spring of 2020, pilot testing of the questionnaire was conducted. We will conduct convenience (snowball) sampling to recruit participants from a target population of rural and urban veterinary practices in Alberta and other Canadian provinces. We will interview veterinary students in academic institutions, and veterinarians and veterinary technicians in small animal, large animal, and mixed animal practice. Participants will be interviewed to gather information on quantitative demographic information and qualitative aspects including antimicrobial prescribing practices; the use of technologies, including "apps" in veterinary medicine; layout and organization preferences for CVMA on Spectrum; and opinions regarding One Health.

ANTICIPATED RESULTS:

We will develop an iOS and Android "app" for veterinary stewardship. Furthermore, through Co-Design consultations, CVMA on Spectrum will become a digital steward for use which will increase adherence to prescription guidelines and reduce antimicrobial resistance in veterinary medicine.

ANTICIPATED IMPORTANT CONCLUSIONS:

The collaborative co-design of Spectrum DVM will support the creation of a reliable and convenient "app" that has the optimal layout and organization best suited for the veterinary community. Based on the successes of Spectrum MD for physicians, we anticipate CVMA on Spectrum will result in improved appropriate antimicrobial utilization for common bacterial infections across commonly treated animal species.

ACKNOWLEDGEMENTS:

Sincere thanks to Helen Buck, Michael Campsall, and Cyrus Greenall at Spectrum Mobile Health Inc, and to Shane Renwick, Jost am Rhyn, and James Dunlop at the Canadian Veterinary Medical Association.

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Abstract 39: Jacob Narbonne

Comparison of indicators for quantifying antimicrobial drug use in Canadian and international salmonid aquaculture from 2004-2018.

Jacob A. Narbonne*[1,2], Brian R. Radke[3], Derek Price[4], Patrick C. Hanington[1], Simon J.G. Otto[1,2,5,6]

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BACKGROUND:

Antimicrobial resistance (AMR) is a unique One Health threat, and salmonid aquaculture industries are increasingly challenged to demonstrate responsible antimicrobial stewardship. Indicators and metrics to quantify antimicrobial use (AMU) data can enhance understanding of use patterns in a comparative manner to inform stewardship policy. The objective of this study is to compare AMU indicators and metrics for Canadian and international finfish aquaculture for antimicrobial drugs.

METHODS:

Total annual salmonid slaughter mass and AMU (total mg) was gathered from Norway, Chile, the United Kingdom (UK), Canada, and British Columbia (BC). We applied the AMU indicator milligrams of active ingredient per Population Correction Unit based on annual salmonid slaughter weights (mg/$\mathrm{PCU}_{\mathrm{Slaughter}}$) using data from these regions for 2004-2018. An additional indicator, mg AMU per adjusted PCU using average treatment weight (mg/$\mathrm{APCU}_{\mathrm{AW}}$) was also applied to annual, anonymized BC AMU data from 2004-2018. The antimicrobials analyzed in this study were: oxytetracycline, florfenicol, sulfadimethoxine + ormetoprim (SO), sulfadiazine + trimethoprim (ST), oxolinic acid, flumequine, and erythromycin.

RESULTS:

When adjusting for annual slaughtered biomass, Chile was consistently the highest user of antimicrobials (Figure 1). The Chilean mg/$\mathrm{PCU}_{\mathrm{Slaughter}}$ was consistently 2-6 times higher than British Columbia and 300-600 times greater than Norway for the entire study period. Norway consistently had among the lowest annual overall AMU based on mg/$\mathrm{PCU}_{\mathrm{Slaughter}}$ estimates. The mg/$\mathrm{PCU}_{\mathrm{Slaughter}}$ decreased in Chile from 2015-2018 despite increasing production, whereas BC fluctuated over the same period. Norway and the UK have both maintained annual mg/PCU levels below 50 mg/PCU throughout the study period. The BC drug-specific mg/$\mathrm{APCU}_{\mathrm{AW}}$ showed greater drops in drug specific use when the species composition of the total slaughter biomass shifted away from Pacific salmon to Atlantic salmon in 2004-2006.

CONCLUSIONS:

Indicators based on PCUs improve the comparability of AMU between regions with different levels of salmonid production. However, PCU-based indicators fail to account for differences in drug potency. If regions use different drugs with marked differences in mg/kg dosing, there can be large resulting differences in mg/$\mathrm{PCU}_{\mathrm{Slaughter}}$ or mg/$\mathrm{APCU}_{\mathrm{AW}}$. Future work will explore other indicators and potential links between AMU and AMR/disease pressure in the context of Canadian Pacific finfish aquaculture. This research will help us to identify useful indicators for reporting AMU data and determine how they can best inform antimicrobial stewardship in the salmonid aquaculture industry.

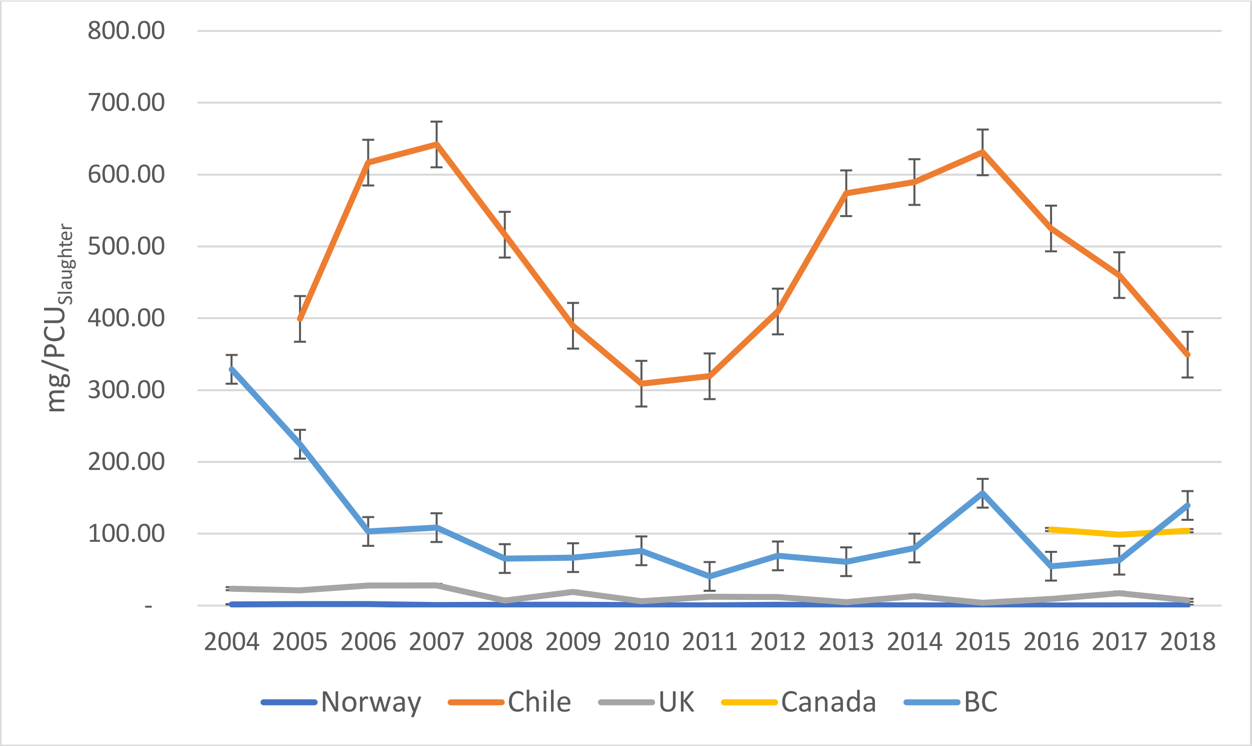

Figure 1. Annual biomass-adjusted overall antimicrobial use (AMU) in top global salmonid producers (mg/PCU$_{\text{Slaughter}}$). Canadian AMU data missing for 2004-2015. Chilean AMU data missing for 2004.

Abstract 40: Julia Louden

Comparison of Antibody Presence of Preconditioning and Traditionally Raised Cattle During the First 40 Days on Feedlot

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INTRODUCTION:

Bovine Respiratory Disease (BRD) is a multifactorial disease that causes significant economic impact on the North American cattle industry each year. Currently, metaphylactic administration of antibiotics is used to mitigate the effects of BRD. However, with antimicrobial resistance growing rapidly, this approach is unsustainable and will become ineffective. Pathogenic colonization of the respiratory tract causes illness in cattle most commonly occurring within the first 40 days after arrival at the feedlot. This period is thought to be crucial due to the stressors cattle are subjected to. Therefore, our approach utilizes specific preconditioning protocols as a way to prepare cattle for life on a feedlot. These protocols gradually introduce calves to various elements of life on a feedlot and include applying a comprehensive vaccination protocol.

MATERIALS AND METHODS:

Preconditioning began at spring processing and continued into the fall with two-stage weaning, gradual introduction to a feed bunk, direct delivery to the feedlot, avoiding cattle markets and an improved vaccination strategy. Upon arrival, preconditioned calves were separated into pens and mixed with traditionally raised calves at various ratios to determine the effects of co-mingling upon preconditioned calves. Cattle were separated into five pens: pen A 100% preconditioned calves, B 75% preconditioned 25% traditionally raised, C 50% preconditioned 50% traditionally raised, D 25% preconditioned 75% traditionally raised, and E 100% traditionally raised cattle. Ten calves from each subset of cattle per pen served as monitoring animals followed throughout the study. Preconditioned calves were sampled during the preconditioning period as well as on the feedlot. Furthermore, both traditionally raised and preconditioned cattle were sampled on arrival at the feedlot, 20 days on feed and, 40 days on feed. Samples collected include deep nasal swabs as well as serum and EDTA blood samples. Serum will be used in enzyme-linked immunosorbent assay (ELISA) to detect circulating antibodies against five viral pathogens commonly associated with BRD: Bovine Coronavirus (BCoV), Bovine Respiratory Syncytial Virus (BRSV), Bovine Viral Diarrhoea Virus (BVDV), Bovine Parainfluenza Virus type 3 (PIV3), and Bovine Herpesvirus 1 (BHV1). Antibody levels will be compared between groups to evaluate if protective antibodies were present and how they varied over time.

ANTICIPATED RESULTS:

It is anticipated that preconditioned calves will have protective antibodies present to BRSV, BVDV, BHV1, and PIV3 as they will have received a booster vaccination 50 days before arrival at the feedlot. Traditionally raised cattle have an unknown vaccination history therefore we suspect a wide variety in antibody titers throughout the study. It is also anticipated that preconditioned calves were better prepared for the feedlot and experience less stress.

ANTICIPATED IMPORTANT CONCLUSIONS:

By supporting the calves' immune system using vaccination and stress reduction, preconditioned calves may experience less BRD leading to a reduction of the need for metaphylactic treatment for calves at arrival, while, at the same time, improving animal welfare.

Abstract 41: Joyce Van Donkersgoed

Canadian Feedlot Cattle Antimicrobial Use and Resistance Surveillance Network: Structure and Function

Joyce Van Donkersgoed[1,5*], Reynold Bergen[2], Calvin Booker[3], Craig Dorin[4], Greg Dimmers[5,6], Nathan Erickson[7], Sheryl Gow[8], Sherry Hannon[3], Steve Hendricks[9], Grace Kuiper[10], Karin Schmid[11], Wendy Wilkins[12]

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Presenting and Corresponding Author*

INTRODUCTION:
In 2019, an antimicrobial use (AMU) and antimicrobial resistance (AMR) surveillance network was initiated in feedlot cattle. The objectives of the network are to 1) provide representative estimates of AMU and AMR in the Canadian finishing feedlot sector; 2) provide a unified approach to monitor trends in AMU and AMR over time; 3) periodically investigate associations between AMU and AMR on a targeted basis in relation to emerging AMR trends; and 4) provide collated industry data for assessment of the potential public and animal health risks associated with AMU in the Canadian finishing feedlot sector to help veterinarians and producers preserve antimicrobial effectiveness and ensure antimicrobial stewardship. The objective of this presentation is to describe the structure and function of the current surveillance system.

MATERIALS, METHODS AND RESULTS:
Most feedlot production in Canada is in Alberta, followed by Ontario and Saskatchewan. The sampling frame includes finishing feedlots with a 1-time capacity >1000 animals that are willing to participate in the surveillance network. The current sampling frame includes 26 Canadian feedlots (total feeding capacity >1 million fed cattle). Feedlots are randomly sampled proportionate to their contribution of fed-cattle production in their province and stratified by 1-time feeding capacity and consulting veterinary practice to ensure representative data across Canada.

