Project Title: Effects of Cattle Feeding Location and use of Tylosin on Expression of Antimicrobial Resistance (AMR) and Liver Abscesses

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Background

The mitigation of antimicrobial resistance (AMR) is a global public health priority. Although it is acknowledged that AMR is a complex issue that is not easily resolved, the utilization of antimicrobial drugs (AMD) in animal agriculture is often implicated as a contributor to emerging AMR microorganisms. Several AMDs considered critically important to human medicine (flouroquinolones, third-generation cephalosporins, and macrolides) are commonly employed in beef production and subsequently, significant pressure is being placed on the industry to reduce and/or eliminate their use. Specifically, the use of Tylosin (Tylan; a macrolide) in the diets of finishing cattle to reduce liver abscess rates has drawn extensive scrutiny, and widespread calls to eliminate it from cattle feeding regimens have been made. As such, efforts to identify effective alternatives are needed. However, the development of alternative prevention strategies is inhibited by a limited understanding of the comprehensive drivers of liver abscesses in finishing cattle (Nagaraja et al., 1999). Briefly, although Tylosin utilization reduces liver abscess rates in finishing cattle, its supplementation does not fully eradicate abscesses. Furthermore, industry wide, there exists tremendous variation in liver abscess rates among cattle fed diets supplemented with Tylosin—suggesting that the onset of an abscess is more complex and likely a result of several microbiological co-factors. Efforts to understand the complexity of the microbiological origins of liver abscesses in cattle fed Tylosin versus those that are not supplemented with Tylosin will facilitate the targeted development of effective alternative mitigation strategies.

In the face of losing this vital tool in beef production, tremendous focus has been placed on identifying an alternative solution that does not sacrifice animal health or productivity. However, in order to competently identify an alternative, a more comprehensive understanding of the microbiological variables influencing liver abscess rates should be established. Therefore, the objective of this study was to utilize newly developed technologies to provide a more comprehensive perspective on the microbiological differences of cattle supplemented with Tylosin versus those fed diets without Tylosin supplementation. With this understanding, it was hypothesized that more targeted and effective Tylosin- alternatives can be developed and implemented in commercial beef production.

Methodology

Study population: Pens of commercial cattle from feedlots within three geographical regions of the U.S. (Colorado, Texas, and California) housing cattle fed diets with and without Tylosin were utilized for this study. The research team worked with each feedlot to identify pens of cattle which were approaching their finishing date. Care was taken to select pens of cattle (n

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= 4/treatment group) with similar characteristics (i.e. breed, weight, harvest date, background, days on feed, etc.). Similarly, to reduce variations due to season, pens of cattle were selected so that harvest dates occurred within the same season (Spring 2016). Cattle from all feedlot locations were fed a high-grain diet and subjected to normal production practices respective to their feeding program.

Feedlot Sample Collection: Pen-floor fecal and soil samples were collected from each experimental unit (EU; pen) within two days prior to harvest using methods described by Yang et al. (2016). Individual fecal pats and soil samples from each pen were composited in sterile sample bags and transported on ice to CSU for analysis.

In-Plant Sampling: Cattle were shipped to commercial processing facilities for harvest within two days following feedlot sample collection. From each pen (n = 4/feedlot), a convenience sample of twenty randomly selected sides were used for collection of subiliac lymph nodes (SLN) immediately after harvest. Lymph nodes and their external fascia were placed in sterile sample bags and transported to CSU in an insulated cooler maintained at 4 to 6°C until further processing.

Similarly, within each pen, abscessed (n = 5/pen) and non-abscessed (n = 5/pen) livers were identified for sample collection. A segment containing the abscess was utilized for abscessed livers while a subsection of the hepatic vein was used for sampling of non-abscessed livers. Liver samples were maintained in sterile sample bags at 4 to 6°C during transportation to and storage at CSU. Additionally, liver scores and abscess rates were recorded for all pens using the methods described by Brown and Lawrence (2010).

Microbial Analysis of Fecal Samples: Fecal samples were subjected to enumeration and isolation of Enterococcus and generic *Escherichia coli* in addition to isolation of *Salmonella* spp using standardized methodologies. Colonies morphologically representative of Enterococcus, generic *E. coli*, and *Salmonella* spp. were placed into tryptic soy broth (TSB) containing 10% glycerol and frozen (-80°C) until determination of antimicrobial susceptibility (described below). Those samples not exhibiting typical *Salmonella* morphology after plating on selective media were considered “negative” and no further work was performed.

