Antibiotic-free production on pathogen occurrence and prevalence of antimicrobial resistance

B. Predicala[1], S. Chekabab[1,2], A. Alvarado[1], D. Korber[3], J. Lawrence[3] and A. Trokhymchuk[4]

Bernardo Predicala

Alvin Alvarado

SUMMARY

This study assessed the impact of Raised Without Antibiotics (RWA) production practices on on-farm antibiotic use, pathogen occurrence, and the prevalence of antimicrobial resistance genes (ARGs) through the whole genome sequencing and analyzing of samples (fecal, manure, sow nasal swab, soil). Some farms that provided antibiotic use data for this study also tested an injection system that automatically records injection events in the barn and its associated meta-data. Based on the results, recommendations for best management practices for various pig production stages were formulated..

Despite encountering technical difficulties, the e-data capture technologies such as the V-ETiC injection system was demonstrated to be a good alternative to recording on paper-based treatment sheets. Compared to non-RWA barns, RWA barns had lower frequency of ARGs and higher frequency of pathogens in piglet feces and in-barn manure samples, both higher ARG and pathogen frequency in sow nasopharynx, and lower frequency of pathogens in sow feces. These results suggest the RWA program is effective in reducing the occurrence of antimicrobial resistance, but may lead to disease outbreaks due to higher pathogen loads, suggesting the need for a long-term surveillance monitoring system of pathogen loads in RWA production systems.

Some of the recommended best management practices to counter the rise of antimicrobial resistance include implementation of appropriate surveillance monitoring and antimicrobial stewardship systems, timely and effective vaccination programs, enhanced disinfection and manure management practices, and improved nutrition and feed programs.

INTRODUCTION

In order to mitigate the risks posed by the expanding antimicrobial resistance threat, more stringent rules governing the use of clinical drugs for the treatment of sick animals have been in place in Canada since December 2018. Consequently, many Canadian producers have proactively implemented procedures wherein animals are raised without the use of antibiotics (RWA) from birth to slaughter. The question is how effective are these intervention measures in actually reducing the total on-farm use of antibiotics, the occurrence of pathogens, and the prevalence of antimicrobial resistance? To answer this question, this study aimed to conduct longitudinal surveillance monitoring of conventional farms still using antibiotics as usual (except in feed) as well as farms that have entered into the RWA program.

One other gap that this study aimed to help address was the lack of a reliable national database on antibiotics use. Presently, pig farmers are required to keep detailed records of antibiotics use in their operations as part of the Canadian Quality Assurance program, but these are mainly paper-based records which are tedious and time-consuming to collect and may be potentially susceptible to errors. This study aimed to utilize available electronic data capture technologies to reduce the record-keeping burden and time-demand on barn staff, while ensuring that high-quality data is collected.

EXPERIMENTAL PROCEDURES

Activity 1: Some farms that provided antibiotic use data for this study were requested to test an injection system that includes a HSW ECO-MATIC syringe paired with the V-ETiC system and radio-frequency identification (RFID) technology. This system automatically records injection events in the barn and the associated meta-data (i.e., pig RFID tag, time, dosage, etc.) for each injection. The system provides automatic recognition of animals via RFID ear tag of the pig, treatments, the drug administered, and dosage.

Activity 2: Fecal and manure samples from 6- or 20-week-old pigs, fecal and nasal swab samples from sows, earthen manure storage samples, and soil samples from the participating farms' immediate environment were taken. Samples were sequenced, analyzed, and profiled using whole-genome sequencing to identify the prevalence of antimicrobial resistance genes (ARGs, also called resistome) and pathogens (also called pathome).

Activity 3: Following the completion of Activities 1 and 2, recommendations for best management practices for various pig production stages were formulated, including possible fine-tuning and modifications to current RWA practices to ensure that

1 Prairie Swine Centre Inc, PO Box 21057, 2105 – 8th Street East, Saskatoon, SK S7H 5N9
2 Department of Chemical and Biological Engineering, College of Engineering, University of Saskatchewan, 57 Campus Drive, Saskatoon, SK S7N 5A9
3 Department of Food and Bioproduct Sciences, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8
4 Prairie Diagnostic Services Inc., 52 Campus Drive, Saskatoon, SK, S7N 5B4

the intent of the program to reduce total antibiotic use, avoid the development and spread of antimicrobial resistance genes, and maintain or improve herd health, are fully realized.

RESULTS AND DISCUSSION

Activity 1: The RFID technology automatically recorded injection events in the barn as well as the associated meta-data for each injection. This data was automatically stored in the V-ETiC mobile app while inside the barn and could then be synchronized and downloaded to the V-ETiC cloud-based software via Bluetooth or WiFi anytime. Various technical issues on the system components and operating program were identified in this study, which resulted in the participating barns discontinuing the use of the device.