Veterinary practices record AMU dispensing data in various software programs to meet the requirements in the Pan-Canadian Framework for Veterinary AMU Oversight. All antimicrobials dispensed by veterinary clinics to participating feedlots per year are tabulated, de-identified and provided to CIPARS.

Most feedlots use computerized animal health software management systems to track all individual animal health morbidity and mortality data (including AMU data from arrival to slaughter) by the animal's unique CCIA and feedlot management tags. CIPARS provides participating feedlot veterinarians with the number of animals to collect specific AMU data from each participating feedlot. Closed lots of fed cattle within feedlot by production year are randomly selected by the vet until the target number of animals is reached. All lot descriptive and AMU data are collated by the enrolling veterinarian, de-identified, and uploaded to system administrators in a non-identified format.

Deep nasopharyngeal swabs are taken in 16 animals per participating feedlot on arrival and rehandling. Samples are taken from different groups and ages of incoming cattle over time. Fecal samples are collected from 10 lots of cattle within 30 days of slaughter per participating feedlot over various seasons. Samples are sent to labs for culture and antimicrobial sensitivity testing. CIPARS analyzes dispensing and feedlot AMU/AMR data. Year 1 results are being compiled and will be reported to participating vets and feedlot producers, network funders, and the industry.

CONCLUSIONS:

The surveillance network is valuable to the Canadian beef industry, veterinarians, federal and provincial governments, and other stakeholders because it helps ensure continued access to efficacious antimicrobials to protect cattle health and welfare. This network demonstrates the beef industry’s social responsibility/accountability to the public/consumers, whilst ensuring global beef trade which is critical for economically sustainable beef production in Canada and food security.

Abstract 42: Sheryl Gow

Canadian Feedlot Cattle Antimicrobial Use Surveillance Network: The Future is Bright

Sheryl Gow[1], Reynold Bergen[2], Calvin Booker[3], Craig Dorin[4], Greg Dimmers[5], Nathan Erickson[6], Sherry Hannon[3], Steve Hendricks[7], Grace Kuiper[8], Karin Schmid[9], , Joyce Van Donkersgoed[10, 11] , Wendy Wilkins[12]

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INTRODUCTION:

Antimicrobial resistance (AMR) is a worldwide health concern that requires focused and ongoing assessment of antimicrobial use (AMU) practices, as well as the monitoring of AMR development in both human and veterinary medicine. Longitudinal data presenting temporal trends in the quantity of AMU and the prevalence of AMR are necessary to help identify emerging issues, support stewardship goals, meet the requirements for AMR and AMU data and demonstrate a national commitment to stewardship. The establishment of an AMU and AMR surveillance system in the Canadian fed-cattle sector in 2019 is contributing scientifically sound data to support the Canadian beef industry and the federal/provincial governments' need for these data, whilst meeting WHO/OIE requirements and the needs of other stakeholders, such as retailers.

MATERIALS AND METHODS:

CIPARS (Canadian Integrated Program for Antimicrobial Resistance Surveillance) has on-going active AMU and AMR sentinel farm surveillance in grower-finisher swine (since 2006), broiler chickens (since 2013), and turkeys (since 2014). In 2019, in collaboration with industry, surveillance in fed beef cattle was initiated. Dairy surveillance was also launched in 2019 in partnership with various academic institutions. AMU data from each commodity is collected systematically for the calculation of quantitative parameters such as number of defined daily doses per animal time, and milligrams of antimicrobial administered adjusting for animal weight and population. These data are collated and provided back to key stakeholders annually.

ANTICIPATED RESULTS:

Examples of the value of AMU surveillance will be provided from CIPARS farm sentinel site program. A discussion on how this knowledge can be valuable to help preserve the use of antimicrobials for animal health and welfare as well as the economic sustainability of animal production will be presented. These data also help the livestock industry and veterinarians identify and collectively address potential emerging AMU/AMR issues. As the feedlot surveillance program gathers additional years of data, it is anticipated that AMU/AMR surveillance in feedlot cattle will support feedlot veterinarians and their feedlot clients as they strive to reduce disease risk, improve treatment efficacy (reduce morbidity and mortality), promote antimicrobial stewardship, support feedlot production sustainability, reinforce consumer and public confidence, and continue the leadership of the Canadian beef industry and feedlot sector in this important area.

CONCLUSIONS:

Canada is an international leader in farm-based surveillance of AMR and AMU which can be used to help support industry sustainability, animal health and welfare, and antimicrobial stewardship. The AMU data that are available are invaluable to the commodities participating in the surveillance system as these data are becoming increasingly required by consumers, retailers and trading partners as evidence of appropriate drug use and to preserve antimicrobial access for animal health.

Abstract 43: Sherry Hannon

A Canadian Feedlot Cattle Antimicrobial Use Surveillance Network: Targeted Research to Ongoing Surveillance

Sherry Hannon[1], Reynold Bergen[2], Calvin Booker[1], Craig Dorin[3], Greg Dimmers[4,5], Nathan Erickson[6], Sheryl Gow[7], Steve Hendricks[8], Grace Kuiper[9], Karin Schmid[10], Joyce Van Donkersgoed [4,11], Wendy Wilkins[12]

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INTRODUCTION:

Globally, widespread antimicrobial use (AMU) in food-producing animals is under increasing scrutiny due to the potential for promotion of antimicrobial resistance (AMR) and its potential implications for human health. Historically, comprehensive data related to the types of antimicrobial drugs (AMDs) used, the extent of AMU, the most common indications for AMU and the demographics of the cattle populations receiving AMDs in Canada have been limited. Segmentation of the cattle industry, fragmentation of the AMD distribution system and loopholes in previous Canadian AMD use policies are likely contributors to information gaps. The Canadian beef cattle industry has a long history of collaboration and support for feedlot cattle-related AMU and AMR research. Historical projects and collaborations have paved the way for the design and successful 2019 launch of the Canadian Fed-cattle Antimicrobial Use Surveillance Program (CanFASP), which includes targeted AMR investigation and AMD dispensing data collection. This presentation highlights both previous and ongoing initiatives which have supported the creation, design, implementation, and continued development and of the CanFASP.

Strong historical and ongoing collaborative efforts amongst industry groups, private veterinary practitioners, feedlot producers, federal government (led by the Canadian Integrated Program for Antimicrobial Resistance Surveillance), provincial governments, initiatives such as the Canadian Animal Health Surveillance System, research funders, academia, and other stakeholders were critical to acquire the knowledge, expertise, and infrastructure to establish the means to collect feedlot cattle AMU data on a large-scale.

RESULTS:

Antimicrobial drugs (AMDs) are important tools for maintaining human and animal health. Prior targeted research projects have provided insight into the complexity of collecting AMU data from the western Canadian feedlot cattle sector, which represents approximately 70% of Canadian feedlot production. Appropriate AMU estimates are required to understand AMU practices, to aid in the interpretation of resistance levels and patterns, to meaningfully assess associated animal and public health risk, and to inform stewardship activities.

CONCLUSIONS:

The CanFASP is an example of how building strong national collaborations amongst many invested stakeholders can fill knowledge gaps, provide data for other commodity and global comparison, and support robust AMU (and AMR) data collection related to feedlot cattle. It is expected that this national AMU surveillance system (specifics of which will be described in other presentations) will continue to promote and support sector sustainability, AMU transparency, and responsibility related to animal care/welfare and public health moving forward. Cultivating collaborations and access to ongoing public and industry funding are key to the continued success of this surveillance network.

Abstract 44: Rahgavi Poopalarajah

Chemical interactions between microorganisms can be exploited to counter antimicrobial resistance and rescue our existing arsenal of antibiotics.

Rahgavi Poopalarajah*,[1] Andrew J. Flewelling,[2] Nathan McCartney[1], Shannon Pyke,[3] Michael D. Parkins,[3] John M. Conly,[3] Kathy D. McCoy,[3] Douglas J. Mahoney,[3] Darren J. Derksen,[2] Joe J. Harrison.[1]

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Bacteria from many natural environments grow in diverse communities in which they compete for limited space and resources. Microbes in these environments can use antimicrobial compounds to thwart their competitors, and therefore, natural antimicrobial resistance may have a role in allowing some bacteria to co-colonize the same spaces as antibiotic producers. Accumulating evidence indicates that some antibiotic-producing organisms have evolved the capacity to subvert the antimicrobial resistance of competitors; however, the scope and prevalence of these interactions among bacteria is not well understood. Here, we identify that a large number of bacterial species have the capacity to produce small molecules that act as natural antibiotic potentiators. We term this ecological phenomenon "counter-resistance." As part of a multi-disciplinary team, I developed a natural product pipeline to search for microbial metabolites that can block carbapenem resistance in *Pseudomonas aeruginosa*, which has been listed by the World Health Organization as a critical priority pathogen for the research and development of new antibiotics. Carbapenems are antibiotics of last resort. This work was enabled by first helping to build the Alberta Microbiota Repository (ABMR), which is an open-source biorepository that contains more than 700 strains of bacteria and fungi, belonging to 354 unique species from 61 genera. These strains were cultivated from various clinical, animal and environmental sources. By high-throughput screening, I tested spent media from ~400 ABMR isolates, in the presence and absence of carbapenem antibiotics, against resistant bacteria. We found that spent media from 21 microbial isolates reduced growth of resistant *P. aeruginosa* by 2.5- to 40-fold, when combined with antibiotics. In collaboration with natural product chemists, we isolated and identified the bioactive compounds from the spent medium of *Neisseria perflava*, *Staphylococcus lugdunensis*, and *Aeromonas media*, using chemical fractionation, mass spectrometry and nuclear magnetic resonance techniques. All of these strains produced oleamide, which is an *N*-acylamide derivative of a monosaturated fatty acid essential in human and animal health. I showed that oleamide itself lacks antimicrobial properties but instead functions to re-establish the activity of carbapenems via checkerboard assays. Structure activity relationship studies identified that the amide group as well as the position of carbon-carbon double bonds are important for the antibiotic adjuvant activity of natural and synthetic *N*-acylamides. A chemical genetic analysis revealed that oleamide compromises the Gram negative outer membrane; however, a Gram positive spectrum of activity indicates that *N*-acylamides target additional resistance determinants in bacteria. Taken together, our results not only identify that many bacteria produce *N*-acylamides that function as natural antibiotic adjuvants, but also reveal that chemical microbe-microbe interactions that drive antimicrobial resistance are far more prevalent than previously estimated. The discovery of oleamide serves as a proof-of-principle for these counter-resistance mechanisms in bacteria from diverse environments. Overall, this research highlights a key knowledge gap in our understanding of antimicrobial resistance and provides information that may be used to devise ecologically-based strategies to mitigate antimicrobial resistance in humans, plants or animals.

Abstract 45: Kayla Strong

Using data from surveillance and research to inform animal health and One Health initiatives

Kayla Strong[1,2] (Presenter, Ph.D. Student)*, Richard Reid-Smith[2,3,4], Carl Ribble[1], Cheryl Waldner[5], Simon J. G. Otto[2,6], Sylvia Checkley[1,2]

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INTRODUCTION:

Enterococcus spp. is a gram-positive bacterium found in the intestinal tract of mammals, including humans, but can cause serious infections when it enters other parts of the body. These infections will become increasingly difficult to treat due to a rise in antimicrobial resistance (AMR). Probable factors associated with AMR include the use of antimicrobials in humans and animals. Current research focuses on how AMR can be transferred via bacteria or genes within the food production system and the potential interventions that may reduce transmission while maximizing animal and environmental health.

Transmission of AMR *Enterococcus* spp. within the Canadian beef production system is investigated through five research questions, based on existing knowledge gaps:

1) What are the human health risks from foodborne AMR *Enterococcus* spp. in Canadian beef products?
2) What factors within beef production contribute to the prevalence of AMR *Enterococcus* spp. in Canadian beef cattle and beef products?
3) What are the appropriate risk mitigation and control strategies to manage foodborne AMR *Enterococcus* in the Canadian beef production chain?
4) How can risk modelling use existing literature to evaluate human health risks from foodborne AMR *Enterococcus* spp. in Canadian beef products?
5) How can users evaluate modelling tools for their effectiveness in communicating human health risks from foodborne AMR *Enterococcus* spp. in Canadian beef products?

