Assessing the impact of intervention measures for reducing antibiotic use

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SUMMARY

Previous activity in this study aimed to evaluate the impact of the Raised without Antibiotics (RWA) program on pig production focused on quantifying the overall antibiotics use both in participating RWA and non-RWA barns. Next steps involved the assessment of prevalence of antimicrobial resistance (AMR) and pathogen abundance, by conducting long-term surveillance of AMR and pathogens using whole genome sequencing (WGS), quantifying the resistome, virulome and bacterial diversity in the RWA and non-RWA barns.

Preliminary results from comparative analysis of the ARGs frequency readouts showed significant differentiation between RWA and non-RWA program effects. Specifically, a significant reduced frequency of ARGs for beta-lactams, multi-drug resistance (MDR), phenicol and tetracycline, was observed in manure samples of RWA barns and for phenicol and tetracycline ARG in RWA-piglet feces. On the other hand, data showed a greater frequency of tetracycline-ARG class in the nasopharynx of sows in RWA barns.

In terms pathogen prevalence, we found a similar pattern between the non-RWA and RWA barns in the piglet feces and the barn manure samples. Overall, our longitudinal study suggests that raising RWA pigs may have an impact on the prevalence of pathogens and AMR. Results also suggest the possibility to correlate RWA practices with variations in specific sets of AMR classes and pathogens – leading to potentially adjusting RWA measures and practices to target the reduction of certain AMR classes and the persistence of specific pathogens.

INTRODUCTION

In response to general concerns about the spread of antimicrobial resistance (AMR) along with increasing public concern regarding the use of antibiotics in livestock production, various measures such as changes to livestock feed regulations have been implemented in Canada. Another strategy available to producers is raising animals without antibiotics (RWA). The question is how effective are these strategies in reducing total on-farm use of antibiotics, the occurrence of pathogens, and the prevalence of antimicrobial resistance?

To answer these questions, we conducted longitudinal surveillance monitoring of conventional farms still using antibiotics (except in feed) and RWA farms. The monitoring strategy focused on three key areas: antibiotics usage, antibiotic resistance, and prevalence of pathogens. Based on the findings, a list of recommendations for best management practices can be developed to help ensure the success of intervention measures, such as the RWA program and regulations for the responsible use of antibiotics.

EXPERIMENTAL PROCEDURES

Activity 1 *– Determining on-farm antibiotic usage patterns and total use*

Each farm participating in the project documented antibiotics in one of two categories:

- Inventory of antibiotics in the farm
- Record of any use of antibiotics for animal treatment, including type of drug, dosage, type and number of animal(s) treated and approximate age, treatment cause, location in the barn, and date and time.

Information related to on-farm antibiotics was collected using treatment sheets that are a part of the CQA requirements or Canadian Pork Excellence Program. Analysis of the data then determined patterns of antibiotic use and total usage of different antibiotics on farm.

Activity 2 *– Surveillance monitoring of prevalence of antimicrobial resistance and pathogens*

The second phase of the project monitored the prevalence of antimicrobial resistance and pathogens in each of the participating farms. Representative fecal and manure samples from 6-week, 12-week and 20-week old pigs, earthen manure storage, and soil samples from the barn's immediate environment were collected from each farm at defined intervals and analyzed. In addition, fecal samples and nasal swabs from sows were included in the monitoring because these animals reside the longest in the barns.

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RESULTS AND DISCUSSION

*A**ctivity 1** - Determining on-farm antibiotic usage patterns and total use*

Treatment records collected included the type of drug, dosage, approximate age of animals, cause of treatment, location in the barn as well as the date of drug administration. Based on the data collected, antibiotics mostly given belonged to four classes: Antifolates (Trimidox), B-lactams (Penicillin G, Ampicillin, Ceftiofur), Tetracycline (Biomycin), and Chloramphenicol (Nuflor).

As expected, most of the antibiotic drugs recorded were from the non-RWA barns, with very little total amount used in RWA barns during the monitoring period (i.e., none in the RWA #1 and #3 barns, and small amount in RWA #2).