Activity 2: Metagenomic analyses revealed that the pathogens present in the samples belonged to five phyla: Firmicutes, Bacteriodetes, Chlamydiae, Actinobacteria and Proteobacteria. Relative to non-RWA barns, RWA barns had higher frequency of pathogens in piglet feces, in-barn manure and sow nasopharynx, and lower frequency of pathogens in sow feces. No differences were observed in the prevalence of pathogens in environment samples collected from the non-RWA and RWA farms.

The resistome profiles showed that the ARGs present in the samples were found to belong to six main classes: Aminoglycosides, β-lactams, Macrolides, Phenicol, Multi-Drug Resistance (MDR) and Tetracyclines. In RWA-piglet feces, the frequency of ARGs was significantly lower for Aminoglycosides, Macrolides, Phenicol and Tetracyclines (Figures 1A, 1C, 1E and 1F) relative to non-RWA. In RWA-barn manure, the frequency of resistance genes to β-lactams, MDR, Phenicol and Tetracyclines (Figures 1B, 1D, 1E and 1F) were also significantly lower relative to non-RWA barn manure. On the other hand, in the RWA-sow nasopharynx, the frequency of resistance genes for β-lactams, MDR and Tetracyclines were significantly higher compared to the non-RWA sow nasopharynx samples (Figures 1B, 1D and 1F). No significant difference was observed in the ARG frequency in sow feces and in barn environment samples between the two types of barns. The resistance to Tetracycline and Macrolide classes were found most frequently in fecal and manure samples. Resistance to the β-lactam class was found more frequently in nasal swabs collected from sows.

Activity 3: Some of the recommended best management practices to counter the rise of antimicrobial resistance include implementation of appropriate surveillance monitoring and antimicrobial stewardship systems, timely and effective vaccination programs, enhanced disinfection and manure management practices, and improved nutrition and feed programs (see Table 1).

Table 1. Examples of recommended best management practices according to production stage and type of operation

Production Stage	Barn Type	Recommended best management practices
Piglet	RWA	1. Continuous surveillance monitoring of antibiotic use, ARGs and prevalence of pathogens. 2. Implementation of effective vaccination program
	non-RWA	1. Antimicrobial stewardship – efficient use of antibiotics 2. Alternatives to antibiotics (e.g., probiotics, prebiotics, synbiotics)
Sow	RWA	1. Continuous surveillance monitoring of antibiotic use, ARGs and prevalence of pathogens 2. Implementation of effective vaccination program 3. Alternatives to antibiotics (e.g., probiotics, prebiotics, synbiotics)
	non-RWA	1. Antimicrobial stewardship – efficient use of antibiotics 2. Use of alternatives to antibiotics (e.g., probiotics, prebiotics)
Manure storage	RWA	1. Continuous surveillance monitoring of antibiotic use, ARGs and prevalence of pathogens 2. Implementation of effective disinfection measures
	non-RWA	1. Antimicrobial stewardship – efficient use of antibiotics 2. Manure treatments (e.g., manure separation, nanotechnology application)

IMPLICATIONS

Results from this project demonstrated that the Raised Without Antibiotics (RWA) program is an effective intervention measure to counter the occurrence of AMR in pig production operations. However, data from this study also showed that the RWA program had varying impacts on the different pig production stages. Hence, these key findings should be applied by reviewing and identifying the specific husbandry practices in each pig production stage, particularly those pertaining to maintaining animal health and administration of pharmaceutical products, that can be modified and/or optimized to further reduce or eliminate the use of antibiotics, and consequently the occurrence of ARGs.

Findings from this study showing that the elimination or reduced use of antibiotics in the production system can give rise to increasing prevalence of certain pathogens, should lead to implementation of continuous, long-term surveillance monitoring of pathogen loads in RWA production systems in order to determine the critical pathogen level before actual disease outbreaks occur. Knowing this will prevent the recurrence of catastrophic disease outbreaks experienced by early adopters of RWA practices, which ultimately led to failure (pulling out) of the program.

Despite the technical difficulties encountered (which should be within the capability of the equipment provider to resolve), the e-data capture technologies such as the V-ETiC injection system was demonstrated to be a good alternative to recording on paper-based treatment sheets. Once fully-debugged and restored to full functionality, this e-data capture technology can be utilized as an integral component of a focused surveillance monitoring system to efficiently and accurately capture, track, manage and monitor the use of any pharmaceutical products and their related meta-data in any type of production barn. In conjunction with the workflow method utilizing a health metadata-based approach for comparing and quantifying WGS data targeting the prevalence of pathogens and antimicrobial resistance developed in this study, this e-data capture technology would essentially improve the quality of the data and offer definitive insights into correlations and trends that were observed in this study.