METHODS:

The research questions will be addressed through five methodologies:

1) Develop a risk profile to assess human health risks from foodborne AMR *Enterococcus* spp. in Canadian Beef products. The risk profile will adhere to Codex 77 guidelines (CAC/GL 77- 2011) to ensure transparency and reproducibility.
2) Identify factors influencing the prevalence of enterococci AMR in the Canadian beef cattle production system, identified through a double-blinded scoping review.
3) Develop an integrated assessment model (iAM) specific to *Enterococcus* spp. AMR within the Canadian beef production system. The iAM will reflect factors collected during the scoping review and follow methodology developed by the iAM.AMR project.
4) Develop an assessment standard for evaluating AMR Transmission Models, based on One Health priorities. The standard will be developed through stakeholder interviews and provide transparency in model assessments.
5) Compare developed and existing AMR transmission models using developed assessment standards. Recommend use for differing modelling approaches.

RESULTS:

Preliminary results suggest monensin, Tylosin, ceftiofur, virginiamycin, and copper supplementation are statistically significant factors influencing the prevalence of enterococci AMR in the Canadian beef cattle production system. Transmission models are anticipated to (1) facilitate interdisciplinary interventions on antimicrobial use and cattle management, and (2) provide platforms to explore interventions in contributing factors.

ANTICIPATED CONCLUSIONS:

Research outcomes will help identify best practices on antimicrobial use in cattle health and beef production practices, directly impacting human and public health. This process will demonstrate the value of existing research and surveillance data for decision-making and policy development. It will also highlight the strengths and weaknesses of using different models for these purposes.

Abstract 46: Etienne De Jongh

One world, one hive: A scoping review of honey bees, climate change, pollutants, and antimicrobial resistance.

De Jongh, E.J. (P)[1], Harper, S.L.[2], Yamamoto, S.S.[2], Wright, C.J.[2], Wilkinson, C.W.[3], Otto, S.J.G.[2]

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p. Presenting Author

INTRODUCTION:
Anthropogenic climate change and increasing antimicrobial resistance (AMR) together threaten the last 50 years of public health gains. Honey bees are a model One Health organism to investigate interactions between climate change and AMR. The objective of this scoping review was to examine the range, extent, and nature of published literature on the relationship between AMR and honey bees in the context of climate change and environmental pollutants.

MATERIALS AND METHODS:
The review followed systematic search methods and reporting guidelines. A protocol was developed a priori in consultation with a research librarian. Resulting Boolean search strings were used to search Embase® via Ovid®, MEDLINE®, Scopus®, AGRICOLA™ and Web of Science™ databases. Two independent reviewers conducted two-stage screening on retrieved articles. To be included, the article had to examine honey bees, AMR, and either climate change or environmental pollution. Data, in accordance with Joanna Briggs Institute guidelines, were extracted from relevant articles and descriptively synthesized in tables, figures, and narrative form.

RESULTS:
A total of 21 articles met the inclusion criteria, with almost half of all articles being published in the last five years (n=10/21). These articles predominantly investigated hive immunocompetence and multi-drug resistance transporter downregulation (n=10/21), susceptibility to pests and disease (n=15/21), especially American foul brood (n=9/21), and hive product augmentation (n=3/21).

CONCLUSIONS:
This review identified key themes and gaps in the literature, including the need for future interdisciplinary research to explore the link between AMR and environmental change evidence streams in honey bees. We identified three potential linkages between pollutive and climatic factors and risk of AMR. These interconnections reaffirm the necessity of a One Health framework to tackle global threats and investigate complex issues that extend beyond honey bee research into the public health sector. It is integral that we view these “wicked” problems through an interdisciplinary lens to explore long-term strategies for change.

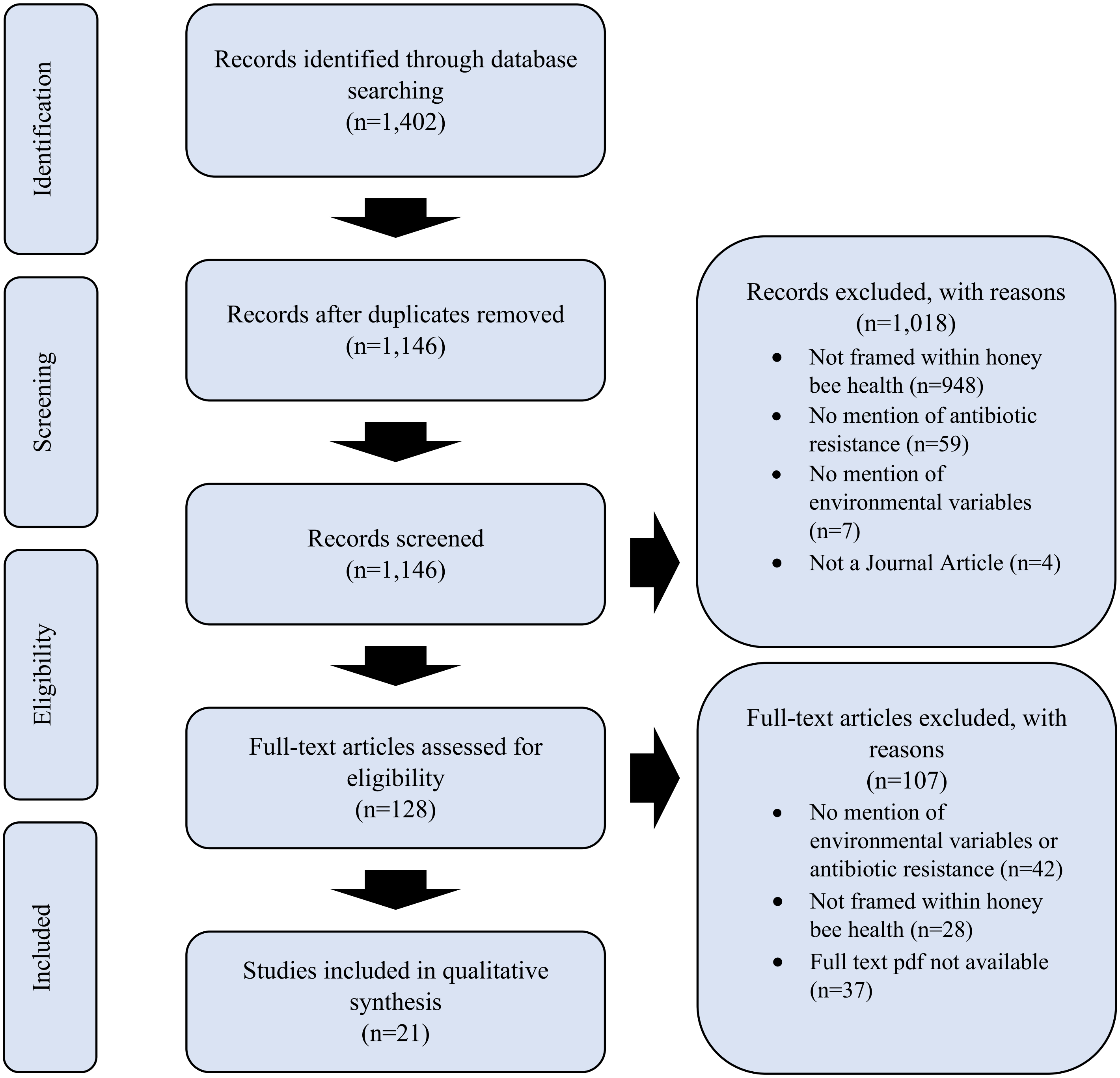

Figure 1. PRISMA flow diagram of the study selection process.

Abstract 47: Matthew Stuart-Edwards

Application of PromethION long-read sequencing for the detection of mobile genetic elements in multi-bacterial sample.

Matthew Stuart-Edwards[1,2], Rahat Zaheer[3], Luke Saville[1,2], Chad Laing[4], Athanasios Zovoilis[1,2]and Tim McAllister[3]

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INTRODUCTION:

Mobile genetic elements, such as Integrate Conjugative Elements (ICE), are a method of horizontal gene transfer in bacteria where new genes can be integrated into the host organism but are also excised, replicated and transferred to other recipients. ICE are of interest as they may contain AMR genes and are therefore a vector for spreading resistance. Identification and tracking of these elements with conventional next generation sequencing techniques is challenging. To detect an ICE that has been integrated with a bacterial genome, a sequencing approach that will produce reads spanning both the genome and the ICE is necessary. To this end, long read sequencing is necessary as short reads indicate the presence, but not point of integration of the ICE into the genome. Long read sequencing such as that provided by the Nanopore PromethION platform enable the opportunity for high throughput identification of ICEs. Here we evaluate for the first time the applicability of this technology platform for this scope.

MATERIALS AND METHODS:

Eight bacterial strains from the Bovine Respiratory Disease complex were sequenced on an Oxford Nanopore Technologies PromethION sequencer both with barcoding the bacteria and pooling the samples to emulate an environmental sampling. After sequencing, a de novo assembler called Flye (doi:s41592-020-00971-x) was used to generate assemblies. The assembler was used in metagenome mode for the mixed samples and individually on each barcoded sample to recreate the bacterial genomes with the integrated ICE sequence.

RESULTS OR ANTICIPATED RESULTS:

PromethION sequencing of the mixed bacteria sample was successful and produced more than 15 million reads and 44.3 billion bases with an average length of 3620 bases. The barcoded sequencing was also successful and produced 9.6 million reads and 23 billion bases with an average read length of 2487 bases. Of interest, the longest read produced was 306,073 bases, which is longer than the sequence of the target ICE.

A NextFlow (doi:10.1038/nbt.3820) pipeline was created to parallelize the Flye assembly and ICE detection process for all barcodes. Flye was executed in metagenome mode for the mixed bacterial samples. The metagenome assembly was unable to properly integrate the ICE and more work is needed to find a better way to use environmental samples. The barcoded samples were successfully assembled with the integrated ICE sequence. Confirming the integration of the ICE was performed by aligning the ICE sequence against the assembled genome with minimap2 (doi:10.1093/bioinformatics/bty191), and the presence was confirmed by Illumina sequencing.

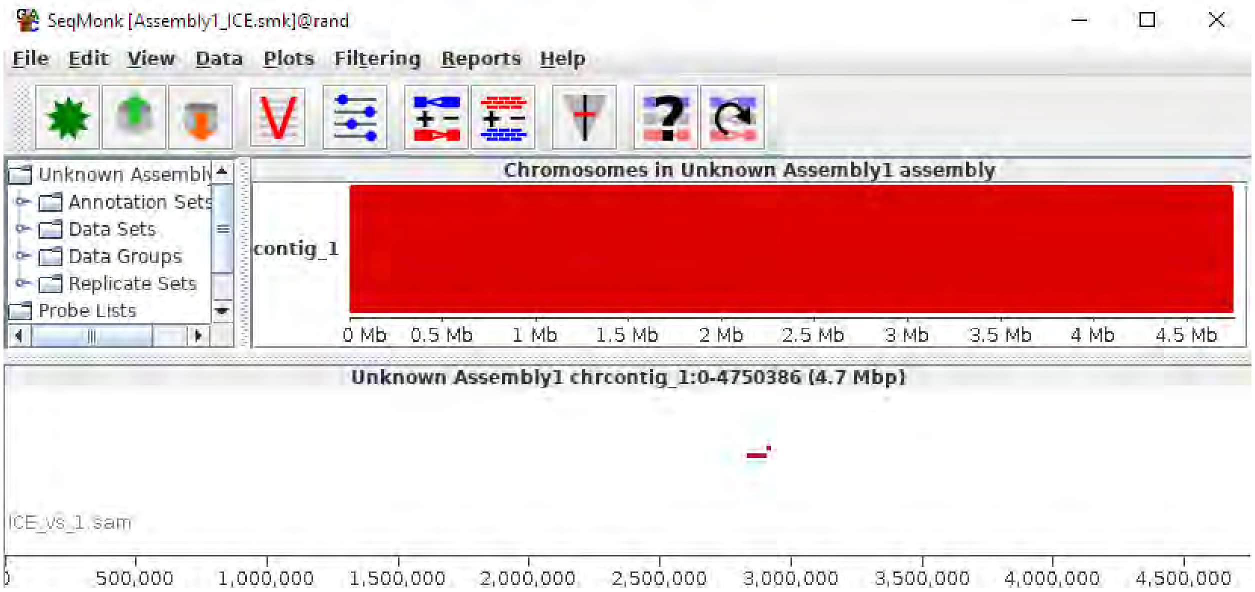

Figure 3:SeqMonk visualization. The assembly of barcode 1 bacteria and the location of the mapped ICE sequence within that assembly showing the integration at around 2.8 Mbp.

