Antimicrobial use in beef feedyard cattle in the United States

by

Katie Jo Hope

B.S., Kansas State University, 2009
D.V.M., Kansas State University College of Veterinary Medicine, 2013

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2019

Abstract

Antimicrobial use is a common occurrence in modern food-animal production. These medications are utilized to prevent, control, and treat a wide variety of infectious diseases in beef cattle. However, as concerns related to antimicrobial resistant bacteria in human and animal health have mounted, countries throughout the world are measuring and monitoring antimicrobial use. These measurements are based on antimicrobial use metrics which generally consist of both a numerator (e.g., weight of product sold or used, number of animals exposed) and a denominator (e.g., animal population, time).

This report is focused on capturing, standardizing, and characterizing antimicrobial use data from 22 beef feedyards in the United States. Data management required navigation of challenges including feedyard recruitment, data acquisition across multiple record-keeping systems, data standardization, and analysis. These 22 feedyards sold a total of approximately 1.2 million head of beef cattle during 2016 and 2017. During their time in the feedyards, these cattle received 2,030,246 regimens of medically important antimicrobials across the categories of in-feed use, control of bovine respiratory disease (BRD), and individual animal therapy. Total antimicrobial regimens of medically important antimicrobials per animal year reported at the study level were 3.65 and 3.17 for 2016 and 2017, respectively. When these regimens are reported by use category; in-feed use contributed 2.91 for 2016 and 2.49 for 2017, control of BRD contributed 0.50 for 2016 and 0.40 for 2017, and individual animal treatment contributed 0.25 for 2016 and 0.28 for 2017. Total milligrams of medically important antimicrobials per kilogram liveweight produced were 44.65 and 30.18 for 2016 and 2017, respectively.

All uses of non-medically important antimicrobials were in the feed; these uses reported as regimens per animal year were 2.78 in 2016 and 2.70 in 2017. Non-medically important

antimicrobial use reported as total milligrams per kilogram liveweight produced were 93.19 and 80.76 for 2016 and 2017, respectively.

To increase granularity, outcome values are also described by study year, use type (in-feed, control of bovine respiratory disease, and individual animal treatment), and antimicrobial class. This level of detail is necessary for interpretation at the individual feedyard level.

Eighteen of the 22 feedyards also participated in a survey regarding their overall antimicrobial use. The survey results were compared to use estimates from the collected data for the year in which the survey was administered. Estimates of medically important antimicrobial regimens per animal year were 3.75 from survey results, compared to 2.98 from the record systems. Milligrams of medically important antimicrobials per kilogram liveweight produced were estimated as 41.67 from the surveys and 31.85 from the collected data.

Estimates of time and resources for completing the project were kept to allow projection of required resources for expansion of the feedyard database. A total of 842 hours were required for data entry, standardization, analysis, and report preparation. Each additional feedyard would require approximately 1.4 days of travel time covering 474 miles and a per diem and lodging cost of $167 per feedyard. Travel time and cost may increase depending on recruitment challenges. The time needed for data management including data import, standardization, and quality control varies between eight and fifteen hours per feedyard depending on record-keeping system.

The question of the application of antimicrobial use metrics related to antimicrobial resistance selection is key. To provide an example, a study was performed using known doses of chlortetracycline in mineral given orally in order to determine related changes in the presence and enumeration of selected antimicrobial resistance genes in fecal samples. The effect of

antimicrobial treatment varied across different resistance genes with time and treatment-related trends.

Antimicrobial use in beef feedyard cattle in the United States

by

Katie Jo Hope

B.S., Kansas State University, 2009
D.V.M., Kansas State University College of Veterinary Medicine, 2013

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2019

Approved by:

Major Professor
Michael D. Apley

Copyright

Abstract

Antimicrobial use is a common occurrence in modern food-animal production. These medications are utilized to prevent, control, and treat a wide variety of infectious diseases in beef cattle. However, as concerns related to antimicrobial resistant bacteria in human and animal health have mounted, countries throughout the world are measuring and monitoring antimicrobial use. These measurements are based on antimicrobial use metrics which generally consist of both a numerator (e.g., weight of product sold or used, number of animals exposed) and a denominator (e.g., animal population, time).

This report is focused on capturing, standardizing, and characterizing antimicrobial use data from 22 beef feedyards in the United States. Data management required navigation of challenges including feedyard recruitment, data acquisition across multiple record-keeping systems, data standardization, and analysis. These 22 feedyards sold a total of approximately 1.2 million head of beef cattle during 2016 and 2017. During their time in the feedyards, these cattle received 2,030,246 regimens of medically important antimicrobials across the categories of in-feed use, control of bovine respiratory disease (BRD), and individual animal therapy. Total antimicrobial regimens of medically important antimicrobials per animal year reported at the study level were 3.65 and 3.17 for 2016 and 2017, respectively. When these regimens are reported by use category; in-feed use contributed 2.91 for 2016 and 2.49 for 2017, control of BRD contributed 0.50 for 2016 and 0.40 for 2017, and individual animal treatment contributed 0.25 for 2016 and 0.28 for 2017. Total milligrams of medically important antimicrobials per kilogram liveweight produced were 44.65 and 30.18 for 2016 and 2017, respectively.

All uses of non-medically important antimicrobials were in the feed; these uses reported as regimens per animal year were 2.78 in 2016 and 2.70 in 2017. Non-medically important

antimicrobial use reported as total milligrams per kilogram liveweight produced were 93.19 and 80.76 for 2016 and 2017, respectively.

To increase granularity, outcome values are also described by study year, use type (in-feed, control of bovine respiratory disease, and individual animal treatment), and antimicrobial class. This level of detail is necessary for interpretation at the individual feedyard level.

Eighteen of the 22 feedyards also participated in a survey regarding their overall antimicrobial use. The survey results were compared to use estimates from the collected data for the year in which the survey was administered. Estimates of medically important antimicrobial regimens per animal year were 3.75 from survey results, compared to 2.98 from the record systems. Milligrams of medically important antimicrobials per kilogram liveweight produced were estimated as 41.67 from the surveys and 31.85 from the collected data.

Estimates of time and resources for completing the project were kept to allow projection of required resources for expansion of the feedyard database. A total of 842 hours were required for data entry, standardization, analysis, and report preparation. Each additional feedyard would require approximately 1.4 days of travel time covering 474 miles and a per diem and lodging cost of $167 per feedyard. Travel time and cost may increase depending on recruitment challenges. The time needed for data management including data import, standardization, and quality control varies between eight and fifteen hours per feedyard depending on record-keeping system.

The question of the application of antimicrobial use metrics related to antimicrobial resistance selection is key. To provide an example, a study was performed using known doses of chlortetracycline in mineral given orally in order to determine related changes in the presence and enumeration of selected antimicrobial resistance genes in fecal samples. The effect of

antimicrobial treatment varied across different resistance genes with time and treatment-related trends.

Table of Contents

List of Figures

List of Tables

Acknowledgements

Thank you to my committee members (Drs. Mike Apley, Ronette Gehring, Brian Lubbers, Dan Thomson, and Dave Renter) for their support, patience, and leadership in this program. I could not have completed this program without their help.

I would also like to thank the staff at Veterinary and Biomedical Research Center (Drs. Kelly Lechtenberg, Keith DeDonder, and DJ Rezac) for allowing me to gain additional valuable experience.

Thank you to Dr. Randy Singer for his invaluable assistance by providing laboratory and sample analysis support.

A final thank you to Dr. Mike Apley and Dr. Nora Schrag for pushing me to succeed and their friendship during this time.

Dedication

I dedicate this dissertation to my family and friends. Without their support, this would not have been possible.

Chapter 1 - Description of antimicrobial use metrics in beef cattle

Introduction

Antimicrobial use is common throughout modern food-animal production. These medications are used to prevent, control, and treat a wide variety of infectious diseases and are highly important to the current beef cattle industry. However, in recent decades there has been increased concern with antimicrobial resistance in both humans and animals.

Concerns of food-animal production contributing to antimicrobial resistance in humans have resulted in multiple reports and actions. A January 2001 report by the Union of Concerned Scientists characterized their view of the overuse of antimicrobials in food animal production by utilizing public information to calculate the antimicrobial use in food animals in comparison to human use.[1] This first analysis in the U.S. was based on data such as reported animal inventories, approved drugs, and their dose, resulting in a comparison of the total kilograms of antimicrobials sold for food animal use and the total kilograms prescribed for human use. No denominator was applied.

In 2006, a new precedent was set when the Food and Drug Administration (FDA) withdrew the approval of the use of enrofloxacin in poultry citing concerns of increased resistance in *Campylobacter* species in association with consumption of poultry products.[2] In 2011, Marshall and Levy supported increased regulation of nontherapeutic antimicrobial use in food-animal production to curtail a "reservoir of resistance genes" based on a series of reports of zoonotic resistant pathogen transfer.[3]

The debate regarding the contribution of food animal antimicrobial use to antimicrobial resistance in humans was further fueled in 2013 by a list of 18 organisms, both bacteria and fungi,

ranked by the Centers for Disease Control and Prevention (CDC) based on level of resistance concern to human health.[4] Most recently, the World Health Organization (WHO) released food animal antimicrobial use recommendations regarding antimicrobial drug classes ranked as important in human medicine.[5] These recommendations were based on low quality evidence, as noted by the authors themselves, but were strongly recommended nonetheless.

The link between antimicrobial use in food-producing animals and antimicrobial resistant bacterial infections in humans is of great concern as food-producing animals are seen as potential origins and disseminators of the organisms of concern.[4] Antimicrobial use concerns related to their suppliers can also be seen in quick service restaurants. McDonalds' released a new antimicrobial use policy for beef in 2018 with a timeline for implementation of reduction targets, as documented by antimicrobial use monitoring, by 2020.[6]

Antimicrobial resistance concerns have not all been about potential zoonotic effects of the use of antimicrobials in food animals. For the commonly isolated bovine respiratory disease (BRD) pathogen *Mannheimia haemolytica*, increased resistance patterns to antimicrobials used frequently to treat bovine respiratory disease (BRD) have been reported by veterinary diagnostic laboratories.[7] A review by DeDonder and Apley noted a trend in decreasing susceptibility of three common BRD pathogens, *Mannheimia haemolytica* included, over the last two decades.[8] Caution should be taken when interpreting these data as the link between laboratory trends and trends in the general cattle population are not well defined.

Advances in technology have allowed for more complete screening of isolated pathogens for resistance to multiple antimicrobials. Whole genome sequencing has identified an integrative conjugative element (ICE) that carries multiple resistance genes, including previously unknown macrolide resistance genes, on one easily transferable unit.[9]

It is first necessary to describe antimicrobial use in order to begin to describe the contribution of antimicrobial use in food animals to antimicrobial resistance in humans and to help in understanding potential selection for resistance to antimicrobials used for prevention, control, and treatment of bacterial disease in food animals themselves. Singer et al. emphasized that the high variability in currently available antimicrobial use data should cause caution to be taken when interpreting potential associations between antimicrobial use and antimicrobial resistance.[10] However, as shown by Hillerton et al., available antimicrobial use data are frequently used to compare use between countries by utilizing antimicrobial use data from various sources and converting to a milligram per kilogram of biomass. [11]

Focusing specifically on cattle in U.S. beef feedyards, approximately 14.4 million head of cattle were on feed as of January 1, 2019 with an estimated 81% of these located in feedyards with a capacity of greater than 1000 head.[12] Some general insights into beef feedyard antimicrobial use were gained when a portion of those feedyards were surveyed by the United States Department of Agriculture (USDA) in 2011. In this survey, approximately 16% of feedyard cattle were reported to have bovine respiratory disease with nearly all of these animals receiving an antimicrobial parenterally, 21.3% of cattle were administered an antimicrobial for control of BRD with an injectable antimicrobial, 18.4% of cattle were treated for BRD with in-feed chlortetracycline, and tylosin was fed to 71.2% of cattle for the reduction in the incidence of liver abscesses.[13]

Detailed measurement of antimicrobial use in feedyards to achieve a sample across the industry is challenging due to differences in recording technology, logistical challenges for data collection and transfer, analytical needs for converting disparate datasets into a uniform reporting platform, and data availability barriers due to concerns about the effects of public access to antimicrobial use data. The subject of this dissertation centers on collecting, managing, and

analyzing data from 22 feedyards which are then compared to the results of surveys administered to a subset of these feedyards. First in the progression of events for reporting and analysis was to determine the metrics to be used.