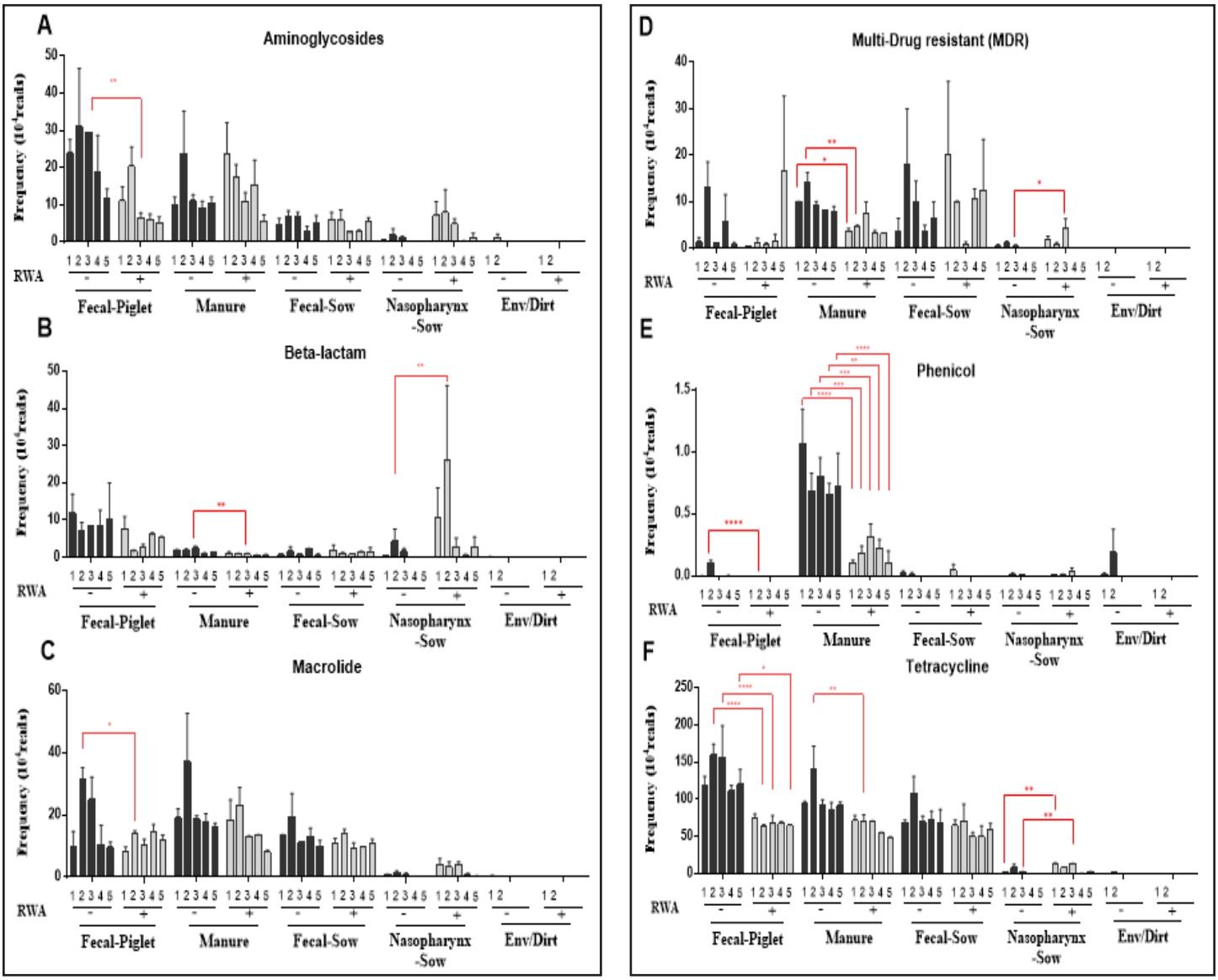

Figure 1. The frequency of ARGs in piglet feces, sow feces and nasopharynx, barn manure, and dirt/soil samples collected near the barn entrance in RWA (+ plus sign) and non-RWA (– minus sign) barns. The ARGs were clustered in six classes: Aminoglycosides (A), β-Lactams (B), Macrolides (C), MDR (D), Phenicol (E) and Tetracyclines (F). * p<0.05 ** p<0.01 *** p<0.005 **** p<0.001

ACKNOWLEDGEMENTS

We would like to acknowledge the financial support for this research project provided by the Agriculture Development Fund through the Saskatchewan Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement. Support provided by the farm owners, management and production staff, and technicians in the participating barns are greatly appreciated. The authors would also like to acknowledge the strategic program funding provided by Sask Pork, Alberta Pork, Ontario Pork, the Manitoba Pork Council, and the Saskatchewan Agriculture Development Fund.