CONCLUSIONS OR ANTICIPATED IMPORTANT CONCLUSIONS:

Our analysis indicates that it is possible to locate and assemble the integrated ICE sequence from a de novo assembly of Nanopore reads of the barcoded bacterial sample, which is a very difficult task with short-read sequencing technologies. Future analysis will focus on publishing a pipeline to automate this process and providing tools for determining the genomic insertion location.

Abstract 49: Karan Varshney

Risk Factors for Incurable Drug-Resistant Tuberculosis: A Systematic Review

Karan Varshney, MPH[1,2,3], Beverly I. Anaele, MPH[3], Rosemary Frasso, PhD, MSc, MSc, CPH[3]

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INTRODUCTION:

Tuberculosis (TB) caused more deaths worldwide in 2018 than any other single infectious disease. In recent years, there has been an upsurge in cases of drug-resistant TB, and strains of TB resistant to all forms of treatment have begun to emerge. There is an urgent need to prevent the spread of TB that is incurable; knowing the risk factors can inform prevention efforts. Hence, the aim of this study was to systematically review the worldwide literature regarding the risk factors for TB that is completely resistant to all forms of treatment. To our knowledge, this is the first systematic review to examine the risk factors for incurable drug-resistant TB.

MATERIALS AND METHODS:

For this systematic review, two researchers independently conducted searches of the literature from Proquest, PubMed, and Scopus using the Preferred Reporting Items for Systematic Review Approach (PRISMA). A research librarian was consulted to create reproducible search strategies. Articles were included if they included at least 10 patients with the highest levels of drug-resistant TB who had poor outcomes, if they examined risk factors, were published after 2010, and were published in English. There were no restrictions based on country of study location. Data extracted from articles included in the review included study characteristics such as location, sample size and study design, as well as patient characteristics including age, sex, comorbidities, prior diagnosis of TB, and other risk factors.

RESULTS:

Searches generated a total of 2825 articles, and of those, 25 were included in the final analysis. We found that the most commonly reported risk for patients developing drug-resistant TB was having a history of TB. Other important risk factors were human immunodeficiency virus (HIV), a history of incarceration, low body mass, being a smoker, alcohol use, unemployment, being male, and being middle-aged. There was limited evidence indicating that being a migrant, being married, having a Beijing genotype of disease, and having had adverse reactions to TB treatment were risk factors.

CONCLUSION:

Increasing treatment adherence rates is urgently needed to control the spread of incurable TB. Prioritizing better treatment access for those with comorbidities is also crucial. Though a meta-analysis was not possible due to differing methodologies of the articles, this review's findings offer valuable insights for the prevention of incurable TB.

Abstract 50: Sani-e-Zehra Zaidi

Can carbapenem resistant Enterobacteriaceae species be isolated from beef cattle and their environment?

Sani-e-Zehra Zaidi[1,2], Krysty Thomas[1], Chad Laing[3], Rahat Zaheer[1], Athanasios Zovoilis[2*], and Tim McAllister[1*]

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INTRODUCTION:

Multidrug resistant bacteria represent a major health threat globally, and are associated with increased morbidity and mortality in humans. Carbapenems are considered the last line of defense for many healthcare-associated infections. One of the greatest concerns are emerging carbapenem resistant Enterobacteriaceae (CRE). At present, antibiotic options for the treatment of carbapenem-resistant Enterobacteriaceae (CRE) are limited, with polymyxins, tigecycline, fosfomycin, and aminoglycosides as the mainstays of therapy. Despite the fact that carbapenems are not approved for use in veterinary medicine, carbapenem resistant Enterobacteriaceae species (*Klebsiella oxytoca , K. pneumoniae, Citrobacter freundii, Enterobacter cloacae, Raoultella spp., E. coli, and Klebsiella intermedia*) have been recovered from surface and sewage water in North America. Additionally, a metagenomic analysis in the United States reported the prevalence of carbapenemase gene (blaKPC-2) in beef cattle raised in both conventional and natural feedlots. The functional blaKPC-2 gene could be transformed and expressed in a *E.coli* TOP10 cell. Therefore, there is a risk that CRE exist within the beef production environment and could potentially be disseminated to humans.

MATERIALS AND METHODS:

This study includes carbapenem resistant bacteria collected from fecal composite from 301 pens (n=156 conventional pens; n=145 natural pens) harbouring approximately 180 cattle per pen from both conventional (n=2) and natural (n=2) feedlots over a two year period (from August, 31 2016 to June, 25 2019) using enrichment. Briefly, 10 – 15 g of feces were collected from ~20 fresh fecal pats within a pen and mixed. A subsample (10 g) of feces was placed in a transport vial containing Cary-Blair media. For enrichment, fecal samples (0.5 g) were inoculated in 1/10 dilution (~0.5 g in 4.5 mL) of EC broth with etrapenem (2µg/ml) followed by overnight incubation at 37°C in a shaking incubator at 250 RPM. After incubation, the samples were sub-cultured on MacConkey agar containing etrapenem (0.5µg/ml). Three colonies from each sample were selected. Bacterial species were characterized by 16S rRNA gene amplification using 27F and 1492R primers followed by sequencing of the PCR amplicon. Phenotypic and genotypic characterization of carbapenem resistant isolates are ongoing.

RESULTS:

A total of 100 carbapenem-resistant isolates were obtained. Based on 16S rRNA, 82% of the isolates (n=68) were *Pseudomonas* with 40.0% of these being *P. aeruginosa (n=27).* Additional genera included *Escherichia* (9.6%), *Acinetobacter*. (4.8%) and *Ochrobactrum* (2.4%). Identification of mechanism of resistance in these isolates is currently in progress.

ANTICIPATED CONCLUSIONS:

This study focused on investigating the prevalence of carbapenemase resistant Enterobacteriaceae in feedlots. Most of the putative carbapenem-resistant isolates obtained in this study were members of the Pseudomonadaceae, although carbapenem-resistant Enterobacteriaceae, including *Escherichia coli* were isolated at lower frequency. Carbapenem-resistant *P. aeruginosa* have been described in a number of clinical settings around the world and are often clonal, with metallo-beta-lactamases identified as one mechanism of resistance. However, the role that Pseudomonads may play in the dissemination of carbapenem-resistant genes to environmental microbiota is unknown. Future work will focus on characterizing the mechanisms and mobility of carbapenem-resistant genes among members of feedlot microbiota.

Abstract 51: Clair Firth

Benchmarking dairy farms – using the Defined Daily Dose (DDD_{vet}) and Defined Course Dose (DCD_{vet}) metrics as defined by the European Medicines Agency

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INTRODUCTION:

Since 2014, farm veterinarians have been required by the Austrian authorities to report the amount of antimicrobial substances that they dispense for use in food-producing animals. Using these data, the Austrian authorities and some production sectors (such as the poultry industry) have begun to provide farm veterinarians and producers with benchmarking data to allow them to anonymously compare their antimicrobial use (AMU) with that of colleagues and between farms.

MATERIALS AND METHODS:

To trial the benchmarking scheme, a pilot study collated antimicrobial use data from 17 veterinary practices covering AMU on 251 dairy farms over a one-year period. Data collected included both antimicrobial use and dispensing data and were therefore more comprehensive than statutory reporting. Animal data (e.g. calving date, movement data) were available for all farms. Metrics for assessing AMU were based on the Defined Daily Dose (DDD_{vet}) unit described by the European Medicines Agency. Dry cow therapy was quantified as Defined Course Doses, DCD_{vet} and corrected with respect to the average calving interval and replacement rate on farm. For the study population, the total DDD_{vet}/cow/year for each farm was calculated, as well as DDD_{vet}/cow/year for a variety of disease indications, such as mastitis, and DCD_{vet}/cow/year for dry cow therapy.

RESULTS:

In order to enable benchmarking between farms, whereby a certain farm can compare their own AMU with that of the median and/or 75th percentile of the AMU of the total study population or their immediate neighbors etc., radar charts (spiderwebs) were created (Fig. 1). In the example shown here, Farm X uses a much higher overall level of AMU for all disease indications than either the median or 75th percentile of the dairy population, with particularly high use for udder disease and to a lesser extent, dry cow therapy. This chart demonstrates ideal areas for the farm veterinarian to discuss a more prudent use of antimicrobials with the farmer, e.g. the use of selective dry cow therapy and methods of mastitis prevention.

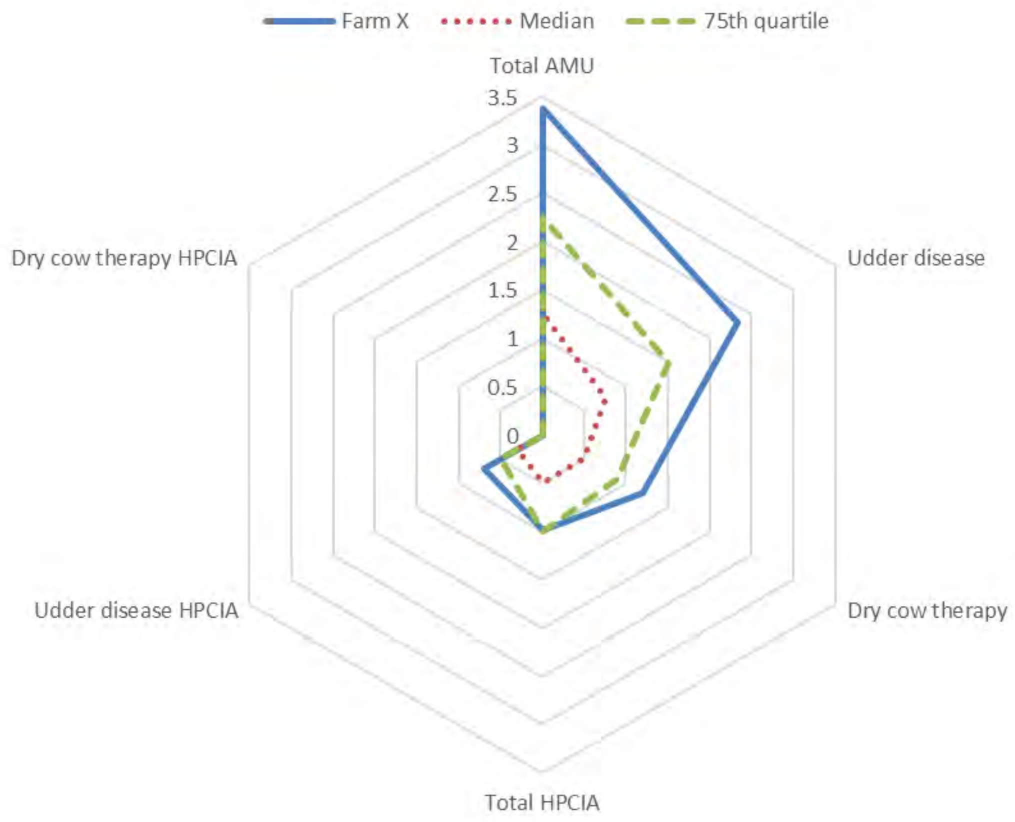

Figure 1: Antimicrobial use radar chart (in Defined Daily Doses/Defined Course Doses) comparing Farm X with the median and 75th percentile of the AMU of the study population

CONCLUSIONS:

The use of benchmarking radar charts provides a simple way for veterinarians and authorities to demonstrate to dairy farms where their AMU is either above or below that of the production sector average. In comparison to the commonly used mg/kg or mg/PCU metrics, DDD_{vet}/cow/year do not encourage the use of HPCIAs. When promoting prudent antimicrobial use, it is, however, essential to remember that animal welfare legislation as well as veterinary ethics, require the treatment of sick animals and the prevention of unnecessary suffering.

Abstract 52: Ayesha Athar

Optimizing the Usability of Hospital Antibiograms: A Step Towards Efficient Diagnostic Stewardship (STRIDE)

A. Athar[1], K.Gonasalves[2]

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BACKGROUND:
In 2018, fifteen Canadians died each day as a direct result of antimicrobial resistance. Trends predict that antimicrobial resistance will kill nearly 400,000 Canadians by 2050. Appropriate prescription of antibiotics is vital to decrease the spread of antibiotic resistance. To prevent and manage bacterial resistance, clinicians must prescribe antibiotics by carefully considering all of the patient and environmental factors. Crucial factors include resistance patterns and susceptibility to pathogens for several antibiotics that are seen within an institution or geographical region. For clinicians to have an overview of the resistance patterns of particular pathogens, Antimicrobial Stewardship Programs in Canada have developed clinical decision-making tools-Antibiograms. Studies have supported that antibiogram consultation and their implementation reduces the number of antibiotic prescriptions in clinical institutions. Despite their availability, there is a significant lack of Antibiogram consultation both in hospitals and in primary care. Our study aims to increase the use of antibiograms by first understanding the needs and concerns of the direct users of this tool such as, physicians, and then to develop a solution that caters to their needs.