The variety of antimicrobial use metrics discussed in the literature is extensive and contributes to the difficulties in discussing use across different species (humans included), production systems, and countries. All of the metrics have benefits and disadvantages and which one is more appropriate than the other is dependent on the utility for a specific query. Evaluation of antimicrobial use requires looking at multiple metrics for the same system if antimicrobial use is to be completely understood.

To facilitate understanding of the importance of the selected metrics, this review is focused on describing antimicrobial use terms that have been applied to beef cattle on feed or another bovine production system where muscle protein production is the final goal (e.g., veal calf farms in Belgium and France).

Methods

A literature search of three databases was performed on February 24, 2019. Keywords were "antimicrobial", "beef", "feedlot", and "use". These keywords and relevant associated words were used for the literature search and are described in Table 1.

The search results are listed below:

- Pubmed: 602 titles returned,
- Agricola: 540 titles returned,
- Web of Sciences (CABI): 2,632 titles returned.

Table 1 - Search Terms

Antimicrobial use	Beef	Feedlot	Use/Metric:
Antimicrobial (s)	cow (s)	feedlot (s)	quantification
antibiotic (s)	cattle	feedyard (s)	use (s)
drug (s)	steer (s)	confinement	measure (s)
treatment	heifer (s)	finish	unit(s)
	bull (s)		sale(s)
	stocker (s)		dose(s)
	feeder (s)		defined dose
	calf		metric(s)
	calves		
	livestock		

All titles were imported into EndNote (version X7.8) on the date of the search for a total of 3,774 titles. EndNote identified and removed 506 duplicate articles. The remaining 3,278 articles were screened by title, plus abstract if necessary. A total of 3,203 articles were removed by this method leaving 75 articles for full text screening. A further 11 articles were removed for the following reasons: full article not available in English (n = 2), no quantification of antimicrobial use (n = 2), <4 antimicrobials reviewed (n = 2), did not include beef cattle (n = 4), and only discussion of pharmacokinetic and pharmacodynamics aspects (n = 1). Seven additional articles previously identified as informative to the literature search were included as well. Figure 1 illustrates the process for article selection.

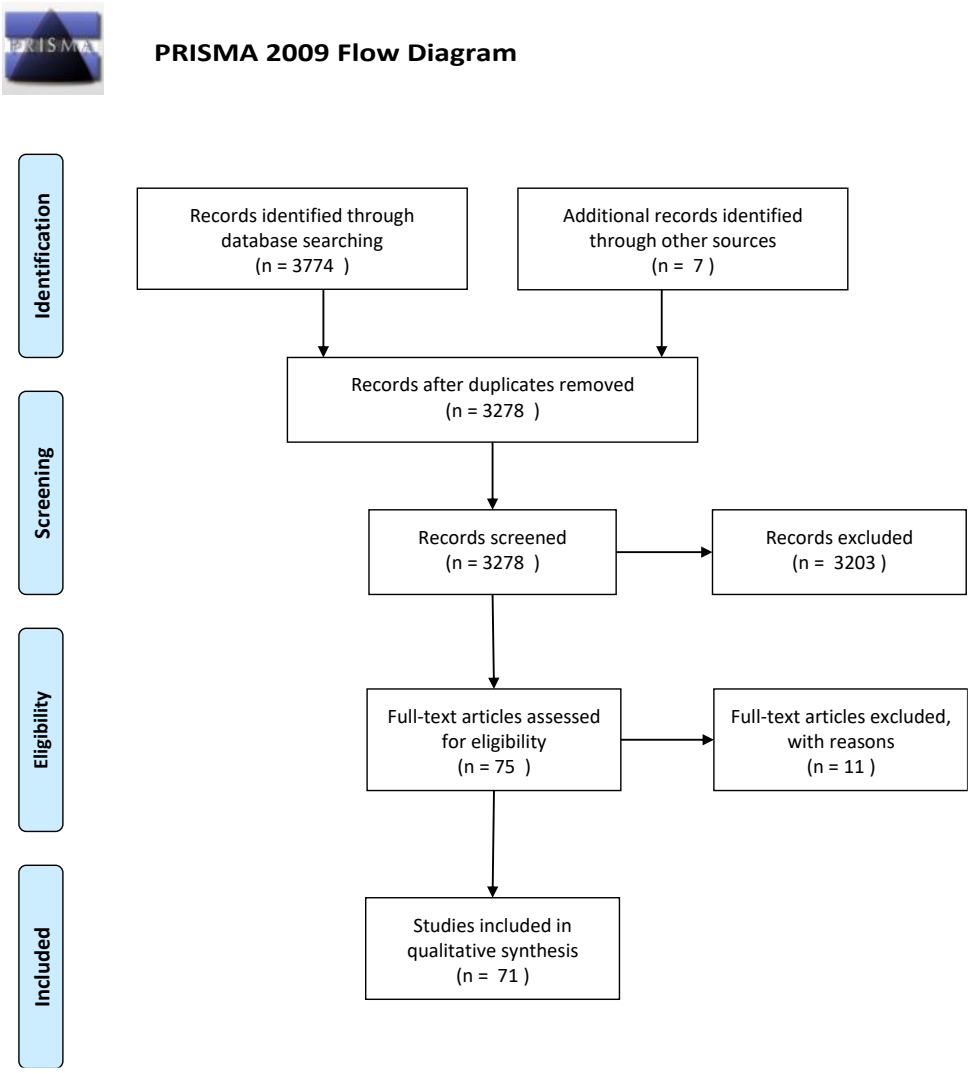

Figure 1 - PRISMA Flow Chart

Types of Metrics Used in Published Articles

For the purposes of this review, the antimicrobial use (AMU) terms were organized into three categories. The first category is numerators, in which the antimicrobial use terms describe data in a manner that identifies an amount of drug product, duration of use, or the number of animals to which the drug is administered. The second category is source, with an emphasis on

the use of sales data. The third category involves denominators, which describe the population to which the numerator would be applied. The majority of the articles are included in more than one category due to having components in more than one of the categories defined above.

Numerators – Measures of Quantity, Duration, and Number of Animals Exposed

A metric that is one of the more recognizable ones due to its use in human medicine is the Number of Defined Daily Doses (nDDD). A defined daily dose (DDD[a]) is a presumed average maintenance dose per day per drug product used for the primary indication in an adult. The World Health Organization (WHO) was the first to define and release this term with the intention of a more global adoption and has published guidelines for assigning DDDs since 1991.[14] It is based off of the Anatomical Therapeutic Chemical Code (ATC) which is a system that divides drugs into specific categories based on multiple properties. A DDD does not necessarily correspond to a label or actually administered dose. A working group formed by the WHO establishes the DDDs for drug products as a way to standardize the variation in doses across countries. One DDD is defined per ATC code and route of administration. A drug product with an injectable and oral formulation would have two DDDs, one for each route. Table 2 describes a compilation of AMU term definitions, starting with DDD.

Another human-specific use measure is the Number of Days of Therapy (nDOT).[15] This measurement is a course measurement, bringing in the element of time exposure rather than strictly drug consumption. A Day of Therapy (DOT) is considered to be a calendar day on which a product

[a] The "n" is added to this abbreviation to indicate reference to the metric, rather than the dose. Inconsistencies in abbreviation methods can be found in the literature. The abbreviation "ADD" is used to refer both to the "defined daily dose" as well as the "number of defined daily doses".

is administered at least once. It does not account for multiple doses given on the same day (e.g. every 8 hours). Calculating the nDOT is an attempt to look at drug exposure as related to potential antimicrobial resistance selection pressure. The nDDD and nDOT metrics are attempts to quantify antimicrobial drug use as the number of doses given and the time that a person was exposed as a proxy for antimicrobial resistance selection pressure.

As interest in monitoring antimicrobial use in food animal production grew, an equivalent indicator to nDDD for use in animals was created. Denmark first reported antimicrobial use as a Number of Animal Daily Dose (nADD) in 2001 as part of their comprehensive antimicrobial use reporting program referred to as DANMAP: The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme.[16] A DANMAP Animal Daily Dose (ADD[b]) is defined as the average maintenance dose of a specific product used for its main indication per day. This term was changed to the Defined Animal Daily Dose (DADD) in the 2013 DANMAP report. A DADD is defined specifically for DANMAP and does not apply to other countries as it is defined specifically based on Denmark's drug record system ADDs.[17]

The ADD can be defined several different ways depending on location and author preference. The ADD definition that is the most similar in concept to the DDD utilized in human medicine is commonly used in papers of European authorship. It is the defined average maintenance dose of a particular drug product per kilogram for a particular animal host species per day, when used for the drug product's main indication.[18-21] The resultant number is often specific to a country or geographic area.[10] In some cases the ADD is defined as an ADD for a specific

[b] The "n" is added to this abbreviation to indicate reference to the metric, rather than the dose. Inconsistencies in abbreviation methods can be found in the literature. The abbreviation "ADD" is used to refer both to the "defined daily dose" as well as the "number of defined daily doses".

treatment weight (e.g. ADD per 200 kg veal calf).[22] However, there is another definition used for ADD that is significantly different. The alternative definition is given as the number of days one dose of a drug product remains in the animal's tissues based on the labeled claims for respiratory disease treatment.[23-26] This approach assigned the number of ADDs resulting from a single administration of an antimicrobial and removes the lack of consideration of antimicrobial potency and formulation differences often associated with the use of ADDs.[21] Checkley, et al., used yet another alternative ADD, based on the DDD, referred to as animal daily dose feedlot (ADDfeedlot). [27] ADDfeedlot was assigned to three commonly used feedlot antimicrobials based on the ADD definition of tissue time discussed above (e.g. a day of ceftiofur sodium = 1 ADDfeedlot). The ADDfeedlot was summed for all enrolled study animals and reported as an overall number of ADDfeedlot used for disease treatment.

In 2015, a request by the European Commission for a veterinary comparable metric to nDDD resulted in the European Medicines Agency (EMA) creating the Defined Daily Dose vet (DDDvet) and the Defined Course Dose vet (DCDvet). A DDDvet is the veterinary equivalent of a DDD created by assuming the average dose per kilogram animal weight per day for an animal species and a DCDvet is the agreed mg amount expressed as mg/kg per treatment course.[28] The determination of a DDDvet or DCDvet is conducted by reviewing product information, including dose, across multiple European countries and assigning the value based on that information. DDDvet and DCDvet are specific to a species, drug product, and route of administration.[29]

Dissimilarity in definitions contributes to the confusion often associated with these various metric descriptions as they are often used interchangeably in the literature with slightly different definitions depending on the study location and authors. The ADD as defined by drug concentrations assumes a relationship between therapeutic duration and antimicrobial selection

pressure. There are major assumptions inherent in the duration of therapeutic effects, let alone the relationship of dose, duration, and route of antimicrobial drug products to resistance selection pressure, which are not well characterized at this point.

Another antimicrobial use metric used in veterinary medicine that is derived from a human metric is the number of prescribed daily doses (nPDD). The prescribed daily dose (PDD) is intended to be a reflection of the actual dose prescribed by a veterinarian.[18] PDD is calculated by the total amount prescribed divided by an average treatment weight and number of animals treated. For a treatment administered to an individual animal, it would simply be the total amount of product prescribed divided by the estimated animal weight at treatment multiplied by one.[19,30] A veal calf study in France used prescribed daily doses (PDD) to create a metric referred to as number of antimicrobial treatments per calf (NTPC). [31] The authors collected the antimicrobial use data from veterinary prescriptions and dispensing information for the farms participating in the study. The PDD was used to describe the dose given per calf per day per product and these values were summed with all other drug classes to create an overall NTPC value for all farms involved. It should be noted, however, that the amount of drug product that is prescribed is not necessarily the amount that is used.