Microbial Analysis of Subiliac Lymph Nodes: In order to reduce the risk of cross-contamination, the external fat and fascia surrounding the lymph node were flame-sterilized prior to trimming. Following sterilization and trimming of all excess external tissues, the SLN was placed in a sterile sampling bag and pulverized using a rubber mallet. Eighty ml of TSB was added to the pulverized lymph node prior to stomaching for 2 min at 200 rpm (Seward Stomacher, Seward UK). The stomached sample was enriched at 37°C for 24 h prior to a secondary enrichment in Tetrathionate (TT) and Rappaport-Vassiliadis (RV) broths to facilitate *Salmonella* isolation. Isolation of *Salmonella* from SLNs was performed as described above. Samples which did not exhibit morphologically representative *Salmonella* were deemed “negative” and removed from the study analyses.

Microbial Analysis of Liver Samples: Liver abscess samples (n = 5/pen) were evaluated as described by Nagaraja et al. (1999). A sub-sample of the purulent material was reserved for later analysis of the microbiome using 16s sequencing.

Antimicrobial Susceptibility of Isolated Microorganisms: Minimum inhibitory concentrations (MIC) of isolated *Salmonella*, Enterococcus, and generic *E. coli* to various antimicrobial drugs were determined using a broth microdilution method (Gragg et al., 2013). Minimum inhibitory concentrations and breakpoints were set using those established by Clinical and Laboratory

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Standards Institute (CLSI; CLSI, 2013) or the National Antimicrobial Resistance Monitoring System (NARMS; FDA, 2011).

Genomic Analysis of the Fecal and Soil Resistome: Approximately 10g of the initial collected sample was placed in a conical vial and stored at -80°C until later analyses (to be completed in the summer of 2016).

16s-Based Determination of the Lymph and Liver Microbiome: Approximately 10g of purulent material from the liver sample and 20ml of enriched lymph node tissue were reserved in conical vials and maintained at -80°C until later analyses (to be completed in the summer of 2016).

Statistical Analysis: Fecal populations, presented as least squares means (log CFU/g), and liver abscess rates were analyzed as a completely randomized design using a commercial statistical analysis software program (SAS vs. 9.4; Cary, NC). Data were analyzed using a model containing Tylosin supplementation (yes/no) as the main effect with pen number and feedyard location as random effects. Means were separated using the PDIFF statement with an α of 0.05.

Findings

The purpose of this study was to evaluate the influence of Tylosin supplementation during the finishing period on the quantitative and qualitative microbiology of pen-floor fecal samples, liver abscesses, and the subiliac lymph nodes. Populations of Enterococcus and generic *E. coli* did not differ ($P > 0.05$) due to Tylosin supplementation.

However, a greater proportion of Enterococcus isolated from the fecal-samples of cattle supplemented with Tylosin exhibited resistance to multiple AMDs when compared to the fecal samples of cattle without previous Tylosin exposure. Similarly, the prevalence of subiliac lymph nodes harboring *Salmonella* spp. was not different among Tylosin supplementation groups. Yet, *Salmonella* isolated from the lymph nodes of cattle supplemented with Tylosin tended to express resistance to more AMDs. It should be noted, however, that over one-half of the *Salmonella* recovered from SLNs originated from cattle in one feedyard—suggesting that environment, instead of Tylosin exposure, has the largest influence on *Salmonella* prevalence in the SLNs.

Implications

These data provide insight into the complex microbiological implications of Tylosin supplementation and antimicrobial resistance. Preliminarily, in support of the original hypothesis, these data suggest that AMR is not simply a result of previous antimicrobial exposure. When combined with the microbiome and resistome counterparts, these culture-based data will provide a more comprehensive scope of the factors influencing liver abscesses and antimicrobial resistance will be established.

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Table 1. Populations of generic *Escherichia coli* and *Enterococcus* (log CFU/g) in composited fecal samples obtained from the pens of cattle fed diets supplemented with Tylosin and the pens of cattle fed diets not supplemented with Tylosin.

Microorganism	Tylosin Supplementation			*P*-value	SEM
	Tylosin	No Tylosin			
Generic *E. coli*	5.94	6.16		0.18	0.19
Enterococcus	4.08	3.81		0.45	0.24

Figure 1. Generic *E. coli* isolated from feedlot pen-floor.

Figure 2. Subiliac lymph node prior to *Salmonella* evaluation.

References

Nagaraja, T. G., A. B. Beharka, M. M. Chengappa, L. H. Carroll, A. P. Raun, S. B. Laudert, and J. C. Parrott. 1999. Bacterial flora of liver abscesses in feedlot cattle fed Tylosin or no Tylosin. J. Anim. Sci. 77: 973-978.

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