METHODOLOGY:
This project is a multicenter mixed-method study conducted at the University of Toronto affiliated hospitals. It consists of two phases. First, a needs assessment will be performed using a cross-sectional survey with both open-ended and close-ended questions inquiring about the attitudes, knowledge and behaviour of clinicians as well as the organizational barriers regarding consultation of antibiograms. Clinicians will complete online surveys within the following hospital units: Antimicrobial Stewardship Program (ASP), Infection Prevention and Control (IPAC), Internal Medicine units, ICU Departments, Emergency Departments, and Surgical Units. In the second phase, with the results of the first phase co-creation sessions will be conducted with the clinicians at one of the participating sites, and based on the identified needs solutions will be developed.

ANTICIPATED RESULTS:
Using an anticipated participation rate of 16%, we hope to survey at least 400 physicians across six sites Survey data will be analyzed in a descriptive manner, reporting counts and percentages for categorical variables and means and standard deviations for continuous variables. Qualitative data will be evaluated using standard coding techniques. Results from this study will be used to co-create potential solutions with clinicians. Future work involves the development of strategies for the implementation of these solutions. This collaborative approach that provides a solution to a genuine need makes this study unique.

ANTICIPATED CONCLUSIONS:
We are hoping that data gathered in this study will add to the existing literature on antibiograms, as well as set a strong foundation for developing hospital-wide empiric antibiotic policies within the University Health Network (UHN). Ultimately, this study will contribute to improving the appropriate prescription of antibiotics and aims to reduce antibiotic resistance.

Abstract 53: Erda Rame Hau

Associations between the presence of antimicrobial residues in fresh milk and farm-level antimicrobial stewardship indicators in six major city centres in India and Nepal: a multicentre study

Erda Rame Hau[1,*], Minu Sharma[2], Jasbir Singh Bedi[3], Nicholas Clark[1], Peter Sly[4], Mannish Kakkar[5], Ricardo Soares Magalhaes[1,3**]

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INTRODUCTION:
Poor antimicrobial stewardship in production animal farming can result in the presence of antimicrobial residues in animal derived foods, which pose a significant public health hazard for the development of antimicrobial resistant bacteria. The presence of antimicrobial residues in animal products for human consumption such as milk can be partly explained by farm-level farming practices and farmer's knowledge and perspectives towards antimicrobial use. This study aims to quantify the association between the presence of antimicrobial residues in fresh milk of peri-urban farms in large urban centres of India and Nepal and farm-level antimicrobial stewardship indicators.

MATERIALS AND METHODS:
A series of cross-sectional surveys were conducted between 2016 and 2019 across a total of 4,656 dairy cattle in 651 farms peri-urban areas of 6 major cities centres in India (n=5) and Nepal (n=1). High-performance liquid chromatographic (HPLC) method was used for the simultaneous determination of sulfamethazine, sulphadiazine, sulphamethoxazole, oxytetracycline, doxycycline, tetracycline, enrofloxacin and chloramphenicol residues in bovine milk. A structured questionnaire and direct interviews were used to collect data regarding farm biosecurity characteristics, farming practices, animal health and hygiene, use of antibiotics in dairy production and attitudes and practices towards antimicrobial usage. Data were summarized using descriptive statistics and antimicrobial stewardship indicators associated with the presence of antimicrobial residues were analysed using generalised linear mixed effects regression models.

RESULTS OR ANTICIPATED RESULTS:
The analysis revealed that the prevalence of antimicrobial residues in fresh milk in the five large urban centres of India ranged from 10% to 16%, while it was 89% in one city of Nepal. In terms of antimicrobial types, tetracyclines were the predominant antimicrobial type found in all cities of India with 54% to 81% of positive samples, while sulfamethazine was the predominant in Nepal found in 69% of milk samples. Our results show significant associations between antimicrobial residues and farming practices and farmers perspectives towards antimicrobial usage. The presence of antimicrobial residues in milk were associated with the distance of farms to veterinary practices, vaccination status of the livestock, ease of access to antimicrobials, use of human antibiotics in animals, perceived animal health risks of not using antibiotics, the perceived benefits of using antimicrobials in improved milk production and the perceived trade-off of affordability of using antibiotics than good farm hygiene and sanitation.

CONCLUSIONS OR ANTICIPATED IMPORTANT CONCLUSIONS:

Our results indicate that antimicrobial residues in fresh milk presents a significant public health hazard in the six major city centres of India and Nepal. These results highlight the importance of farm-level antimicrobial stewardship programs that target improved antimicrobial farming practices and also delivering adequate knowledge to farmers on antibiotics usage and their impact on animal and human health.

Abstract 54: Theresa Oduol

The Effect of Diagnostic Interventions on Antimicrobial Prescribing Patterns for Uncomplicated Canine Urinary Tract Infections

Theresa Oduol[a], Casey Cazer[b], DVM, PhD, Patrick Carney[c], DVM, PhD, Laura Harrington[a], PhD (Corresponding)

[a] Department of Entomology, Cornell University College of Agriculture and Life Sciences, [b] Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, [c] Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine

The emerging threat of antimicrobial resistance within veterinary medicine can be mitigated through evidence-based medicine. Antimicrobials are commonly prescribed for canine urinary tract infections (UTIs) with or without evidence of the causative agent and its susceptibility to different antimicrobials. Typically, veterinarians diagnose uncomplicated UTIs using either urinalysis (UA) only, urine culture with antimicrobial susceptibility testing, or clinical signs only. We extracted data from Cornell University's Hospital for Animals electronic medical records to investigate how different diagnostic interventions shape the prescribing patterns (antimicrobial type, dosage, duration) for uncomplicated canine UTIs in adult dogs with no predisposing conditions. We aim to compare adherence to International Society of for Companion Animal Infectious Disease (ISCAID) antimicrobial use guidelines between canine UTIs diagnosed with either a urine culture, urinalysis or just clinical signs. Our expected results are that ISCAID antimicrobial guidelines are followed more closely when veterinarians diagnose and treat a UTI using a urine culture along with antimicrobial susceptibility testing (AST). This is because urine cultures identify the causative agent while AST determines which treatment is best suited for eliminating the infection. Therefore, veterinarians are equipped with the tools to best determine the best treatment for patients based on this information. Our work will emphasize the importance of appropriate prescribing to mitigate the risk of relapsed infection, overprescribing, and the emergence of antimicrobial resistance. More importantly, it further conveys why the use of diagnostic tools in assisting medical professionals yields better outcomes for the patient.

Abstract 55: Shannon Pyke

Effects of compounds produced by commensal bacteria on antibiotic-resistant *Pseudomonas aeruginosa* infections

Shannon Pyke[a*], Christina Ohland[a], Rahgavi Poopalarajah[b], Joe Harrison[b], Kathy D. McCoy[a]

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[b]*Department of Biological Sciences, University of Calgary.*

*Presenting author

INTRODUCTION:

The discovery of antibiotics is considered to be one of the most significant medical discoveries of all time. However, in recent years the emergence and spread of antibiotic-resistant bacterial strains has become a global health threat, prompting the World Health Organization to publish a global priority list of antibiotic-resistant bacteria. This includes *Pseudomonas aeruginosa,* a Gram-negative pathogen that causes life-threatening healthcare-associated infections. There is urgent need to discover new and effective antibacterial therapies to combat antimicrobial resistance. One such strategy is to identify naturally occurring small molecules, called antibiotic adjuvants, which do not act as antibiotics on their own, but are able to increase the efficacy of antibiotics.

MATERIALS AND METHODS:

This project will investigate the ability of small molecules produced by commensal bacteria to increase the susceptibility of antibiotic-resistant pathogens to existing antibiotics. Human isolates of the commensal bacteria *Neisseria perflava* and *Staphylococcus lugdunensis* have been identified *in vitro* as producers of specific metabolites that reduce the growth of antibiotic-resistant *P. aeruginosa* when given in combination with antibiotics (Dr. Joe Harrison's lab – unpublished data). In this project we will colonize germ-free C57BL/6 mice with human isolates of *N. perflava* and *S. lugdunensis* or the human pathogen *P. aeruginosa* via oral gavage to investigate whether bacterial production of an antibiotic adjuvant *in vivo* can potentiate the effects of antibiotics on the pathogen *P. aeruginosa.*

We hypothesize that the bacteria-derived metabolites identified through *in vitro* screens will increase the sensitivity of *P. aeruginosa* to antibiotic treatment *in vivo*.

RESULTS:

Currently, we have successfully monocolonized mice with *S. lugdunensis* and *P. aeruginosa*, as shown by full length 16S rRNA gene sequencing of DNA isolated from mouse fecal samples. Bacterial loads were stable over a one month period after monocolonization as shown by fecal bacteria culture. Stool samples were plated on LB media and LB media containing the antibiotic meropenem to confirm that *P. aeruginosa* remained antibiotic-resistant. To determine the colonization dynamics during bicolonization, *P. aeruginosa* monocolonized mice have now been co-housed with *S. lugdunensis* monocolonized mice. Bicolonized mice will then be treated with meropenem antibiotics, to determine whether the presence of metabolites produced by *S. lugdunensis* will increase the susceptibility of *P. aeruginosa* to antibiotics.

In contrast to *S. lugdunensis* and *P. aeruginosa, N. perflava* has not been able to stably monocolonize germ-free mice. We will therefore attempt to bicolonize *N. perflava* with *P. aeruginosa* or *S. lugdunensis* to determine whether the presence of other bacteria can aid in colonization.

In further studies we will administer the purified identified metabolite to *P. aeruginosa* monocolonized mice concurrently with meropenem to directly determine antibiotic adjuvant activity.

CONCLUSIONS:

Through the discovery of antibiotic adjuvants produced by commensal bacteria, this study will pave the way for microbial-based therapies to combat the rise of antibiotic resistance and allow existing antibiotics to be more effective.

Abstract 56: Januana Teixeira

Employing a One Health and model location approach to ascertain risk, and to identify key reservoirs and transmission mechanisms of antimicrobial resistant *Campylobacter jejuni* towards effective mitigation

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*Post-doctoral fellow and presenter

INTRODUCTION:

Campylobacteriosis, incited by *Campylobacter jejuni* is a prevalent enteric disease in Canada and elsewhere in the world. Adjustments for under-reporting have estimated the per capita rates in Canada to be 450 cases/100K. The primary treatment option for acute campylobacteriosis, when indicated, is the administration of antibiotics, primarily macrolides and fluoroquinolones. The possibility of increasing rates of treatment failure due to resistance development is a significant issue globally. Moreover, the epidemiology of campylobacteriosis is currently enigmatic. Poultry is considered to be a primary reservoir, and handling and/or consumption of undercooked poultry the principal mechanisms by which *C. jejuni* is transmitted to people. However, the bacterium is found in an array of animal and environmental reservoirs. Emerging evidence is implicating beef cattle as an important reservoir of *C. jejuni* infecting people, although not likely via consumption of meat. The transmission pathway of high risk subtypes of *C. jejuni* among cattle, poultry, and people is currently unresolved. Primary goals of our research are to employ a regional One Health approach using a model study location in conjunction with molecular epidemiological methodologies to ascertain risk, and to identify key reservoirs and transmission mechanisms of antimicrobial resistant *C. jejuni* towards effective mitigation.

MATERIALS AND METHODS:

Southwestern Alberta (SWA) was chosen as the model study location. This location has high rates of campylobacteriosis, a single public diagnostic facility, a moderately sized human population (<200K) representing a mixture of urban (60%) and rural (40%) residents, and high densities of livestock. *C. jejuni* isolates from diarrheic humans, primary livestock species (e.g. beef cattle and poultry), and surface waters in the region were isolated and fingerprinted by Comparative Genomic Fingerprinting (CGF). CGF data along with metadata (e.g. phenotypic resistance to antibiotics) were accessioned into the Canadian *Campylobacter* CGF database (C3GFdb).