Another metric is the number of used daily doses (nUDD). Measures of the number of UDD was initially reported in a swine antimicrobial use study by Timmerman, et al.[32] In order to calculate a UDD, more granular information is needed than for calculating an ADD or a PDD. Calculation of the UDD requires the amount of product given during a defined time period which is then divided by the number of animals being treated and a weight at time of treatment. UDD is intended to reflect the dose that was actually given on farm to an animal and often deviates from an ADD.[19,30,33]

Another common method of presenting antimicrobial use data is by weight of active substance. This is usually reported on a milligram or kilogram basis. The data source for reporting active ingredient by weight is often sales data with results being reported in a milligram per population correction unit (PCU) or biomass equivalent which are discussed in more detail in the denominator section. Milligram of active ingredient sold is commonly used due to the relatively easy access to antimicrobial sales data. [11,34-37] In some cases, the active ingredient is reported minus the salt (e.g. tetracycline instead of tetracycline HCL), which in the 2008 DANMAP report had the effect of reducing reported mass of active ingredient by 3.4%. [38]

Treatment cost is occasionally used as a metric of antimicrobial use.[33] In beef feedyards, treatment cost refers to the dollar amount of all antimicrobial costs per individual animal along with metaphylaxis (control of bovine respiratory disease) treatments, then divided by the number of animals in the lot at arrival.[39] It is important to note that when looking at treatment cost as an antimicrobial use metric, changes in cost of drug products can vary significantly between drug class and years.

Numerators: Sales Data

One of the frequently mentioned methods of reporting antimicrobial use in the literature is through reporting sales of drug products. Antimicrobial sales information generally refers to the tracking of sales of specific drug products. One of the larger antimicrobial use monitoring programs in terms of number of countries included is the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) program.[40] This program, initiated by the European Commission and European Medicines Agency (EMA), involves the collection of antimicrobial

sales data for major food-producing animal species. The most recent ESVAC report includes sales data reported in some form from 30 European countries.

In the United States, another use of antimicrobial sales monitoring is that mandated in 2008 by Section 105 of the Animal Drug User Fee Act (ADUFA), which requires drug sponsors to annually report the amounts (in kilograms) of antimicrobial drugs approved for use in food-producing animals in the United States. The FDA takes this described information and summarizes it prior to making it available in a public report; the first such report summarized antimicrobial sales data for 2009. In 2016, a new reporting provision was added to Section 105 of ADUFA, requiring drug sponsors to provide estimates of species-specific sales data for the major food-producing species (cattle, swine, chickens, turkeys). Data for all other species are included in an 'other' category.[41]

The applicability of sales data depends on what is being evaluated. If the goal is to measure antimicrobial use contribution to the development or continuation of antimicrobial resistance in specific populations, it is unsuitable. However, if the interest is in simply monitoring drug distribution (with variable and undetermined relationship to use) over time, then sales data may be sufficient.[30,33] However, interpretation of antimicrobial sales data is almost always fraught with caveats and should be done with caution. A difference should be noted in interpreting country-level sales data compared to specific food animal production facilities. The proportion of drug product sold that is actually used is unknown at a national sales level. A single production unit (e.g., feedyard or dairy) would be more apt to track drug product shrinkage and use related to purchases due to the economic implications.

Antimicrobial use data can also be used to create estimates that are reported as total amount of antimicrobial drug products without consideration of any specific drug properties and provided

with no estimate of the population over which it was used, oftentimes citing reports of antimicrobial sales information.[42,43]

Table 2 – Selected Antimicrobial Use Terms as Used in the Literature

Antimicrobial Use Terms	Definition
Defined Daily Dose (DDD) (human)	Assumed average maintenance dose per day for a drug used in its main indication in adults (70 kg). [14]
Defined Daily Dose for animals (DDDvet)	Assumed average dose per kilogram animal per species per day. [28]
Defined Course Dose for animals (DCDvet)	Assumed average dose per kilogram animal per species per treatment course. [28]
Drug mass in kilogram	Kilograms of active ingredient
Animal Daily Dose (ADD)	Dose required to treat a typical animal for one day. [44]
	Average maintenance dose of a specified drug per kilogram for the main indication in a specified species. [18]
	The number of days that a single dose remains at therapeutic levels in a target tissue of an animal based on label claims for treatment of bovine respiratory disease. [45]
Animal Daily Doses feedlot (ADDfeedlot)	The number of actual antimicrobial treatments given at the label dose of the antimicrobial as calculated by a self-defined ADD. [27]
Animal Defined Daily Dose (ADDD)	Number of days of treatment for an animal based on an assumed average maintenance dose. [45]
Defined Animal Daily Dose (DADD)	Average maintenance dose of a specific product used for its main indication per day.[17]
Animal Course Dose (ACD)	Daily dose multiplied by the duration of treatment. [44]
Prescribed Daily Dose (PDD)	Average daily dose prescribed. [18]
	Dose on veterinarians' prescription record divided by the average live weight at the beginning of treatment. [19]

Used Daily Dose (UDD)	Actual administered dose per day per kilogram of animal weight of a drug. [32]
Treatment Incidence (TI)	Number of animals per 1000 that are treated daily with one dose, could be defined as one ADD, UDD, or PDD. [19]
Days of Therapy (DOT) (human)	Administration of one drug product on any given day without consideration of the dosing schedule on that day.[15]
Treatment Cost	Summary of bovine respiratory disease treatment drug costs at the lot (control of bovine respiratory disease) and individual animal level (individual treatment) summed then divided by the number of head in the lot at arrival. [39]
Number of Animal Daily Doses (nADD)	Number of ADDs used per animal per year.[20]
Denominators	
Number of Animals Treated	Count of animals treated with antimicrobial drug.
Target Animal Biomass	Calculated by multiplying the estimated number of animals in the target species by the average weight of that species.[46]
Population Correction Unit (PCU)	Calculated mass of live and slaughtered animals multiplied by an estimated weight at treatment for each country, 1 PCU = 1 kg. [36]

Treatment Incidence – A Proxy for Disease Incidence

Pardon et al. looked at three metrics and compared farm ranks based on the different metrics. The ADD, UDD, and PDD were used to calculate a treatment incidence (TI).[19] Treatment incidence is calculated as follows:

$$\frac{\text{total amount of drug product (mg)}}{\text{ADD,UDD,or PDD } \left(\frac{\text{mg}}{\text{kg}}\right) * \text{number of days animals were at risk} * \text{weight of animals at risk (kg)}} * 1000$$

The calculated value is the number of animals per 1000 animals that are treated daily with the drug product as calculated by the selected metric.[32] Treatment incidence can be viewed as a substitute for disease pressure on a particular farm; as it is a metric based on the amount of drug product used

combined with a "dose" per animal, and time at risk.[19,32] In 2003, Catry et al. recognized the importance of capturing actual treatment regimens. The information included in a regimen would be dose, treatment interval, duration of treatment, and product formulation.[47] All information provided in a complete treatment regimen could have an influence on antimicrobial selection pressure.

Denominators – Measures of Reference Population and Time

In order to appropriately apply any of the numerators discussed above in a manner that allows interpretation, the antimicrobial quantity data should be attributed to a population. In human medicine this information is often presented as patient days, time spent in the hospital, or even as admissions to the hospital. These serve as estimates of populations exposed and durations of exposure to selection pressure from antimicrobial use.[48] In contrast to human hospitals, food animals are even less of a homogenous population. The variety of production animals, systems, geography, and record systems make collecting population information for antimicrobial use in food-animal production very complex. Even within a single species and production system type, populations can range from animals on an individual farm to multiple farms in the same area; the number of animals per site also varies greatly.

Starting with the highest level of population description (the least granularity) requires discussing the ESVAC report denominator, the population correction unit (PCU). A PCU is an estimated denominator of all livestock present in a specific country. It is calculated by adding the following inputs,

- the number of animals slaughtered multiplied by the estimated average weight at the time of treatment

- the number of livestock (beef cows, dairy cows, ewes, etc) required to produce the portion of slaughtered animals multiplied by the estimated average weight at treatment
- the number of animals imported and exported by the estimated average weight at treatment.

For all categories listed above, the average weight at treatment varies depending on the production class and age of animals to which it is being applied.[11,37,49]

In an effort to create a similar denominator in the United States, in August of 2017 the FDA requested comments for the calculation of a biomass denominator to use in association with antimicrobial sales data. FDA proposed a U.S.-specific method that takes into account domestic animal populations and weights and that will better fit the circumstances of animal production in the United States. The current proposed method of calculation is similar to a PCU, but would look at estimated slaughter weights and estimated annual average weights instead of average weight at time of treatment. The proposal put forth by the FDA incorporates a milligram (mg) of drug sold (ADUFA data) per target animal biomass (TAB). As of April 2019, the final decision has not been publicly released.[46]

$$\text{mg/TAB} = \frac{(\text{sum of all sales of a specific antimicrobial drug class (mg) for a given target animal species})}{(\text{estimated number of animals in the target species} * \text{estimated average weight (kg) of that species})}$$

Carmo et al. also calculated a population estimate by using an average weight at treatment following the ESVAC method.[34] However, they referred to it as a biomass estimation rather than a PCU due to only looking at specific species rather than all livestock present in the countries. The PCU and biomass calculations are efforts made to provide a basis to compare antimicrobial use to

a weight of a population; it is very important to know to what time period the mass estimate applies. If known, the weight can also be portrayed as a total weight of animals in a defined period. Standardized weight of the population on farm can be used to calculate a total weight of animals present on a farm for a specific period.[50]

Two other denominators often encountered in the literature present a more granular population or population-time component by reporting data as per animal or per animal day, respectively. Animal days are most often utilized for studies that directly track the number of animals included. Animal days is the aggregate time for which an animal was at risk of being exposed to the antimicrobial. The animal day is used to represent the number of antimicrobial exposures during a specific risk of exposure period (e.g., 2 ADD per 1000 animal-days).[33] An exposure period can be used to represent a whole population (e.g. all animals within a particular system) or a specific portion (e.g. all animals in pens participating in a study).[23,51] In a study by Bosman et al., nADD per production cycle was used. Production cycle was defined as the average time of the feeding period and was approximately 175 days in this particular study. Production cycle is similar to animal days as it is intended to reflect a time period over which an average animal could be exposed to antimicrobial use.[21,31]

Published Data Collection Methods

There are several approaches that have been employed to collect antimicrobial use data. A common method is using a national compulsory reporting system. Compulsory reporting may include mandatory reporting of active drug products sold, veterinarian drug sales, and prescriptions.[21,34,50] These data are most often captured through some form of electronic data transfer and then presented in a consolidated public report.[40,41,44]

Another published technique of data collection is employing surveys and questionnaires. Questionnaires are the list of questions answered by respondents. Surveys are the questions combined with the process of administering and collecting the information. Questionnaires and surveys are sometimes synonymous with each other as some form of a questionnaire is often utilized for survey completion to ensure consistency across all parties. This method can be completed in person, via phone, or online.[52,53]

Carson, et al. compared two forms of antimicrobial use capture. The participating beef cattle farms were asked to keep a treatment diary of all animals that were treated during the study period and were provided with a garbage can to collect all empty drug containers. On average, the participants recorded 60% of their parental antimicrobial use compared to what was captured by the provided garbage cans.[54]

When study authors are looking at a few select farms or feedyards, another collection method is a direct download from the feedyard electronic animal health systems. In general, this technique provides the most granular use data as it is tracked by individual animal treatments and may include the drug product, animal weight, and dose, depending on the system.[24-27,39,45]

Combinations of Metrics are Needed for a Complete View of Use

Comparisons

Studies in veterinary medicine have shown that prescribed daily doses (PDD) [18] and used daily doses (UDD) [19] can vary significantly from the DDD. A study looking at a comparison of nDDD and days of therapy (DOT) in human hospitals noted that if the DDD of an antimicrobial either over or under estimates a PDD or UDD then a reduction in nDDD may not translate to a reduction in overall drug use.[55] Another study by Bond, et al. discussed antimicrobial use between

The Netherlands and Denmark; their data showed that using a milligram of drug (sales data) per population correction unit (PCU) metric compared to the national mandated collection data overestimated the difference between the countries.[20] This is an important point to note, especially since the animal demographics between the two countries are relatively similar. Another country level comparison was conducted by Carmo, et al. looking at antimicrobial use patterns in Switzerland and Denmark for cattle and swine. This study reviewed use trends for six years (2007-2013) in both species by collecting data from a combination of sources. Swiss data were sourced from sales data combined with research information while the Danish data came from the VetStat program; data were presented in a milligram per biomass for both.[34] Associating antimicrobial use across countries with vastly different collection techniques should be done with caution, as differences could be attributed to multiple factors including geography, production systems, prescribing practices, and disease pressure.