- In Non-RWA #1, 79% of antibiotics used are Antifolates, 20% are B-lactams, 1% are Tetracyclines, and 0% Amphenicol.
- In Non-RWA #2, 40% of antibiotics are Antifolates, 11% are B-lactams, 43% are Tetracyclines, and 0% are Amphenicol.
- In Non-RWA #1, 25% of antibiotics are Antifolates, 60% are B-lactams, 0% are Tetracyclines, and 6% are Amphenicol.

***Activity 2** - Surveillance monitoring of prevalence of antimicrobial resistance (AMR) and pathogens*

Samples were sequenced and analyzed using Whole Genome Sequencing (WGS) technique (involving processing and analysis of raw samples, quality control protocols, and outputs that include frequency of bacterial species, resistome (antimicrobial resistance genes), and pathome (abundance of pathogens)), which is a widely available tool with demonstrated potential for AMR surveillance. The overall methodology is illustrated in Figure 1 (Chekabab et al., 2020).

***Activity 3** - Identification and quantification of drug residues related to AMR*

To determine the presence of residual drug components that might related to AMR, aliquots of manure and soil samples collected from the participating barns at the start and the end of the study were sent for analysis using Liquid Chromatography coupled with tandem Mass Spectrometry (LC/MS/MS).

All samples were tested for a total of 29 drugs which are routinely tested and quantified at the lab, including: Acetaminophen, Benzoylecgonine, Chloramphenicol, Ciprofloxacin, Clindamycin, Cocaïne, Codeine, Cotinine, Enrofloxacin, Erythromycin, Fluoxetine, Lincomycin, Methamphetamine, Norfloxacin, Norfluoxetine, Ofloxacin, Oxolinic Acid, Pentoxifylline, Pipemidic Acid, Sulfabenzamide, Sulfadimethoxine, Sulfadoxine, Sulfamerazine, Sulfamethazine, Sulfamethoxazole, Sulfapyridine, Sulfaquinoxaline, Sulfathiazole and Trimethoprim.

Additionally, our samples were tested and quantified for tetracycline and tested for the presence/absence of additional antibiotic drugs including: Apramycin, Streptomycin, Dihydrostreptomycin, Bacitracin, Tilmicosin, Erythromycin, Tiamulin, Lincomycin, Tylosin, Tylvalosin, Neomycin, Virginiamycin M1, Virginiamycin S1, Penicillin G, Spectinomycin, Tetracycline and Chlortetracycline.

Results of the analysis summarized in Table 1 show only the specific drugs detected in samples. Overall, the LC/MS-MS data showed the presence of Acetaminophen in both RWA and non-RWA manure samples. Tilmicosin, Tiamulin, Spectinomycin, Tylosin were also detected in both manure and soil samples in both RWA and non-RWA barns.

IMPLICATIONS

Analysis of the resistome and pathome over time (statistical analysis between group of samples) for all samples sequenced and analyzed clearly show different separate clusters of types of samples – fecal, manure, and nasopharynx – with respect to the presence of ARGs and pathogen prevalence.

We observed a significant decrease in ARGs-frequency in RWA manure samples for beta-lactams, MDR, phenicol and tetracycline. Furthermore, RWA piglet feces had reduced phenicol and tetracycline AMR levels. On the other hand, sampling of the nasopharynx of RWA sows showed a greater frequency of tetracycline-ARG.

In the analysis of total prevalence of pathogens, we found a similar pattern between the non- RWA and RWA barns in the piglet feces and the barn manure samples. The frequency of pathogens in the fecal-piglets and manure was not significantly different between RWA and non-RWA farms. However, the RWA-Sows tended to have fewer pathogens in feces and more in nasopharynx compared to non-RWA.

Based on these findings, our longitudinal study suggests that raising pigs using RWA practice may have impact over time on the prevalence of pathogens and AMR, which may differ depending on the animal development stage and on the type of sample.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support for this research project provided by the Saskatchewan Agriculture Development Fund and the Canada-Saskatchewan Growing Forward 2 Bi-lateral Agreement. As well, the support provided by the production staff and technicians in the participating barns was 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.