RESULTS:

Approximately 10,000 *C. jejuni* isolates from SWA were recovered and fingerprinted. To ascertain the risk to people from *C. jejuni* resistant to antibiotics, temporal changes in resistance for isolates recovered from livestock and people were examined. Strikingly, resistance to medically important fluoroquinolone antibiotics (ciprofloxacin) increased ten times in the ≈1,300 diarrheic people examined over a 15 year period (2004-2018), peaking at ≈30%. The majority of infections with FQR *C. jejuni* isolates were determined to be domestically acquired, and primarily by subtypes that are prevalent in beef cattle, but also found in poultry reservoirs. Fluoroquinolone use is restricted in cattle and poultry production in Canada, and our findings indicate that the mechanisms of FQR emergence and transmission are complex. We are currently using SWA and the data within the C3GFdb in conjunction with empirical experimentation (mammalian and avian models) to identify mechanisms of FQR emergence and transmission. In this regard, we have used CGF and corresponding metadata to select, sequence, and analyse ≈3,000 relevant *C. jejuni* genomes.

CONCLUSIONS:

Our findings indicate that elucidation of the dynamics of emergence and transmission of resistant *C. jejuni* strains within and from cattle and chicken reservoirs should be prioritized. Further, our research highlights the advantage of applying a regional 'One Health' approach.

Abstract 59: Jason Vanstone

The Viral Prescription Pad - A Mixed Methods Study to Determine the Need for and Utility of an Educational Tool for Antimicrobial Stewardship in Primary Health Care

Christine Lee[1], Maryam Jafari, MPH[2], Regan Brownbridge[3], Casey Phillips, BSP, PharmD[4], Jason Vanstone, PhD[5†]

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[†]Presenting and Corresponding Author

INTRODUCTION:
In order to combat rising rates of antimicrobial resistant infections, it is vital that antimicrobial stewardship become embedded in primary health care (PHC). Despite the high use of antimicrobials in PHC settings, there is a lack of data regarding the integration of antimicrobial stewardship programs (ASP) in non-hospital settings. Our research aimed to determine which antimicrobial stewardship interventions are optimal to introduce into PHC clinics beginning to engage with an ASP, as well as how to optimize those interventions. This work became focused specifically around management of viral upper respiratory tract infections (URTIs), as these infections are one of the main sources of inappropriate antibiotic use.

METHODS:
This mixed methods study of sequential explanatory design was developed through three research projects over three years in Regina, Saskatchewan, Canada. First, a survey of PHC providers was performed to determine their perceived needs from a PHC-based ASP. From this work, a "viral prescription pad" was developed to provide a tool to help PHC providers engage in patient education regarding appropriate antimicrobial use, specifically for URTIs. Next, interviews were performed with family physicians to discuss their perceived utility of this tool. Finally, we performed a public survey to determine preferences for the medium by which information is received regarding symptom management for viral URTIs.

RESULTS:
The majority of PHC providers responding to the initial survey indicated they were improperly equipped with tools to aid in promoting conversations with patients and providing education about the appropriate use of antimicrobials. Following dissemination of the viral prescription pad and semi-structured interviews with family physicians, the viral prescription pad was deemed to be a useful educational tool. However, about half of the physicians interviewed indicated they did not actually provide a viral prescription to patients when providing advice on symptom management for viral URTIs. When asked about their preferences, 76% of respondents to the public survey indicated they would prefer to receive written or a combination of verbal and written information in this circumstance.

CONCLUSIONS:
PHC providers indicated a need for educational tools to promote conversations with patients and provide education about the appropriate use of antimicrobials. Viral prescription pads were regarded by family physicians and patients as useful tools in facilitating discussion on the appropriate use of antimicrobials. PHC providers should exercise caution in opting out of providing written forms of information, as many respondents to the general public survey indicated their preference in receiving both verbal and written information.

Abstract 60: Danielle Julien

Antimicrobial Stewardship Program Virtual Care and Tele-stewardship: Development of An Evaluative Tool for Participants and Preliminary Findings from the Pilot Testing

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INTRODUCTION:

Currently, Alberta Health Services (AHS) Acute Care Antimicrobial Stewardship Program (ASP) does not perform virtual care between sites. The Virtual care pilot project focuses on rural and remote outreach across the province of Alberta to facilitate antimicrobial stewardship activities that are not developed or as readily available/accessible as in major urban centres. Integration of AHS approved virtual technologies will be integrated into the new collaborative care workflow between the ASP Provider, regional center pharmacist, and also at the bedside with the patient. These technologies allow the identified patient subpopulation to be admitted to regional facilities closer to home while enabling their access to and benefits from antimicrobial stewardship. Virtual care has adopted the Telehealth Usability Questionnaire (TUQ) as a standardized survey tool for evaluation which is designed to measure patient, provider, and caregiver satisfaction during virtual sessions. Our research question for this pilot project was: *Can we build a platform to enable capacity building by developing the Alberta Tele-stewardship Network*?

Our aim is to implement and evaluate ASP Virtual care, a tele-stewardship inpatient consultation and collaborative care service between one ASP Provider and three regional centres in Alberta.

Four objectives support this aim:

1. Determine the impact of virtual rounds on rural and remote antimicrobial prescribing.

2. Evaluate rural and remote patient and provider access to the ASP stewardship.

3. Describe characteristics of patient/family and clinician participants in ASP Virtual Rounds.

4. Conduct an evaluation on participant satisfaction and report on the pilot testing .

Our initial phase of work will be presented focusing primarily on the fourth objective of this pilot project.

MATERIALS AND METHODS:

Pilot testing of ASP Virtual care was conducted during the summer and fall of 2020. In December of 2020, the first mobile platform tele-stewardship three-way pilot consultation in the province was conducted, thereby accomplishing a significant project milestone. A quantitative questionnaire was constructed to evaluate pilot participant satisfaction. This included assessment of usefulness, ease of use/learnability, interface quality, reliability and satisfaction/future use of the mobile technology. Questions for the survey tool were developed in consultation with stakeholders from Alberta Health Services and adapted with permission from Parmanto *et al*. Some questions were modified slightly to capture the health care provider's experience. The questionnaire was pretested on two separate occasions during the summer of 2020 and made available in online and paper formats to a non-random sample of participants from July 29 to December 17, 2020. Prior to pilot consultations, two multi-disciplinary education sessions were conducted with project stakeholders and participants.

RESULTS OF INITIAL PILOT EVALUATION:

Virtual consultations were conducted at four sites: Foothills Medical Centre (Calgary), Peter Lougheed Centre (Calgary), Chinook Regional Hospital (Lethbridge), and the Red Deer Regional Hospital Centre (Red Deer). Overall, the majority of pilot participants strongly agreed the ASP Virtual care consultation supported the care of the patient, was simple and easy to use, was an acceptable way to provide healthcare services and they were satisfied with the Virtual care system and would use it in the future.

ANTICIPATED IMPORTANT CONCLUSIONS:

Based on the preliminary pilot evaluations, we anticipate ASP Virtual care will be an accessible, effective system that will support the creation of a reliable and convenient tool. This tool has the strong potential to facilitate antimicrobial stewardship activities efficiently between rural/remote hospital centres and major urban hospital centres. The development of the ASP Tele-stewardship and Virtual care platform will facilitate capacity building and optimal use of antimicrobials by clinicians in human medicine, with transferable potential for the veterinary medicine.

REFRENCES:

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Abstract 61: Kamal Acharya

Promoting antibiotic stewardship among veterinary prescribers using the OPEN stewardship platform: an evaluation of usability, utility, and impact.

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INTRODUCTION:

A reduction in unnecessary antimicrobial use in both humans and animals can result in a reduction in the prevalence of antimicrobial-resistant pathogens. An effective antimicrobial stewardship strategy to reduce antibiotic use in hospitals and clinical settings is to provide prescribers with periodic feedback reports on their individual antibiotic prescribing integrated with antibiotic prescribing guidelines. However, this strategy requires substantial investment in infrastructure and capacity development, which is not feasible in most veterinary clinics that operate as small businesses with a narrow profit margin. We have developed an online OPEN Stewardship platform capable of generating individualized feedback reports, thereby facilitating the implementation of antibiotic stewardship in veterinary clinics. In this study, we will assess the usability and utility of the OPEN Stewardship generated feedback reports amongst veterinarians and determine the impact of the reports on their antibiotic prescribing patterns.

MATERIALS AND METHODS:

We are enrolling a total of 80 veterinarians from Ontario, Canada in this study. The treatment cohort, comprised of 20 dairy veterinarians and 50 companion animal veterinarians, will receive three feedback reports on their antibiotic prescribing over a six-month period. After reviewing the feedback reports, participants will evaluate the usability and usefulness of various elements of the feedback reports by completing three follow-up online surveys. The impact of the feedback reports on veterinary antibiotic prescribing will be assessed using an interrupted time-series approach, comparing pre-intervention and post-intervention outcomes. A control cohort (n=10) will also be observed in order to ascertain and control for potential temporal trends in outcomes.

ANTICIPATED RESULTS:

The analysis of post-feedback surveys will indicate the ease of use and potential usefulness of the feedback reports amongst veterinarians. Additionally, participants' input will be used for further refinement of the feedback reports and improvement of the online stewardship platform. We hypothesize that the pre- and post-intervention antibiotic prescribing data will indicate that the provision of recurring feedback reports on antibiotic prescribing will result in a reduction of antibiotic prescribing in the treatment cohorts.

ANTICIPATED CONCLUSION AND IMPACT:

We anticipate that the veterinarians will regard the feedback reports generated by the OPEN Stewardship platform as a valuable antibiotic stewardship tool in clinical veterinary settings. We expect that the availability of a free, and open access platform such as OPEN Stewardship to administer and automate antibiotic stewardship in veterinary settings will promote wider adoption of stewardship initiatives and reduce unnecessary antibiotic use.

Abstract 62: Zeeshan Qadar

A call to action: pharmaceutical disposal in the context of antimicrobial resistance in Canada.

Sheikh Muhammad, Zeeshan Qadar

The National Collaborating Centre for Infectious Diseases, University of Manitoba

INTRODUCTION:

Antibiotics are among the most commonly prescribed pharmaceutical agent groups in the world, including in Canada. The accumulation of these agents in the environment can contribute to antimicrobial resistance (AMR). Apart from discharge to drainage systems by manufacturing, pharmaceuticals can enter the environment through improper disposal (in sinks and toilets) and human excretion. Wastewater treatment systems are not capable of completely removing these residues or preventing them from entering a water supply and spreading to agricultural and surface waters. Various means and approaches to pharmaceutical disposal are in place in Canada, but collect only a fraction of unused and expired antibiotics and other pharmaceuticals. A review of literature was undertaken to increase understanding of disposal strategies of pharmaceuticals in Canada, as well as promising practice examples from European countries in the context of AMR in human health.

MATERIALS AND METHODS:

A search for peer-reviewed literature published in Canada was undertaken, employing key databases (PubMed, CINAHL, EBSCO-Host, Medline-Ovid, ProQuest, Scopus and Web of Science). In addition, a systematic search of grey literature and government reports from Canada was performed, as well as selective review of European sources.

RESULTS OR ANTICIPATED RESULTS:

Canada lacks a national guideline and program for pharmaceutical disposal, such as those implemented in several European countries. Only British Columbia, Manitoba, Ontario and Prince Edward Island have regulated medication return programs, whereas other provinces and territories have voluntary programs, administered and monitored by their respective pharmacy associations. Moreover, in Canada, there is no formal environmental surveillance for these agents, no standard guidelines for acceptable concentrations of pharmaceuticals in drinking water, nor policies regarding clinical evaluation packages (physician's samples) exist in jurisdictions.

CONCLUSIONS OR ANTICIPATED IMPORTANT CONCLUSIONS:

Canada needs further harmonization across different pharmaceutical programs and should consider best practices from other jurisdictions. A strategical shift is needed towards environmentally safe pharmaceutical products (least bio accumulative) and a nationwide monitoring policy for environmental risk assessment on antibiotics as one strategy to reduce AMR.

Abstract 63: Diego B. Nobrega

Prevalence of Antimicrobial Resistance Genes and their Association with Restricted Antimicrobial Use in Food-Producing Animals: A Systematic Review and Meta-Analysis

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[1]Presenting author, [2]Corresponding author

INTRODUCTION:

There is an ongoing debate involving potential effects of restrictions of antimicrobial use in the prevalence of antimicrobial resistance (AMR) in bacteria. Genetic determinants of resistance such as AMR genes are strongly associated with AMR. In theory, a resistant bacterial population not under selective pressure of antimicrobials is expected to slowly revert to a susceptible state. Yet, it remains uncertain whether reduced exposure to antimicrobials will result in a lower prevalence of AMR genes, as bacteria may retain such elements even in absence of antimicrobial use. Therefore, our was to determine whether a restricted antimicrobial use in food-producing animals will result in a decreased prevalence of AMR genes in bacteria isolated from animals and humans.