Comparison of different antimicrobial use metrics on the same data has shown that farms can appear to change in the apparent amount of antimicrobials they use by changing the metric used. An example of this was a study done on 15 white veal farms in Belgium. The authors showed that the farm rank could change based on whether they utilized a treatment incidence based on nADD, nPDD, nUDD, or an nADD based on a standard treatment weight of 164kg.[19] In systems that assign penalties based on antimicrobial use data, these differences could be the deciding factor whether an individual farm does or does not receive sanctions which could have severe economic consequences for the producers.

To Benchmark or Not to Benchmark

According to Merriam-Webster, the definition of benchmark is "something that serves as a standard by which others may be measured or judged".[56] This basic definition can serve as a basis for regulations that can drastically change industries, which is why it is very important that the purpose for data collection is clear from the onset of collection. Arbitrarily collecting data for a use that is decided upon later is not an efficient or effective use of time or resources and may mismatch granularity and quality of data with the final interpretive outcome. Data collection should be initiated with a clear goal.

The current main driver of reviewing antimicrobial use in food-producing animals is the concern with antimicrobial resistance in both human and animal health. The use of medically important antimicrobials for growth promotion in food animals has raised concerns for several years. In 2000 and 2001, Barton discussed the potential problems associated with using medically important antimicrobials for growth promotion such as antimicrobial residues, antimicrobial resistance in animals, and antimicrobial resistance transferring from animals to humans.[57,58] In 2004, Phillips, et al., studied available antimicrobial use data from food animals, specifically growth promotion use, linked to antimicrobial resistance data in humans.[59] A review by Murphy et al. looked for publications that contained information on antimicrobial resistance and factors that could provide selection pressure.[60] However, the authors cautioned that despite the general agreement that widespread antimicrobial use can provide selection pressure, the ability to gauge the magnitude of it is not well understood.

The question of why the use data is being collected is the most important aspect of determining which metric is appropriate. The appropriate approach almost always involves a combination of metrics to gain a complete view of antimicrobial use. Assigning arbitrary targets

(e.g., total kg drug purchased) to meet without regard for animal welfare and health concerns may lead to catastrophic welfare issues.

The Connection of Benchmarking and Policy

Benchmarking can be done in a useful and productive manner, but caution must be taken not to allow metrics to create an illusion of uniformity among production systems that are not equivalent. Utilizing antimicrobial use information can assist veterinarians and producers in identifying production systems, or specific areas within these systems that could benefit from a more in-depth investigation of challenges leading to antimicrobial use. Benchmarking can also be utilized on a national level to identify production systems which significantly depart from the norm for antimicrobial use dependent on the granularity of data collected.

Examples of antimicrobial use metrics used in a metrics-based reduction program include Denmark's DANMAP report and The Netherlands MARAN report. The Danish began a benchmarking system referred to as the "Yellow Card" program, which also sets antimicrobial use limits. A farm that is above a set limitation is subject to sanctions from the Danish Veterinary and Food Administration.[34]

In 2012, the FDA released Guidance for Industry (GFI) #209 (The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals) as an informative document on recommendations regarding antimicrobial use in animals. The recommended principles of judicious use outlined in GFI #209 included limiting medically important antimicrobial drugs to uses in food-producing animals that are considered necessary for assuring animal health, and limiting such drugs to uses in food-producing animals that include veterinary oversight.[61] As further encouragement of judicious antimicrobial use, the FDA recently released

goals for the next five years.[62] Some of the objectives listed include revising drug product labels to more closely adhere with antimicrobial stewardship by requiring a duration of use be specified, placing all medically important antimicrobial use under veterinary oversight, increasing awareness of antimicrobial stewardship in companion animals, and encouraging new development of non-antimicrobial products. The trends in ADUFA sales data, presumably expressed in relation to a biomass denominator, may be one of several factors that FDA may use to indicate the success of these initiatives.

A concern that still remains is the availability of antimicrobial use data for risk assessments. Risk assessments are oftentimes required for FDA approval of a new animal drug application. FDA GFI #152 (Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to their Microbiological Effects on Bacteria of Human Health Concern) outlines an approach which drug sponsors may use to evaluate the microbial food safety of antimicrobial new animal drugs intended for use in food-producing animals. Singer and McEwen discussed the need for antimicrobial use data to enhance the validity of risk assessments, especially those that model human health impact.[63]

Attitudes Towards Antimicrobial Use in Food Animal Production

It must be recognized that the available antimicrobial use metrics will be used in discussion of concerns related to antimicrobial use in food animals. This concern is often characterized in relation to categories of medical importance established in FDA GFI document #152.[64] This document provided guidelines for new animal drug applications for food-producing animals. The drug product is classified into one of three categories depending on the importance of the drug class to human medicine. Each of the regulatory categories result in different approval requirements (currently GFI #152 indicates categories of important, highly important, and

critically important to human health - this GFI is currently under revision and these categories may be changed or updated).[65] Scott, et al. recently discussed the need for standardization between the designations of antimicrobial importance by the WHO, FDA, and EMA.[66]

Antimicrobial use in the animal is related to concerns about environmental antimicrobial contamination and selection pressure as well as food safety.[67] Related to these concerns, antimicrobial stewardship discussions have been implemented in veterinary medicine with renewed interest in the last few years; areas of emphasis include disease prevention, proper diagnosis, drug selection, and judicious antimicrobial use. The information provided by these discussions is intended to augment the education received in a college of veterinary medicine.[68,69]

As requested by the WHO during the development of the Global Action Plan on AMR, the World Organisation for Animal Health (OIE) began amassing information on antimicrobial resistance in animals which includes tracking antimicrobial use.[70] Two reports, most recently in 2017, detailing the efforts to track antimicrobial use in animals at a supra-national level have been released by the OIE.[71]

The perceptions of pressure from consumers, regulatory agencies, competing producers, veterinary organizations, beef packers, and beef retailers have an impact on antimicrobial use decisions by veterinarians and producers in beef cattle production.[72,73] Use decisions often consider that using antimicrobials is not only an economic decision, but that there are both moral and ethical considerations that precede the decision to treat an animal.[74] Scott et al. discuss the moral and ethical considerations that are involved in a decision to use antimicrobials in animals. Surveys of producers and veterinarians indicate they believe they are using antimicrobials appropriately and do not cause harm to human health.[75] This environment of the intersection, and

perhaps collision, of science and real and perceived norms creates a charged environment in which to develop and use antimicrobial use metrics.

References

1. Mellon M, Benbrook C, Benbrook KL. *Hogging it: Estimates of Antimicrobial Abuse In Livestock*: Union of Concerned Scientists, 2001.
2. Chiller TM, Angulo FJ, Nelson JM, et al. Fluoroquinolone-Resistant Campylobacter Species and the Withdrawal of Fluoroquinolones from Use in Poultry: A Public Health Success Story. *Clinical Infectious Diseases* 2007;44:977-980.
3. Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev* 2011;24:718-733.
4. Department of Health and Human Services Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013, Accessed 8-15-2019 at, https://www.cdc.gov/narms/resources/threats.html
5. World health Organization. WHO Guidelines on Use of Medically Important Antimicrobials in Food—Producing Animals. 2017, Accessed 8-15-2019 at, https://www.who.int/foodsafety/areas_work/antimicrobial-resistance/cia_guidelines/en/
6. McDonalds. Antibiotic Use Policy for Beef and Dairy Beef, 2018, Accessed 8-15-2019 at, https://corporate.mcdonalds.com/corpmcd/scale-for-good/our-food/animal-health-and-welfare.html
7. Lubbers BV, Hanzlicek GA. Antimicrobial multidrug resistance and coresistance patterns of Mannheimia haemolytica isolated from bovine respiratory disease cases—a three-year (2009–2011) retrospective analysis. *Journal of Veterinary Diagnostic Investigation* 2013;25:413-417.
8. DeDonder K, Apley M. A literature review of antimicrobial resistance in Pathogens associated with bovine respiratory disease. *Animal health research reviews* 2015;16:125-134.
9. Michael GB, Kadlec K, Sweeney MT, et al. ICE Pmu1, an integrative conjugative element (ICE) of Pasteurella multocida: analysis of the regions that comprise 12 antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy* 2011;67:84-90.
10. Singer RS, Reid-Smith R, Sischo WM. Stakeholder position paper: Epidemiological perspectives on antibiotic use in animals. *Preventive veterinary medicine* 2006;73:153-161.
11. Hillerton JE, Irvine CR, Bryan MA, et al. Use of antimicrobials for animals in New Zealand, and in comparison with other countries. *New Zealand Veterinary Journal* 2017;65:71-77.
12. United States Department of Agriculture National Agricultural Statistics Service ASB. Cattle Inventory Report, 2019, Accessed 8-15-2019 at, https://www.nass.usda.gov/Charts_and_Maps/Cattle/index.php
13. Dargatz DA, Lombard JE. Summary of BRD data from the 2011 NAHMS feedlot and dairy heifer studies. *Anim Health Res Rev* 2014;15:123-125.

14. World Health Organization. Guidelines for ATC classification and DDD assignment 2019, Accessed 8-15-2019 at, https://www.whocc.no/atc_ddd_index_and_guidelines/guidelines/
15. Polk RE, Fox C, Mahoney A, et al. Measurement of adult antibacterial drug use in 130 US hospitals: comparison of defined daily dose and days of therapy. *Clinical infectious diseases* 2007;44:664-670.
16. Flemming Bager DLM, Sigrid Andersen. DANMAP 2001 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark In: Programme TDIARMaR, ed, 2001;68.
17. Agersø Y, Bager F, Boel J, et al. DANMAP 2013: use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 2014.
18. Jensen VF, Jacobsen E, Bager F. Veterinary antimicrobial-usage statistics based on standardized measures of dosage. *Preventive Veterinary Medicine* 2004;64:201-215.
19. Pardon B, Catry B, Dewulf J, et al. Prospective study on quantitative and qualitative antimicrobial and anti-inflammatory drug use in white veal calves. *Journal of Antimicrobial Chemotherapy* 2012;67:1027-1038.
20. Bondt N, Jensen VF, Puister-Jansen LF, et al. Comparing antimicrobial exposure based on sales data. *Preventive Veterinary Medicine* 2013;108:10-20.
21. Bosman AB, Wagenaar JA, Stegeman JA, et al. Antimicrobial resistance in commensal Escherichia coli in veal calves is associated with antimicrobial drug use. *Epidemiology and Infection* 2014;142:1893-1904.
22. Fertner M, Toft N, Martin HL, et al. A register-based study of the antimicrobial usage in Danish veal calves and young bulls. *Preventive Veterinary Medicine* 2016;131:41-47.
23. Benedict KM, Gow SP, McAllister TA, et al. Antimicrobial Resistance in Escherichia coli Recovered from Feedlot Cattle and Associations with Antimicrobial Use. *PLoS One* 2015;10:e0143995.
24. Noyes NR, Benedict KM, Gow SP, et al. Mannheimia haemolytica in Feedlot Cattle: Prevalence of Recovery and Associations with Antimicrobial Use, Resistance, and Health Outcomes. *Journal of Veterinary Internal Medicine* 2015;29:705-713.
25. Noyes NR, Benedict KM, Gow SP, et al. Modelling considerations in the analysis of associations between antimicrobial use and resistance in beef feedlot cattle. *Epidemiol Infect* 2016;144:1313-1329.
26. Rao S, Van Donkersgoed J, Bohaychuk V, et al. Antimicrobial drug use and antimicrobial resistance in enteric bacteria among cattle from Alberta feedlots. *Foodborne Pathog Dis* 2010;7:449-457.
27. Checkley SL, Campbell JR, Chirino-Trejo M, et al. Antimicrobial resistance in generic fecal Escherichia coil obtained from beef cattle on arrival at the feedlot and prior to slaughter, and associations with volume of total individual cattle antimicrobial treatments in one western Canadian feedlot. *Can J Vet Res* 2008;72:101-108.
28. European Surveillance of Veterinary Antimicrobials (ESVAC). Principles on assignment of defined daily dose for animals (DDDA) and defined course dose for animals (DCDA)(EMA/710019/2014), 2015, Accessed 8-15-2019 at, https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/european-surveillance-veterinary-antimicrobial-consumption-esvac