MATERIALS AND METHODS:

The electronic databases Agricola, AGRIS, BIOSIS Previews, CAB Abstracts, MEDLINE, EMBASE, Global Index Medicus, (AIM, LILACS, IMEMR, IMSEAR, WPRIM, WHOLIS and SciELO), ProQuest and Science Citation Index were searched for potential original studies, which were subsequently included in this systematic review if they met the following eligibility criteria: 1) population studied included food animals and/or humans, 2) intervention was a restriction in use of antimicrobials in food animals, 3) presence of a comparator group from same population that did not undergo such an intervention, or wherein an intervention is more recent in comparison to the experimental group, and 4) outcome defined as presence or number of AMR genes reported from each group. Based on availability of data, multilevel mixed-effects models were used to meta-analyze multiple outcomes reported within a study. Three meta-analyses were conducted to summarize findings from studies reporting the following AMR genes or group of genes: *van*A, *mec*A and $bla_{\text{CTX-M}}$.

RESULTS:

Four-hundred and thirty manuscripts were reviewed, from which 53 were included in the systematic review. Restricted use of avoparcin in food-producing animals was strongly associated with a reduced prevalence of the *van*A gene. Nevertheless, there was a clear distinction between cross-sectional and longitudinal effects, with the later showing an attenuated effect towards prevalence of the *van*A gene. For the $bla_{\text{CTX-M}}$ family, effects of restrictions were species-dependent; restricted antimicrobial use in poultry and swine were unlikely to impact prevalence of $bla_{\text{CTX-M}}$ genes in bacteria. In contrast, reduced antimicrobial use in cattle was associated with reduced prevalence of $bla_{\text{CTX-M}}$ genes. For the *mecA* gene, prevalence was 0.64 times (95% confidence interval 0.44-0.94) for samples obtained from groups with restricted antimicrobial use in comparison to samples from conventional farming. Finally, for the majority of remaining AMR genes reported from studies (with exception of beta-lactamases other then $bla_{\text{CTX-M}}$), restricted antimicrobial use was associated with a decreased prevalence of genetic determinants of resistance.

CONCLUSIONS:

In summary, the available body of scientific evidence supported the idea that restricting use of antimicrobials in food animals was associated with a lower presence of AMR genes in bacteria. These findings provided further mechanistic support to recent WHO guideline recommendations regarding antimicrobial use in food producing animals, and further information to support potential global policies addressing appropriate use of antimicrobials in food animals.

Abstract 64: Stephany Barrera Almanza

Prevalence and antibiotic resistance of *Salmonella enterica* found in dairy systems from 13 states in Mexico.

Barrera-Almanza, S*[1] ., De Donato-Capote, M[1] ., Vazquez-Flores, S[1] .

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Salmonella enterica is one of the most important infectious pathogens in humans and animals worldwide. These bacteria are ubiquitous in both hosts and environment, which facilitates its transmission. *Salmonella* is considered a serious threat due to its virulence and carriage of antibiotic resistance (AR) genes, and it is necessary to take immediate action to control the pathogen. In Mexico, there is no information about the prevalence of *Salmonella* spp. in cattle, farm environment, and its AR patterns. The objective of this study was to determine the prevalence of *Salmonella* isolates in dairy systems and to evaluate its resistance to the most common antibiotics used to treat salmonella infections.

Samples were collected with Q-swabs in a cross-sectional study from feces of healthy newborn calves (127) and healthy periparturient cows (116); the environmental study consisted of samples of maternity beds (134) from 55 dairy farms. The study took place in Aguascalientes, Baja California, Chihuahua, Coahuila, Durango, Mexico State, Guanajuato, Hidalgo, Jalisco, Queretaro, San Luis Potosi, Tlaxcala, and Zacatecas in Mexico. Samples were enriched in selenite cystine broth, and then grown in Salmonella-Shigella agar. Identification was performed by biochemical tests and *Salmonella* genus was confirmed by PCR amplification of the *ipa*B gene. The Sero-quick kit was used to identify *Salmonella* isolates at serogroup level (A, B, C, D, E, F, and G). Antimicrobial susceptibility tests were conducted by disc diffusion method on Muller-Hinton agar according to the Clinical Laboratory Standards Institute (CLSI) guidelines, using 5 antibiotics: ampicillin (10µg), ciprofloxacin (5µg), levofloxacin (5µg), amikacin (30µg), and gentamicin (10µg).

From the 55 dairies sampled, 35 had at least one isolate, resulting in a 63.6% prevalence with 57 *Salmonella* spp. isolates. Stratified analysis indicated that 10 out of 127 newborn calves were positive in eight dairies, 16 periparturient cows were positive out of 116 samples in 14 dairies, and 31 isolates out of 134 sampled maternity areas (23.1% of positivity) ($p < 0.05$). PCR amplified the *ipa*B gene in all 57 isolates. The most frequent serotype was C (41.03%), followed by D (30.8 %), and then B (17.9%), E and F (5.1% each), and G (2.6%). Ampicillin resistance was identified in a 23.3% of the isolates, 10% were resistant to ciprofloxacin, 6.7% to levofloxacin, 6.7% to amikacin, and 13.33% to gentamicin.

Salmonella spp. presence was moderate in dairy cattle. The highest frequency of *Salmonella* spp. was in maternity areas with manure bedding. Environmental contamination can be a result of *Salmonella* shedding from periparturient cows, which can survive outside the host in suitable conditions for prolonged periods. *Salmonella* serogroups D and C accounted for 77% of the isolates and they were widespread among the different farms. The resistance among these isolates were similar to the reported by CDC that found resistance in 100,000 non-typhoidal *Salmonella* isolates from 1.2 million infections per year. Isolation of various serogroups of *Salmonella* in different samples indicate the wide distribution of this bacterium in seemingly healthy animals, underlining the necessity of surveillance programs for *Salmonella* in dairy farms.

Abstract 65: Alikhan Mansuri

Identification of Susceptibility Biomarkers for Rapid Diagnosis of *Pseudomonas aeruginosa* and *Candida albicans*.

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Infectious diseases are one of the top ten leading causes of death in Alberta. The most common therapy includes antibiotic treatment; however, antibiotics have been made ineffective against several pathogen strains due to antibiotic resistance. Therefore, it has become clinically important to diagnose and differentiate between resistant and sensitive strains to establish effective treatment efficiently. At the Lewis Research Group, we present a rapid diagnostics method, which shortens diagnosis time from 24-48 hours to just 4 hours post positively flagged blood bottle. This method involves the identification of susceptibility biomarkers, or extracellular metabolites that are differentially abundant between the resistant and sensitive strains. Here, this project sought to identify susceptibility biomarkers for *Pseudomonas aeruginosa* (bacterium) and *Candida albicans* (yeast). Strains were grown for four hours, and the extracellular medium was analyzed using LC-MS to detect and quantify metabolites. For the bacterium, several biomarkers were found, including uracil and 10-hydroxydecanoate, which showed dramatically different levels between resistant and sensitive strains. The yeast also showed promising biomarkers, including D-ribose and glucose. This study has revealed several, reliable biomarkers that are detectable in just 4 hours. With much quicker diagnosis, patients can receive treatment quicker, thus improving their chances of survival from an infectious disease.

Abstract 66: Karen Liljebjelke

Genomic analysis of an integrative conjugative elements conveying multi-drug resistance and metal tolerance in *H. somni* from feedlot cattle

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INTRODUCTION:

Histophilus somni is a gram-negative bacterial inhabitant of the upper-respiratory microbiome of cattle. An opportunistic pathogen, *H. somni* contributes to bovine respiratory disease (BRD) in feedlot cattle, along with other members of the Pasteurellaceae family; *Haemophilus somnus* and *Pasteurella multocida*. Antimicrobials are routinely used for control and treatment of this economically important disease in feedlot cattle. Antimicrobials are administered via feed to groups of high-risk animals, or individually by injection. Antimicrobial drugs in tetracycline and macrolide classes are most commonly administered, although drugs in beta-lactam, cephalosporin, florquinolone, sulphonamide, and phenicol classes are also used. Knowledge of antimicrobial resistance (AMR) trends in pathogens is essential for making management decisions, prudent use of antimicrobials and antimicrobial stewardship in beef cattle production. Examination of the role of mobile genetic elements (MGE) in maintaining and disseminating AMR through horizontal gene transfer (HGT) is crucial for understanding AMR. MGE carrying AMR have been previously identified in *M. somnus* and *P. multocida*. The objective of this study was to use comparative genomics to examine MGEs and AMR genes (ARG) in whole genome sequences of *H. somni* isolated from feedlot cattle.

MATERIALS AND METHODS:

Short-read WGS were generated using the Illumina MiSeq 500 platform. The draft whole genome was assembled *de novo* from raw FASTQ files using SPAdes v3.13.0. *In silico* bioinformatic methods (PGAP) were used to annotate the genome. Antimicrobial-resistant genes were identified using the annotation tools of the comprehensive antibiotic resistance database (CARD) (https://card.mcmaster.ca/). The genome was probed for plasmid-associated gene sequences using the tool Recycler (https://github.com/Shamir-Lab/Recycler). Prophages and mobile genetics elements (MGE) including integrative and conjugative elements (ICE) were identified in the WGS using Virsorter (https://github.com/simroux/VirSorter) and VRprofile (https://tool-mml.sjtu.edu.cn/STEP/STEP_VR.html).

RESULTS:

Examination of whole genome sequences (WGS) from *H. somni* isolates from feedlot cattle revealed a large mobile genetic element (MGE) carrying six antimicrobial resistance genes (ARGs). A novel Integrative Conjugative Element (ICE) of 72,914 bp length was identified as a member of the ICEHin1056 family. The ICE encodes for 79 proteins including a heavy metal tolerance gene (*mco*) and six ARGs: *tet(H), floR, Sul2/folP, APH (3'')-Ib, APH (6)-Id, and APH (3')-Ia*. The ARGs confer resistance to the tetracycline, phenicol, sulphonamide, and aminoglycoside classes of antimicrobials. The integration site is a tRNA-leu gene (CAA). The ICE has a 21-nucleotide (identical) direct repeat sequence. The ICE encodes for a complete Type IV secretion system (T4SS).

CONCLUSIONS:

The ICE in *H. somni* is similar in sequence to those previously found in other closely related bacteria associated with BRD in feedlot cattle. The *H. somni* ICE carries ARGs which confer resistance to antimicrobials in the tetracycline, florquinolone, sulphonamide and aminoglycoside classes. Tetracyclines are the most frequently used antimicrobial drugs used to control and treat BRD. Fluroquinolones and sulphonamides are also used to control and treat BRD. The discovery of a novel Integrative Conjugative Element carrying multi-drug resistance and metal tolerance in *H. somni* is important considering that antimicrobials are used in a variety of ways to prevent, control, and treat BRD, an important disease in feedlot cattle.

Abstract 67: Mai Farghaly

Examination of the horizontal gene transfer dynamics of an integrative and conjugative element in *Histophilus Somni*

Mai Farghaly[1], Michael Hynes[2], Mohammad Nazari[1], Neil Rawlyk[3], Frank van der Meer[1], Sylvia Checkley[1], Karen Liljebjelke[1*]

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INTRODUCTION:

Histophilus somni is a gram-negative bacterium that causes histophilosis in cattle and contributes to bovine respiratory disease (BRD). These diseases are significant causes of morbidity and mortality in feedlot cattle. Integrative and conjugative elements (ICEs) are autonomous-transferred mobile genetic elements that play a significant role in disseminating antimicrobials between bacteria via horizontal gene transfer (HGT). One recently sequenced ICE from *H.somni* isolated from feedlot cattle Alberta named *ICEHs02* is 72,914 base pairs in length and comprises 79 genes, including the tetracycline, aminoglycosides, florfenicol, sulfonamide, and multicopper oxidase resistance genes. This study aimed to investigate the host range of *ICEHs02* and to assess the effect of antimicrobial stressors on the frequency of the transfer of the ICE.

MATERIALS AND METHODS:

In vitro conjugation assays were conducted to examine the frequency of transfer of *ICEHs02* into other bacteria. PCR and sequence analysis were used to confirm the presence of *ICEHs02* and its circular intermediate in the recipient strains. Susceptibility testing of the *ICEHs02*-carrying recipients were conducted by broth microdilution. The effect of the antimicrobials on the excision rates and transfer frequency was investigated by mating assays and qPCR.

RESULTS:

ICEHs02 was shown to transfer into *H.somni* and *Pasteurella multocida* strains. PCR assays confirmed the ICE-associated core genes, accessory genes, and the excised circular form in the transconjugants. Susceptibility testing confirmed the functional activity of the *ICEHs02*-associated resistance in the recipient strains. The copy numbers of *ICEHs02* per chromosome exhibited a significant increase of ~37 fold after tetracycline exposure, and ~ 4 folds increase after ciprofloxacin treatment. The transfer rates were increased significantly upon tetracycline and ciprofloxacin induction.

CONCLUSIONS:

This study emphasized the importance of ICEs in the dissemination of antimicrobial resistance (AMR) between bacteria and demonstrated the effect of antimicrobial stressors on the transfer rates of ICEs.

Abstract 68: Annie (Nghi) Nguyen

Molecular mechanism underlying host range of *Tequintavirus* that infecting Shiga toxin producing *Escherichia coli* (STEC)

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[2]Department of Biological Sciences, University of Calgary

INTRODUCTION:

The virulent *Tequintavirus* (i.e. T5-like phages) a member of *Siphoviridae* family, infects and co-evolves with a number of *Escherichia coli* strains.

Tequintavirus has shown to be a suitable model to study phage – host interaction due to their unique two-step interaction process and their potential to be used as a biocontrol agent against Shiga toxin producing *Escherichia coli* (STEC). STEC are a diverse group of foodborne pathogens causing life threatening disease in human worldwide, especially in North America, Europe and Japan with clinical complications including severe stomach cramps, diarrhea and/or hemolytic uremic syndrome, etc. Cattle and their environment are the primary reservoir of those pathogens. STEC are transmitted to humans through contamination of raw meat products, fecal contamination of water or cross-contamination to other food. Host range is determined by a range of molecular interactions between phage and host throughout the infection cycle. To be infectious, a phage must be able to attach to bacterial cells by attaching to bacterial surface receptors to initiate the infection process. As surface structures are among the most variable elements of a bacterial cell, and nearly every surface structure can serve as a phage receptor, the initial interaction of phages and their receptors is an important determinant of phage host range. Phage tails are molecular machines responsible for bacterial recognition and attachment. *Tequintavirus* infection is a two-step process, which is initiated by reversible adsorption of tail fiber proteins (TFPs) to the O antigen of the lipopolysaccharides (LPS) and followed by irreversible attachment to the outer member proteins (OMPs) via the receptor binding protein (RBP) from phage tail. Therefore, I predict that extensive gene arrangements may occur in phage TFPs and RBP to response to distinct STEC hosts.

MATERIAL AND METHODS:

Previously, we have isolated and identified 7 members of *Tequintavirus* (AKFV33, AHF125, AXO26A, AXO103A, AXO103B, AXO103C, AXO45B) that have strong lytic activity but different host range against STEC serogroups. All phages were subjected to whole genome sequencing and bioinformatics analysis to examine their genomic features and identify non-homologues variance, followed by microplate phage virulence index assay to screen their host range and examine their lytic capability. Furthermore, six tail proteins of AKFV33 and AXO103A, with know host range and lytic activity will be overexpressed and examined their binding activities with surface receptors of bacterial cells.

PREMILINARY RESULTS:

The preliminary result showed that all contained genomes of 106.7 – 110.0 kb encoding 150 – 163 open reading frames and 22-24 tRNAs. Comparative genomic analysis suggested that all newly founded phages share high similarity with the *Tequintavirus* reference phage AKFV33 (>92% nucleotide identity). However, the tail genes encoding for L-shaped tail fiber proteins (TFPs) and receptor binding proteins (RBPs) are highly diverse compared to other known host attachment proteins. Bioinformatic analysis on protein sequences of the newly found proteins suggests that low similarity in TFPs and RBPs of our phages (30 – 65%) may lead to the differences in host range recognition and attachment.

SIGNIFICANCE:

This study will provide novel insights regarding gene variance and generate new knowledge regarding the molecular mechanisms underlying host recognition and infection of STEC phages.

Abstract 69: Md Murad Mufty

Transformation of environmental DNA to *Pseudomonas aeruginosa,* in the presence of amoebae in the biofilm environment

Md Murad Mufty (Presenter)1 and Nicholas J. Ashbolt (Corresponding author)2, 3

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INTRODUCTION:
Pseudomonas aeruginosa is an opportunistic Gram-negative pathogenic bacterium abundant in the environment and healthcare, engineering, and premise plumbing water system. It causes serious infections, particularly in immunocompromised individuals. Free-living amoebae (FLA) are a critical group of the microbial protozoal community that influences biofilm-associated bacteria (e.g., *P. aeruginosa*) piped-water environment. *P. aeruginosa* can acquire resistance through gene transformation which plays a significant role to transmit and spread antimicrobial resistance. *P. aeruginosa* is an amoebae-resistant microorganism, can survive and grow within free-living amoebae hosts in biofilm in recreational water, like hot-tub and whirlpool. Despite of clinical significance, limited information available regarding the role of amoebae to disseminate antibiotic resistance transfer to *P. aeruginosa* in biofilm environment.

MATERIALS AND METHODS:
The study was carried out to investigate the environmental DNA transfer to *P. aeruginosa,* randomly isolated from hot tub and whirlpools in central-southern Alberta, Canada, as a model for biofilm-related growth and selection for antibiotic-resistance and transfer. Using fluorescent microscopy, we examined in-situ interactions of *P. aeruginosa* with *Acanthamoebae polyphaga* and *Willaertia magna,* two water-related FLA species in drinking water biofilms. We observed the transformation of plasmid eDNA to *P. aeruginosa* in the presence of those two FLAs.

RESULTS:
Both *A. polyphaga* and *W. magna* showed no immediate interactions with *P. aeruginosa* when introduced to the same natural biofilm environment. *A. polyphaga* encysted within nine days after introduction to the tap-water biofilms and mostly persisted by forming cysts until 90 days. *However, W. magna trophozoites* exhibited a time delay in feeding on *P. aeruginosa* and were observed to have limited internalization of *P. aeruginosa* cells after 18 h from their introduction, supporting limited intracellular growth. We found free-living amoebae promote eDNA transformation to environmental *P. aeruginosa* isolates. In our comparative transformation study, in the absence of amoebae, it takes at least 49 days to transform the environmental DNA to *P. aeruginosa*; meanwhile, it takes only 9 to 11 days in the presence of amoebae. In the presence of *A. polyphaga*, the transformation happened after 9 days, and for *W. magna* it took 11 days.

CONCLUSIONS:
The overall results indicate that the presence of FLAs within the same niche as *P. aeruginosa* may prompt plasmid DNA transformation.

Abstract 70: Mihret Girum

Single Polyvalent Phage Efficacy in Mixed APEC Strain Cultures

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INTRODUCTION:

Avian pathogenic Escherichia coli (APEC) has raised concerns within the poultry industry due to the increasing presence of antibiotic resistance. The development of systemic APEC infections in poultry can lead to colibacillosis which results in lesions and diseases that affect agricultural yield. Impacts of antibiotic resistance have created a demand for identifying safe and effective alternatives. Ongoing research has shown that naturally abundant bacteriophages possessing traits such as self-replication and high lytic activity, are viable treatment alternatives.

Bacteriophages are also characterized by their narrow host ranges; phage specificity for specific strains, while advantageous, limits the implementation of phage therapy in environments that possess multiple pathogenic species or strains. To address this concern, phage cocktails exhibiting capacity to target multiple strains, have shown success in diverse bacterial environments. Although effective, phage cocktails are limited due to complex pharmacological effects of interference between phages in mixture. Thus, identification of polyvalent phages that can lyse multiple strains is of interest within the industry. This project aims to evaluate the lysing capability of polyvalent phages in mixed APEC strain broth cultures. The phages utilized in the study are isolated from City Sewage and target E. coli of serogroups O1, O2 and O78.

MATERIALS AND METHODS:

Microplate Phage Virulence Assay

To identify host range of O78-infecting phages, a microplate phage virulence assay has been conducted. The assay tested the lysing efficiency of single phages at series multiplicity of infections (MOI's, 1.00E-08─ 0.1) against APEC serogroups O1, O2 and O78. Results indicated the most viable phage for subsequent broad host range activity experimentation, labelled as SW1.

Phage Virulence Index Assay

The dynamic interaction between phages and APEC strains will be determined using virulence index assays. The phage's lytic activity over wide range of MOI's (1.00E-07─1) and against single and mixed strains will be tested on microplates and assessed by comparing optical density measurements over a period of 5 hours. This data will provide a quantitative assessment of virulence for each phage against individual strain and mixture of 3 serogroups over time.

Broth Culture

Lastly, to compare efficacies of broad host range phage treating bacterial mixtures, a phage-treated mixture of APEC O1, O2 and O78 will be subsampled at 4, 7, 10 and 24 h to monitor bacterial survival and phages amplification. Selective medium and PCR will be used for differentiation of bacterial serotypes. Overlay plaque assay will be used for phage enumeration.

CURRENT AND ANTICIPATED RESULTS:

The microplate virulence assay has identified three phages with broad host range. Preliminary data from small scale broth culture experiments demonstrated that polyvalent O78-infecing phages were able to inhibit growth of O1, O2 and O78 serogroup bacterial mixtures, despite their relatively low virulence against O1 and O2. Ongoing work aims to determine if this efficacy will be consistent in a large-scale broth culture and the mechanism underlying this efficacy.

SIGNIFICANCE:

Understanding the behavior of broad- host-range phages in mixed bacterial cultures will provide a basis for utility of single bacteriophage preparation for effective APEC prevention and treatment.

Abstract 74: Catrione Lee

Characterization of the Genomic Context of Antimicrobial Resistance Genes in Metagenomic Datasets with *de novo* and Targeted Assemblies of Agriculturally Relevant Microbiomes

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INTRODUCTION:
Antimicrobial resistance (AMR) is a rising concern in the One Health continuum. Concerns of the transmissibility of resistance genes from bacterial communities to another, between the One Health continuum sectors – which may act as reservoirs – have been raised. One method of tracking antimicrobial resistance through the environment is to use metagenomic sequencing of various agricultural and environmental microbiomes to search for antimicrobial resistance genes (ARGs) and characterizing their genomic contexts such as mobile genetic elements (MGEs), like integrative conjugative elements (ICE) and plasmids.

MATERIALS AND METHODS:
A dataset of 43 publicly available Illumina-sequenced shotgun metagenomic samples with 100-bp paired-end reads was selected from a previously published study. The number of samples per environment was as follows: 20 feedlot fecal composite, 13 catch basin downstream of feedlots, 4 manured agricultural soil samples, and 6 municipal sewage influent. The taxonomic classification of each sample's sequenced reads was determined using the Kraken2 classifier against the NCBI nt database. For a focused analysis, a single fecal composite sample was selected to troubleshoot and optimize analysis. Targeted assemblies for analyzing ARGs' genomic context and searching for ICEs were accomplished through the tool MetaCherchant, with the supplied by the databases MEGARes2 and ICEberg2, respectively. *De novo* metagenomic assemblies were performed for all samples reads with the pipeline nf-core MAG, including two different assemblers, Megahit and metaSPADES. Detection of ARGs and ICEs was accomplished through ABRicate analysis with the MEGARes2 database and subsequent BLAST search with the ICEberg2 database. Further annotation of MAGs was used to characterize the genomic context.

RESULTS:
We analyzed 43 datasets with two assembly algorithms for a total of 86 *de novo* metagenomic assemblies. Metagenome-assembled genomes (MAGs) were selected to further analyze ARGs and genomic context due to completeness and contamination. We also studied the ability of targeted assemblies to reconstruct the genomic context of ARGs. Based on our analyses, we have a profile of the ARGs, MGEs, genomic context of agriculturally relevant microbiomes.

CONCLUSIONS:
Our work identified ARGs in metagenomic datasets, and we explored their genomic context. Our work will recommend the best assembly program based on MAG assembly qualities, completeness, and contamination. Furthermore, our work studied the potential and limitations of short-read sequencing for characterizing ARGs' genomic context through both the targeted and the *de novo* assemblies. Insights into profiles of ARGs, MGEs, and other genomic context features will be presented. Targeted assemblies are sensitive enough to detect ARG-associated ICEs but may not necessarily provide enough context for those elements' characterization. *De novo* assemblies are robust enough to detect ICE-associated ARGs, albeit in smaller quantities, and can characterize them via more informative annotations and associations. However, there is a significant underrepresentation of ARGs and ICEs within the selected fecal composite sample, indicating a problem in the methodology. This could be due to an unoptimized sequencing strategy for this type of analysis and/or an incorrect selection of tools for the chosen analysis.