29. European Medicines Agency European Surveillance of Veterinary Antimicrobal Consumption (ESVAC). Defined daily doses for animals (DDDvet) and defined course doses for animals (DCDvet), 2016, Accessed 8-15-2019 at, https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/european-surveillance-veterinary-antimicrobial-consumption-esvac
30. Werner N, McEwen S, Kreienbrock L. Monitoring Antimicrobial Drug Usage in Animals: Methods and Applications. *Microbiology Spectrum* 2018;6:25.
31. Jarrige N, Cazeau G, Morignat E, et al. Quantitative and qualitative analysis of antimicrobial usage in white veal calves in France. *Preventive Veterinary Medicine* 2017;144:158-166.
32. Timmerman T, Dewulf J, Catry B, et al. Quantification and evaluation of antimicrobial drug use in group treatments for fattening pigs in Belgium. *Preventive veterinary medicine* 2006;74:251-263.
33. Collineau L, Belloc C, Stark KDC, et al. Guidance on the Selection of Appropriate Indicators for Quantification of Antimicrobial Usage in Humans and Animals. *Zoonoses and Public Health* 2017;64:165-184.
34. Carmo LP, Nielsen LR, Alban L, et al. Comparison of Antimicrobial Consumption Patterns in the Swiss and Danish Cattle and Swine Production (2007-2013). *Frontiers in Veterinary Science* 2017;4:11.
35. Chantziaras I, Boyen F, Callens B, et al. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. *Journal of Antimicrobial Chemotherapy* 2014;69:827-834.
36. Grave K, Greko C, Kvaale MK, et al. Sales of veterinary antibacterial agents in nine European countries during 2005–09: trends and patterns. *Journal of antimicrobial chemotherapy* 2012;67:3001-3008.
37. Grave K, Torren-Edo J, Muller A, et al. Variations in the sales and sales patterns of veterinary antimicrobial agents in 25 European countries. *Journal of Antimicrobial Chemotherapy* 2014;69:2284-2291.
38. Danish Programme for Surveillance of Antimicrobial Consumption and Resistance in Bacteria from Food Animals Food and Humans (DANMAP). DANMAP 2008-use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark, Accessed 8-15-2019 at, https://www.danmap.org/
39. Cha E, Abigail J, David R, et al. Implementing structural equation models to observational data from feedlot production systems. *Preventive veterinary medicine* 2017;147:163-171.
40. European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Sales of veterinary antimicrobial agents in 30 European countries in 2016, Accessed 8-15-2019 at, https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/european-surveillance-veterinary-antimicrobial-consumption-esvac
41. Food and Drug Administration, Center for Veterinary Medicine. Antimicrobials sold or distributed for use in food-producing animals in 2017, 2018, Accessed 8-15-2019 at, https://www.fda.gov/animal-veterinary/cvm-updates/fda-releases-annual-summary-report-antimicrobials-sold-or-distributed-2017-use-food-producing
42. McEwen SA, Fedorka-Cray PJ. Antimicrobial use and resistance in animals. *Clinical Infectious Diseases* 2002;34:S93-S106.

43. Cameron A, McAllister TA. Antimicrobial usage and resistance in beef production. *Journal of Animal Science and Biotechnology* 2016;7.
44. French Agency for Food Environmental and Occupational Therapy (ANSES) Agency for Veterinary Medical Products (ANMV). Sales survey of veterinary medicinal products containing antimicrobials in France in 2016, https://www.anses.fr/en/thematique/veterinary-medicine-anmv
45. Benedict KM, Gow SP, Reid-Smith RJ, et al. Metrics for quantifying antimicrobial use in beef feedlots. *Can Vet J* 2012;53:841-848.
46. U. S. Food and Drug Administration Center for Veterinary Medicine. FDA's Proposed Method for Adjusting Data on Antimicrobials Sold or Distributed for Use in Food-Producing Animals, Using a Biomass Denominator, 2017, Accessed 8-15-2019 at, https://www.fda.gov/animal-veterinary/cvm-updates/fda-proposes-biomass-method-obtain-animal-antimicrobial-sales-and-distribution-data-based-animal
47. Catry B, Laevens H, Devriese LA, et al. Antimicrobial resistance in livestock. *Journal of Veterinary Pharmacology and Therapeutics* 2003;26:81-93.
48. Filius P, Liem TY, van der Linden PD, et al. An additional measure for quantifying antibiotic use in hospitals. *Journal of Antimicrobial Chemotherapy* 2005;55:805-808.
49. European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Trends in the sales of veterinary antimicrobial agents in nine European countries, 2005-2009.
50. Bos MEH, Taverne FJ, van Geijlswijk IM, et al. Consumption of Antimicrobials in Pigs, Veal Calves, and Broilers in The Netherlands: Quantitative Results of Nationwide Collection of Data in 2011. *Plos One* 2013;8:9.
51. Pardon B, De Bleecker K, Hostens M, et al. Longitudinal study on morbidity and mortality in white veal calves in Belgium. *Bmc Veterinary Research* 2012;8.
52. Di Labio E, Regula G, Steiner A, et al. Antimicrobial resistance in bacteria from Swiss veal calves at slaughter. *Zoonoses and Public Health* 2007;54:344-352.
53. Busani L, Graziani C, Franco A, et al. Survey of the knowledge, attitudes and practice of Italian beef and dairy cattle veterinarians concerning the use of antibiotics. *Veterinary Record* 2004;155:733-738.
54. Carson CA, Reid-Smith R, Irwin RJ, et al. Antimicrobial use on 24 beef farms in Ontario. *Canadian Journal of Veterinary Research* 2008;72:109.
55. Dalton B, Sabuda D, Conly J. Trends in antimicrobial consumption may be affected by units of measure. *Clinical Infectious Diseases* 2007;45:399-400.
56. Benchmark. *Merriam Webster*. https://www.merriam-webster.com/dictionary/benchmark.
57. Barton MD, Hart WS. Public health risks: Antibiotic resistance - Review. *Asian-Australasian Journal of Animal Sciences* 2001;14:414-422.
58. Barton MD. Antibiotic use in animal feed and its impact on human healt. *Nutrition research reviews* 2000;13:279-299.
59. Phillips I, Casewell M, Cox T, et al. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy* 2004;53:28-52.
60. Murphy CP, Carson C, Smith BA, et al. Factors potentially linked with the occurrence of antimicrobial resistance in selected bacteria from cattle, chickens and pigs: A

scoping review of publications for use in modelling of antimicrobial resistance (IAM.AMR Project). *Zoonoses and Public Health* 2018;65:957-971.

61. Food and Drug Administration Center for Veterinary Medicine. Guidance for Industry 209: The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animal, 2012;26.

62. Food and Drug Administration Center for Veterinary Medicine. Supporting Antimicrobial Stewardship in Veterinary Settings: Goals for Fiscal Years 2019 – 2023 2018, Accessed 8-15-2019 at, https://www.fda.gov/animal-veterinary/cvm-updates/fda-releases-five-year-plan-supporting-antimicrobial-stewardship-veterinary-settings

63. McEwen SA, Singer RS. Stakeholder position paper: the need for antimicrobial use data for risk assessment. *Preventive veterinary medicine* 2006;73:169-176.

64. FDA Center for Veterinary Medicine GFI #152 Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern. https://www.fda.gov/media/69949/download, 2003.

65. Food and Drug Administration Center for Veterinary Medicine. Supporting Antimicrobial Stewardship in Veterinary Settings - Goals for Fiscal Years 2019-2023. https://www.fda.gov/media/115776/download, 2018.

66. Scott HM, Acuff G, Bergeron G, et al. Critically important antibiotics: criteria and approaches for measuring and reducing their use in food animal agriculture. *Annals of the New York Academy of Sciences* 2019;1441:8-16.

67. de Passille AM, Rushen J. Food safety and environmental issues in animal welfare. *Revue Scientifique Et Technique-Office International Des Epizooties* 2005;24:757-766.

68. Lloyd DH, Page SW. Antimicrobial Stewardship in Veterinary Medicine. *Microbiology Spectrum* 2018;6.

69. Speksnijder DC, Jaarsma ADC, van der Gugten AC, et al. Determinants Associated with Veterinary Antimicrobial Prescribing in Farm Animals in the Netherlands: A Qualitative Study. *Zoonoses and Public Health* 2015;62:39-51.

70. Shallcross LJ, Davies SC. The World Health Assembly resolution on antimicrobial resistance. *Journal of antimicrobial chemotherapy* 2014;69:2883-2885.

71. World Organisation for Animal Health (OIE). OIE annual report on antimicrobial agents intended for use in animals 2017, Accessed 8-15-2019 at, http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/AMR/Annual_Report_AMR_3.pdf

72. McIntosh W, Dean W. Factors Associated with the Inappropriate Use of Antimicrobials. *Zoonoses and Public Health* 2015;62:22-28.

73. Hockenhull J, Turner AE, Reyher KK, et al. Antimicrobial use in food-producing animals: a rapid evidence assessment of stakeholder practices and beliefs. *Veterinary Record* 2017;181:510-+.

74. McIntosh WMA, Schulz S, Dean W, et al. Feedlot Veterinarians' Moral and Instrumental Beliefs Regarding Antimicrobial Use in Feedlot Cattle. *Journal of Community & Applied Social Psychology* 2009;19:51-67.

75. Scott HM, Midgley G, Loneragan GH. Antimicrobials in Animal Agriculture: Parables and Policy. *Zoonoses and Public Health* 2015;62:3-9.

Chapter 2 - Methods of Characterization of Antimicrobial Use Data in 22 Beef Feedyards in the United States

Introduction

In March 2016, the FDA requested proposals for collection of antimicrobial use data in the four main food-animal production industries: beef cattle, dairy, swine, and poultry (chickens, and turkeys). Cooperative agreements were awarded in August 2016 and recruitment and data collection for beef and dairy began in 2017. Beef cattle in feedyards which were sold in 2016 and 2017 are the focus of this report. Aspects of the project reported here include availability and type of records kept by each feedyard, data capture and management methods, and antimicrobial use data.

The United States Department of Agriculture National Animal Health Monitoring System (USDA NAHMS) conducts surveys of commodities on a rotating basis. The most recent feedyard survey took place in 2011. [1] According to Feedlot 2011 Part IV, at least 75% of feedyards with a capacity of over 1,000 head recorded the date, drug product, and amount of injections given. In the same report, electronic record systems were briefly discussed. Feedyards with a capacity greater than 8,000 head all reported having an electronic record-keeping system while 70.4% of smaller feedyards (1,000 to 7,999 head) had electronic record-keeping systems. Electronic record systems vary greatly in the amount of information recorded and the effort necessary to capture the data and convert them to a standardized format.

It is important to recognize that the feedyard antimicrobial use study reported here does not comprise a statistical sample of the feedyard industry in the United States. The cooperating

feedyards represent samples of convenience which were accessed through direct contacts and intermediaries. Efforts were made to achieve both geographic and feedyard size distribution.

Glossary of Terms used in report:

Feedyard and Feedlot: These terms are commonly used synonymously, indicating a facility where cattle are held in confinement and fed a high-concentrate feed. In all cases in this report, these terms refer to open air facilities with dirt floors, feed bunks accessible in the pen, and *ad libitum* water availability. To avoid confusion with the term "lot", only the term feedyard is used to describe a facility in this report.

Lot: While sometimes used as a shorter term for "feedlot", in this report this term refers to an economic unit to which cattle are assigned on arrival. The lot may be housed in a single or in multiple pens and may move to different pens during their time in the feedyard, but most typically the lot designation will stay the same.

Close out: This is a document created at the time all financial accounting is finalized for a lot which has been sold. The close out contains information on the number of cattle purchased and sold, mortality, feed consumption, and all costs associated with the feeding period for the lot.

Days on feed (DOF): Each day an animal is in the feedyard constitutes one day on feed. For the purposes of this report, the <u>total DOF</u> is referring to the sum of the DOF for each animal in the lot (e.g., 100 head of cattle in the feedyard for 10 days would be 1000 DOF). This total DOF divided by 365 is the basis for calculating the total animal years in a lot; combining the total DOF values for all lots closed out in a given year at a feedyard results in the total DOF for that feedyard in that year.

Head-in: The number of cattle initially assigned to a lot.

Head-out: The number of cattle sold from a lot.

In-weight: Average weight per animal upon arrival at the feedyard.

Out-weight: Average weight per animal upon departure from the feedyard.

Pay weight: This is the weight for which a financial transaction occurred for either purchase (pay weight-in) or sale (pay weight-out).

Ration: A ration designates a particular feed mixture based on multiple factors; the relevant factor for this report is the presence or absence of antimicrobials and the inclusion rate of these antimicrobials. Cattle arriving at a feedyard are not yet acclimated to high energy concentrate feed-stuffs. During the acclimation period, the cattle feed is "stepped-up" in energy content, typically by moving through rations of increasing energy content or by adjusting the blend of a low energy starting ration with a higher energy finish ration. These step-ups are identified by different names. This is important in the analysis of feed records due to varying timeframes for introducing in-feed antimicrobials such as tylosin (medically important) or monensin (non-medically important). It was important to note the start and end of rations with differing in-feed antimicrobial inclusions during both survey administration and during data analysis of feed records.

Recruitment Methods

Participants for this study were recruited through several different channels. These included investigator relationships, beef producer organizations, and feedyard consulting veterinarians. No minimum data recording capabilities were required to participate in order to include all types of feedyards. Initial discussions with potential participants were conducted by email or phone. These methods were employed to identify interested locations after which an on-

site visit was scheduled. At this visit the cooperative agreement executive summary was provided along with a data sharing agreement detailing what types of information were requested and the potential delivery format of the data. Recruitment involved a variety of individuals depending on the feedyard; these contacts included owner/operators, feedyard managers, and cattle managers. Some feedyards elected to participate and signed the agreement at the initial site visit, others elected to discuss internally prior to signing. Some feedyards decided to not participate upon discussing the project, and some feedyards decided to not participate after initial enrollment was completed. If a feedyard elected to discontinue participation once data collection had ensued, their data were removed from all electronic devices and any hard copies were destroyed.

Characteristics of Cooperating Feedyards

Numbers by State

Participating feedyards were located in one of the five highest reported feeder cattle production states: Colorado, Iowa, Kansas, Texas, and Nebraska.[2] Cattle on feed on January 1 for 2016 and 2017 are described in Table 3.

Table 3: Size Characteristics of States of Participating Feedyards

Year	2016	2017
Cattle on Feed on January 1	10,683,484	10,610,404
By State		
Colorado	910,000	940,000
Iowa	1,230,000	1,160,000
Kansas	2,230,000	2,300,000
Texas	2,440,000	2,430,000
Nebraska	2,520,000	2,470,000

Source: United States Department of Agriculture National Agricultural Statistics Service

Data Collection

Types of Systems

Feedyards that were able to access some type of record of antimicrobial use for their animals were eligible for enrollment in the cooperative agreement. Antimicrobial administration records varied from expansive electronic data capturing programs to drug product purchase invoices from veterinarians and distributors. All of the commercially available record systems encountered in this study had the capability of tracking individual animal data. Whether or not the information was captured, however, was dependent on the individual feedyard.

The feedyards varied substantially on the level of detail provided in the data. The ability to accurately track the amount of antimicrobials used by the feedyard depended on the way the drug utilization was recorded. Eighteen of the participants provided individual animal identification, drug product, animal weight at time of treatment, and dose administered. For the other four participants, data consisted of doses reported for a number of animals, number of milliliters of a specific drug product billed to a lot, or the total amount of drug product purchased in a specific time period.

The different record systems encountered in the participating feedyards are described in detail in the next section and the number of feedyards that provided data per data system are shown in Table 4. In order to avoid recognition of individuals who participated in this study and any perceptions of promoting any particular data-capturing system, none of the specific data-capturing systems are named beyond the description below.

Data System Descriptions

Electronic system #1: This system provided records as described in the bulleted list below. Ten feedyards provided data from system #1. Data were obtained by direct receipt of files from the

data system provider, or by receipt of files from an intermediary which routinely downloaded and evaluated the data. Files were received as comma-separated-value (CSV) files. Initial evaluation and standardization of the data occurred after data import into R (version 3.5.1).[c] The packages "tidyverse" and ggplot2 were used extensively.[d] The imported data consisted of four main areas:

1. Lot Data
    - Lot identification
    - Head in
    - Head out
    - Date in
    - Date out
    - Lot days on feed
    - In-weight
    - Out-weight
2. Feed Data
    - Lot identification
    - Number of head fed by day
    - Daily feed consumption by ration
    - Total days on feed (accounting for death loss)
3. Control of bovine respiratory disease (BRD) by lot
    - Date treated
    - Number treated
    - Drug
    - Dose (mL)
4. Individual animal treatment by lot
    - Lot identification
    - Individual animal identification
    - Disease indication
    - Animal weight
    - Drug
    - Dose (mL)
    - Date treated

---

[c] R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

[d] Hadley Wickham (2017). tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse. H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Electronic system #1 combined with a custom system: Data for two feedyards were received as lot and feed data contained in a file from the System #1 provider combined with data for control of bovine respiratory disease (BRD) and individual animal treatment from a custom data system.

Electronic system #2: System two also has capabilities to capture lot data, feed data, and individual animal treatment data. In this project, the one feedyard utilizing this system used it for individual animal treatment data capture along with System #1 for lot and feed data. Data received from System #2 were the same as for System #1 for control of BRD and individual animal treatment.

Electronic system #3: This system was used by four feedyards. An electronic file download was not available in a format which allowed conversion to a CSV file for entry into the study database. Data were downloaded as PDF files for one feedyard and were received as paper printouts for 3 feedyards. These data were then manually entered into spreadsheets in the study format. The detail of individual animal treatments for this system varied as to what was recorded and available. The range was from detailed individual animal treatment records (2 feedyards) to cost of antimicrobials billed to each lot within the feedyard (2 feedyards). In the instance of cost recording only, a standard regimen dose informed by producer-provided estimated weight at treatment was calculated to derive the number of regimens administered from the billed amount.

Custom record systems: Two feedyards directly provided data as spreadsheet files derived from electronic record systems which had been developed in-house. A third feedyard cooperated with an outside record keeper who provided the data in a spreadsheet format. Two of the custom systems provided the data in a format which was capable of being converted to CSV files with minimum manipulation. Output from the other custom record system required significant

manipulation to achieve a more standardized format. The final format of custom record system data prior to entry into R for analysis closely resembled that described for System #1 above.

Manual entry – Purchase records, use records, custom record systems: Two unique situations presented themselves in this category, with one feedyard represented per situation.

1. **Purchase data only for in-feed supplements and treatment drugs.** Days-on-feed (DOF) were estimated by reported average DOF multiplied by the reported number closed out in 2016 and 2017. The total amounts of in-feed antimicrobials were calculated by amount of drug product purchased. The number of treatment regimens were estimated by the amount of drug purchased combined with reported average weight at treatment and the label regimen. The amount purchased in a calendar year from July 1 to June 30 (the study year) was associated with cattle closed out in a calendar year based on the nature of purchases and sale dates of the majority of cattle fed in this feedyard. The in-feed antimicrobial contained in the supplement would have been used for a short period after purchase; however there were no treatment records to allow assignment of a time frame to the use of the individual animal treatment antimicrobials. Uses of purchased antimicrobials were attributed to the study year period in which they were purchased.

2. **Custom record system for lot data with cost reporting for drugs charged to the lot for each month.** The lot closeouts provided total DOF, in and out-weights, and amount of feed supplement or chlortetracycline charged to the lot. Inclusion rates in the supplement and cost of chlortetracycline were utilized to derive total amount of drug used. The days of chlortetracycline use were derived by dividing the amount used by an estimated standard dose, which was calculated from the label dose and reported mean weight at the time of receiving chlortetracycline. For individual treatments, the amount of drug in milliliters per specific drug

product per lot was reported. The label regimen was used along with a reported weight at treatment to derive an estimated standard dose. The estimated standard dose was then divided into the total amount of drug used to derive the estimated number of regimens.

Table 4: Electronic System Characterization by Feedyard

Record System	# Feedyards
Electronic system #1 direct or indirect	10
Electronic system #1 combined with custom system	2
Electronic system #2 combined with system #1 - Direct	1
Electronic system #3 - manual entry from provided records	4
Custom record system direct or indirect	3
Records for purchase or number treated, some in combination with custom record systems - Manual entry	2

Methods of Data Capture

Data were received by multiple methods. Many of the feedyards utilized a data intermediary to securely transfer data to the study authors for manipulation and analysis. The type of intermediary varied from feedyard consulting groups to electronic system IT personnel. Fifteen of the participating feedyards provided data in this manner. The remaining seven feedyards provided data directly from their personal records.

Types of Data Acquired

Individual animal treatments

Individual animal treatment records including animal identification, antimicrobial product, dose, and animal weight at time of treatment were captured for 18 feedyards. Of these 18 feedyards, two of the feedyards provided antimicrobial product and animal weight at time of treatment, but did not provide a dose. Doses were then calculated based on the given weight at treatment with a label dose. Three feedyards provided antimicrobial doses administered per individual animal, but did not report weight at time of treatment. One feedyard provided the number of animals treated with dose administered per animal; however, individual animal identification and weight at time of treatment were not available.

Antimicrobial product was provided in total milliliters per specific drug product per lot for two feedyards. One feedyard provided total antimicrobial drug purchased for the feedyard during the study period.

Control of bovine respiratory disease

Sixteen feedyards provided antimicrobial use information for control of BRD. Fifteen of the feedyards provided date of administration, number of animals, and the dose administered per animal. The remaining feedyard provided a total number of milliliters of antimicrobial given per lot.

Control of BRD information was unavailable for six feedyards. These feedyards may not have administered any antimicrobials for control of BRD during the study period or the information may not have been identified separately from individual animal treatment. Personal confirmation and evaluation of records supports the conclusions that these six feedyards did not administer antimicrobials for control of BRD.

In-Feed

All participants used medically or non-medically important antimicrobials in feed, therefore, all feedyards provided feed data. Nine feedyards provided daily ration information which included lot number, date fed, ration name, amount fed per day, and amount of antimicrobials fed per day. Twelve feedyards supplied feed data as the total amount of feed fed with an inclusion rate of antimicrobial products. Total amount of supplement billed to the feedyard was provided for one feedyard.

Data Management

Once the data were received from an individual feedyard, they went through a process outlined in Figure 2. The data were allocated to four separate areas (lot data, feed data, control of BRD, and individual animal treatment) as discussed in *Data System Descriptions*. Data standardization and quality control followed; this process allowed for filters to be applied at each level, permitting potentially erroneous data to be removed from each of the four areas. The feedyard data were grouped by data form similarity, such as feedyards that used the same electronic record-keeping system, so that the same logic checks could be applied across the datasets and compared in parallel. Once the data went through the initial filtering, they were re-joined together with the lot file. The lot information file was considered the gold standard for including individual animal therapy, in-feed usage, and control of bovine respiratory disease. Data from these three areas were required to match a lot number given in the feedyard lot file closed out in the correct year. An additional step was performed to combine a master drug table with individual therapy, control, and in-feed category data. The master drug table provided information about the drug products that was necessary for calculations such as drug class, milligram per milliliter of

injectable products, and grams of active product per pound of product for in-feed antimicrobials. After combining the cleaned data, a secondary system of checks were performed. This second step required graphical outputs to visualize data. Data points that were outliers or did not match with known levels were rechecked for any input errors. Upon completion of the second data quality control step, the data were graphed from a single master data file containing information for all feedyards.

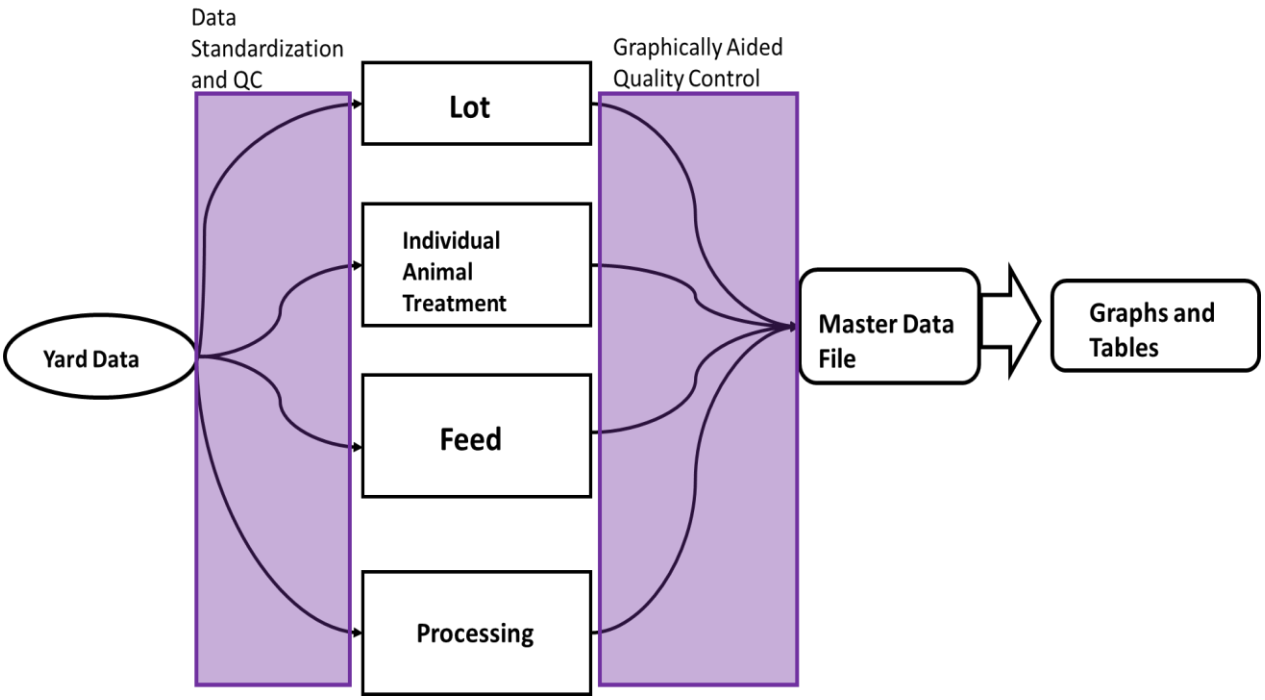

Figure 2 - Data Flow Diagram

Standardization of Data

Data import and standardization procedures

Data were imported in one of three ways. Direct download via specific electronic system IT, indirect download through a data intermediary, and manual entry into an Excel spreadsheet. Post-importation into R, the column headers were renamed to standardized names across all

feedyards, additional necessary identification were added, and data were filtered as described below. The information added to each feedyard was a study identification number, study year with which the data corresponded, and type of data record (feed, control, or treatment). Quality assurance, data handling, and analysis procedures were reviewed by the USDA Center for Epidemiology and Animal Health (CEAH) through multiple in-person meetings between the FDA Cooperative Agreement personnel and USDA CEAH personnel.

Filtering to remove erroneous data

Filters were applied at each level to reduce inclusion of blatantly erroneous data. This approach resulted in removal of data; the estimated proportion of data removed is reported with the area-specific discussions below.

Lot data filters

Lot data displayed occasional deviations from the norm which possibly indicated either the use of a lot for accounting purposes across multiple cattle situations or erroneous entries/transfer. In order to eliminate these outliers, filter limits were applied. Approximately 10% of lot data rows were removed from feedyard-provided data. This included lots that were included in the feedyard data transfer but were outside of the study period of interest, which was cattle closed out in 2016 and 2017. Removal of lot data required that feed, control, and treatment records for cattle in those lots were also removed. The filters removed data rows that fit the following criteria:

- Average weight-in of more than 1500 pounds
- Average weight-out of more than 1800 pounds
- Less than 9 head per lot
- Less than 7 days on feed
- Greater than 365 days on feed
- Year closed-out did not match study period

- Any lots missing information needed for calculation of numerators or denominators such as lot number, number of animals, days on feed, or out weight.

Individual animal therapy filters

Filters in this category focused on the integrity of the individual animal data. Individual animal therapy filters resulted in an estimated 53% of individual animal records being removed. A record was one product or procedure per animal; any antimicrobial use for that animal was retained. The removal of rows appears high as it reflects the removal of any non-antimicrobial procedures that were performed on an animal. The filters removed individual animal treatment data based on the following criteria:

- Treatment weight of less than 50 pounds
- Dose of zero milliliters
- Non-antimicrobial items
- Administration of vaccines, non-steroidal anti-inflammatories, steroids, dehorning, castration, deworming, ear tag applications, chute charge, and implants
- Data rows that were missing information needed for calculation of numerators or denominators such as drug product, dose, or treatment date

Control of bovine respiratory disease filters

Filters for control of BRD were focused on removing non-antimicrobial items after all feedyard data were combined into one file. The removal of non-antimicrobial items accounted for approximately 74% of the data rows since administration of antimicrobials for control of BRD typically occurs at the time of initial processing and the records contained numerous other entries.

Examples of non-antimicrobial items include vaccines, dehorning, castration, deworming, ear tag applications, and implants.

In-feed filters

Filters for in-feed antimicrobial use were focused on removing data that were ineffective for calculating outcome parameters. In-feed use filters resulted in an estimated 17% of feed records being removed. The filters removed data meeting the following criteria:

- The number of head fed was 0
- An amount of drug product fed of zero milligrams
- Ration inclusion rates were not provided
- Unavailable ration information was cause for exclusion from the project

Logic checks

Benchmark graphing was conducted for each feedyard after data standardization to check logical value of data as compared to other feedyards in the study and to published data. In some instances, markedly disparate results led to review of data management steps and errors in calculation formulas or data transfer were discovered. Any errors due to systematic analysis flaws were corrected across all data sets.

Assumptions in Data Analysis

Multiple key assumptions are considered in reporting these data. An important initial assumption is that the authors received all antimicrobial use data for cattle present in the feedyards during the requested time frame. There is economic incentive for tracking total antimicrobial quantities used as these data are necessary for calculating the cost of gain and allocating input costs to areas such as processing, feed, and treatment of disease. Data sources used for this report were

the same as utilized by the feedyards, and in some cases their consulting veterinarians and nutritionists, to evaluate economic performance. The importance of capturing these data are even more significant when the feeding process is performed for customers on a custom feeding basis where all costs are billed to the customer. The majority of the feedyards in this study performed at least some custom feeding.

The authors also followed the assumption that all treatments provided with specific information of animal identification, product, and animal weight, but not the individual animal dose, were administered at the label dose of the product as per protocol. Two feedyards provided individual animal therapy data in this manner.

Feed data ranged from detailed micromachine allocations in individual feed batches to reported inclusion of ingredients on a ration weight basis. In the case of reported inclusion rates, the assumption was made that this inclusion rate was achieved as an average over the days the ration was fed.

Output Calculation Methods

Numerators

Regimens

All antimicrobial use metrics have limitations as discussed in Chapter 1. To report antimicrobial use in a manner that is as unbiased but descriptive as possible, the characteristics of antimicrobial administration in this study are reported as regimens and weight of active substance. In the case of regimens, the descriptions are necessary to provide information which in the future may be used for evaluation according to parameters that may provide insight into antimicrobial resistance selection pressure.

A major challenge was whether to assign a duration of exposure to the various single injection antimicrobials used in feedyards. Currently, there are widely disparate levels of evidence to support quantification of the therapeutic duration for an antimicrobial administered for therapy or control of bovine respiratory disease. There is an even greater paucity of data to support an estimation of the duration of effect on resistance selection for either respiratory or enteric pathogens. For these reasons, it was decided to describe the characteristics of the regimens as administered rather than arbitrarily assign what would essentially be interpreted as a number of daily doses per single injection administration. The characteristics of parameters that define a regimen in this report are listed in Table 5.

Table 5- Regimen Term Definitions

Factor	Description
Drug Product	Active antimicrobial substance. For the majority of regimens used in a feedyard, it will be a single antimicrobial.
Route	Route by which the treatment was delivered. Common antimicrobial routes in feedyards include: parenteral (intramuscular, subcutaneous, intravenous) and oral (in-feed, water).
Total Amount	Total number of milligrams administered for the entire regimen. (number of administrations * mg/administration)
Amount per Administration	Dose of antimicrobial administered at a single time point. This is recorded as total amount (in milligrams) of drug administered, rather than a mg/kg dose.
Number of Administrations	The number of times that drug is administered to the animal for the same disease incident.
Time Frame	The amount of time between first and last administration. For example, if an animal received 1 injection one time, the interval is 0 hours. If they received a total of 2 injections, the first on day 1 and second on day 3 then the time frame is 2 days

Calculation of in-feed regimens required the availability of dates of antimicrobial products fed. This could be calculated by daily ration information with the number of head fed per day, or by total ration fed with a consistent inclusion rate combined with the lot in-date, out-date, and number of animals present. Regimens for control of BRD were calculated from the lot number, number of animals, and antimicrobial product(s) administered. Individual animal treatment regimens were calculated from animal identification, antimicrobial product, and a record of reason for treatment.

Regimens could not be described for the three categories of feedyard data without the information listed. New regimens administered to the same animal for the same disease were identified when treatment with the same antimicrobial occurred more than two days after the previous treatment or whenever administration of a new antimicrobial occurred even within the seven day period.

Milligrams

The total milligrams of antimicrobial use were calculated by drug class for medically important antimicrobials for three different areas: individual animal therapy, control of BRD, and in-feed use. Milligrams of non-medically important antimicrobials were calculated only for in-feed use as this is the only category in which they were used.

Milligrams for control of BRD were calculated by taking the dose of the antimicrobial product in milliliters and multiplying it by the number of head and concentration of the product. Individual animal treatment milligrams were calculated by taking the dose of the antimicrobial product in milliliters and multiplying it by the concentration of the antimicrobial product. In-feed calculation of total milligrams was performed in a different manner as the antimicrobial inputs were in weight of drug per amount fed. The weight of antimicrobial was standardized to

milligrams from various inputs such as grams per ton, milligrams per head per day, and kilograms of product fed. The total milligrams were then calculated by summing the milligrams per antimicrobial product.

Denominators

Animal Year

Reporting animal year from feedyard records is relatively straightforward as the number of days an animal resides in a feedyard is captured in most record systems for economic purposes. Total head days are routinely reported in lot closeouts by feedyards; in these cases the head days represent a method where the days in the lot are counted for animals even if they did not finish the feeding period. If total head days for the lot were not reported by the producer or feedyard based on this calculation, total head days were calculated by taking days on feed for each lot and multiplying this value by the head out. Calculation in this manner will underestimate the actual total head days as it is not accounting for cattle which started the feeding period and did not finish. However, the error introduced by this method will be much less than the typical lot death loss, which in these feedyards was usually 0.5% or less; much of this mortality occurs earlier in the feeding period for respiratory disease (typically 50% of mortalities in feedyards) which means that mortalities contribute a small proportion to the overall head days. Any error in this method would be conservative from an antimicrobial use perspective in that it would tend to underestimate the animal year denominator, thereby inflating the regimen per animal year total.

Once total head days were calculated, they were then divided by 365 to produce the animal year denominator. Estimates at the study and feedyard level were both calculated in this manner. Study level animal year is calculated from the sum of the total head days across all feedyards and

divided by 365; while feedyard level animal year is calculated from the sum of the total head days for each feedyard divided by 365.

Kilogram of animal liveweight sold

Reporting kilograms of animal produced is also relatively straightforward as it is the main economic driver for feedyards. Total kilograms liveweight sold is routinely reported in lot closeouts by feedyards. It can be denoted one of two ways, total out weight and pay weight out; total out weight is the weight of the cattle when they leave the feedyard while pay weight out is the purchase weight by the abattoir. The difference in total out weight and pay weight is due to the application of shrink, which is the loss of weight during transport. Shrink is oftentimes estimated at 4%. Total out-weight was used for this report due to the lack of consistency on reporting pay-weight in the various record-keeping systems.

Once total out-weight was calculated for all feedyards, study level and feedyard level kilogram of liveweight produced were calculated in similar manners. Study level kilogram liveweight produced takes the sum of the total out-weight for each feedyard while the feedyard level takes the sum of the total out-weight per lot.

It should be noted that kilograms liveweight sold does not account for any animals that were exposed to an antimicrobial treatment and died. Mortalities create the effect of applying the milligram numerator over a smaller kilogram liveweight sold. The resistance selection impact of a treatment on an animal that does not leave the premises, other than possibly to rendering, is not fully understood.

Final Combined Metrics

In this report, two outputs are reported. The overall outputs are total milligram of active substance per total kilogram (mg/kg-LW) of animal liveweight sold and antimicrobial regimens per animal year (Reg/AY).

Conclusion

Data management involved multiple steps to capture, standardize, and analyze the antimicrobial use data across disparate data types. Quality assurance protocols were incorporated at multiple steps to assure that data management did not introduce errors and to identify data which did not pertain to antimicrobial use. Results and discussion are reported in Chapter 3.

References

1. United States Department of Agriculture Animal and Plant Health Inspection Service. National Animal Health Monitoring System Feedlot 2011: Part IV: Health and Health Management on US. Feedlots with a Capacity of 1000 or More Head, 2013, https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/monitoring-and-surveillance/nahms/nahms_feedlot_studies

2. USDA. Cattle Inventory Report In: National Agricultural Statistics Service ASB, ed, 2017.

Chapter 3 - Antimicrobial Use in 22 United States Beef Feedyards

Introduction

As discussed in Chapter 1, there are a multitude of metrics that have been used in the published literature to describe antimicrobial use in food-animal production. Each of these metrics have nuances associated with them, and none are singularly capable of providing a complete picture of antimicrobial use across a food animal industry, or even within an individual production unit. The goal of this project was to report antimicrobial use in the most transparent manner possible with available data from a convenience sample of participating feedyards. The authors intentionally refrained from specifying particular metrics as the best or more useful and focused on characterizing the data gathering and management processes for the available record systems as well as assuring accuracy of descriptions from available data.

Beef cattle in 22 feedyards which were sold in 2016 and 2017 are the focus of this report. Aspects of the project reported here include antimicrobial use data in two combined metrics, milligrams per kilogram of liveweight sold (mg/kg-LW) and regimens per animal year (Reg/AY). These metrics are reported in multiple levels of granularity.

Metric values are reported at both the study level and the feedyard level. Reporting of values at the study level combines individual lot data across all feedyards and calculates one value for each outcome metric. Reporting at the feedyard level first calculates values for each feedyard from the lots within that feedyard, and then expresses the outcome metrics as central tendencies of the individual feedyard values with descriptions of variance. The agreements signed with

participating feedyards prohibit the public reporting of individual feedyard values, even when identity is masked; therefore, reporting in a manner which would describe individual feedyard values is not presented here.

For the purposes of this report, a combination of method of administration and intent were used to derive the following use categories.

- Medically important antimicrobials
    - In-feed use
    - Individually administered to a group for control of BRD
    - Individually administered use for treatment of any disease
- Non-medically important antimicrobials
    - In-feed use

Materials and Methods

The materials and methods for the results are reported in Chapter 2.

Results

Description of Feedyard Characteristics

For this report, 22 feedyards provided antimicrobial use data. The feedyards represented five states: Colorado, Iowa, Kansas, Nebraska, and Texas. As shown in Table 6, in 2016 the 22 feedyards represented 599,289 head of beef cattle closed-out with an increase to 667,295 head of beef cattle closed-out in 2017. The size of individual feedyards ranged from less than 2,500 head closed-out per year to more than 100,000 head. The percent of USDA reported annual steer and

heifer slaughter represented by the participating feedyards was 2.4% and 2.6% for 2016 and 2017, respectively.

Table 6 - Description of Participating Beef Feedyard Characteristics

Year	2016	2017
Total Cattle Closed Out by Year	599,289	667,295
Percent of USDA Reported Annual Steer and Heifer Slaughter for that year	2.4%	2.6%
Feedyards Classified by Total Head Closed Out by Year		
≤ 2,500	3	1
2,501 to 5,000	2	4
5,001 to 10,000	4	3
10,001 to 25,000	5	4
25,001 to 50,000	4	6
50,001 to 75,000	1	1
75,001 to 100,000	2	2
> 100,000	1	1

Description of Medically Important Antimicrobial Use Data at the Study Level

The reporting approach progresses through 3 levels of granularity

1. Total mg of antimicrobial per kg of animal liveweight sold (mg/kg-LW) and antimicrobial regimens per animal year (Reg/AY)
2. Total mg/kg-LW and Reg/AY reported by antimicrobial class
3. Total mg/kg-LW and Reg/AY reported by antimicrobial class within use categories of in-feed, control of bovine respiratory disease, and individual animal treatment.

The first level of granularity is not informative for any purposes related to antimicrobial stewardship. The lack of information related to antimicrobial class and reasons for use provide no meaningful guidance as to antimicrobial classes or use categories. This level is reported here due

to being easily calculated from the individual antimicrobial class values and to illustrate the differences in information content at different levels of granularity.

The second level of granularity begins to adjust for differences in potency of the different antimicrobial classes although there is still no information as to route and reason for use. Even within antimicrobial classes, especially the individual animal administered injectable macrolides, the milligrams of antimicrobial uses may be heavily influenced by the product selected and even the dose selected within the label range for an individual product.

The third level of granularity gives the most information on use category and also allows consideration of each antimicrobial class within each use category. Even more granular data are available for most feedyards, depending on record system, which would allow evaluation of these data by source of cattle, in-weight, time of year, and multiple other factors affecting the need for, and outcomes of therapy. Even at the third level of granularity, there is the need to understand the regimens being used as described in the latter portion of this chapter.

Note that tables 7, 8, 9 and 10 contain data related to both mg/kg-LW and Reg/AY. For illustration of the increasing levels of granularity, the mg/kg-LW metrics from each of these tables will be discussed first in relation to the corresponding figures, then the same progression will be made for Reg/AY.

Milligrams of Medically Important Antimicrobials per Kilogram Liveweight Sold

Figure 3 and Table 7 display the medically important antimicrobial metric of mg/kg-LW sold. Figure 3 includes illustration of antimicrobial classes within the totals to demonstrate the hidden granularity when only a total milligram drug value is reported. Total medically important mg/kg-LW were 44.65 for 2016 and 30.18 in 2017.

Table 7 - 2016 and 2017 Total Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold Calculated at the Study Level for all Uses of Medically Important Antimicrobials

Year	Reg/AY	mg/kg-LW
2016	3.65	44.65
2017	3.17	30.18

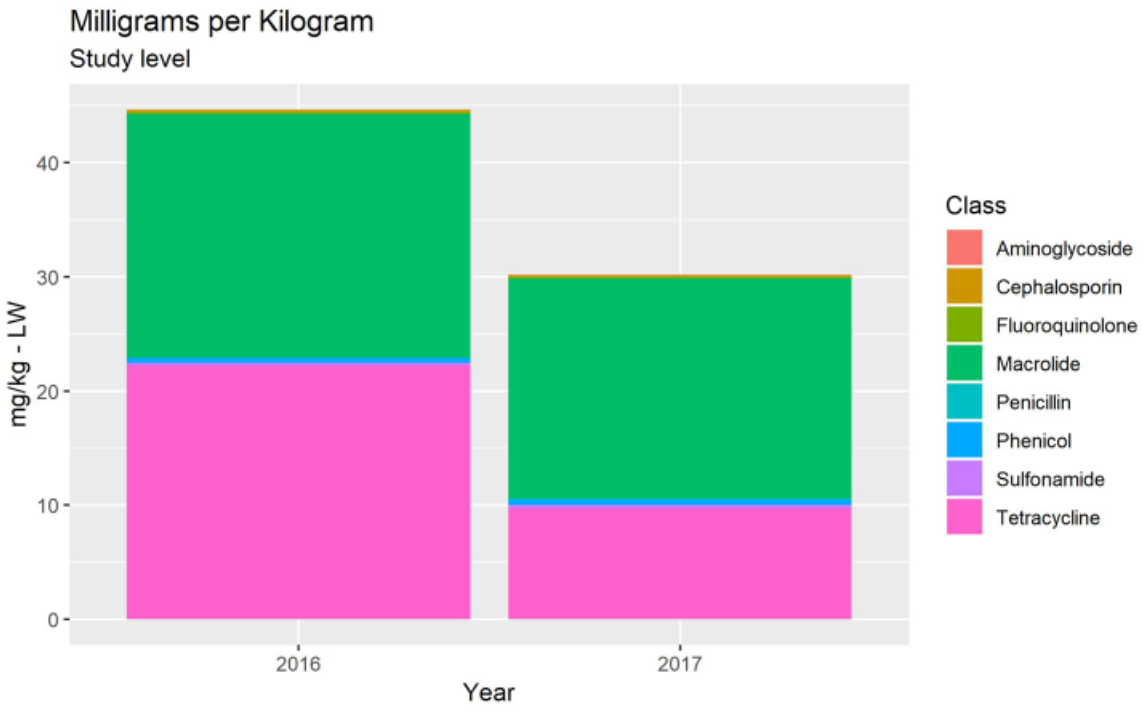

Figure 3 - Total Milligrams per Kilogram Liveweight Sold Calculated at the Study Level for all Uses of Medically Important Antimicrobials

When medically important antimicrobial mg/kg-LW values are reported by drug class and year (Figure 4, Table 8), the most notable numerical change occurred in the tetracyclines between 2016 and 2017 with a numerical decrease from 22.21 mg/kg-LW to 9.73 mg/kg-LW, respectively. The default decimal place setting for Table 8 was two decimal places, with an increase to 3 decimal places for the aminoglycoside class to capture values for both study years. Medically important

antimicrobial class mg/kg-LW values for 2016 and 2017 ranged from 0.03 and 0.03 for aminoglycosides to 21.33 and 19.31 for macrolides, respectively. The aminoglycoside use consisted of oral neomycin. It should be noted that the values in Table 8 and Figure 4 represent the combined values for all use categories.

Table 8 - 2016 and 2017 Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold by Class for Medically Important Antimicrobials Calculated at the Study Level

Year	Class	2016	2017	Feedyard Count
Reg/AY	Aminoglycoside	0.007	0.004	2
	Cephalosporin	0.14	0.10	20
	Fluoroquinolone	0.07	0.06	20
	Macrolide	2.34	2.39	22
	Penicillin	0.01	0.01	13
	Phenicol	0.06	0.05	21
	Sulfonamide	0.005	0.01	9
	Tetracycline	1.02	0.56	21
mg/kg-LW	Aminoglycoside	0.03	0.03	2
	Cephalosporin	0.21	0.14	20
	Fluoroquinolone	0.16	0.14	20
	Macrolide	21.33	19.31	22
	Penicillin	0.05	0.07	13
	Phenicol	0.40	0.45	21
	Sulfonamide	0.27	0.31	9
	Tetracycline	22.21	9.73	21

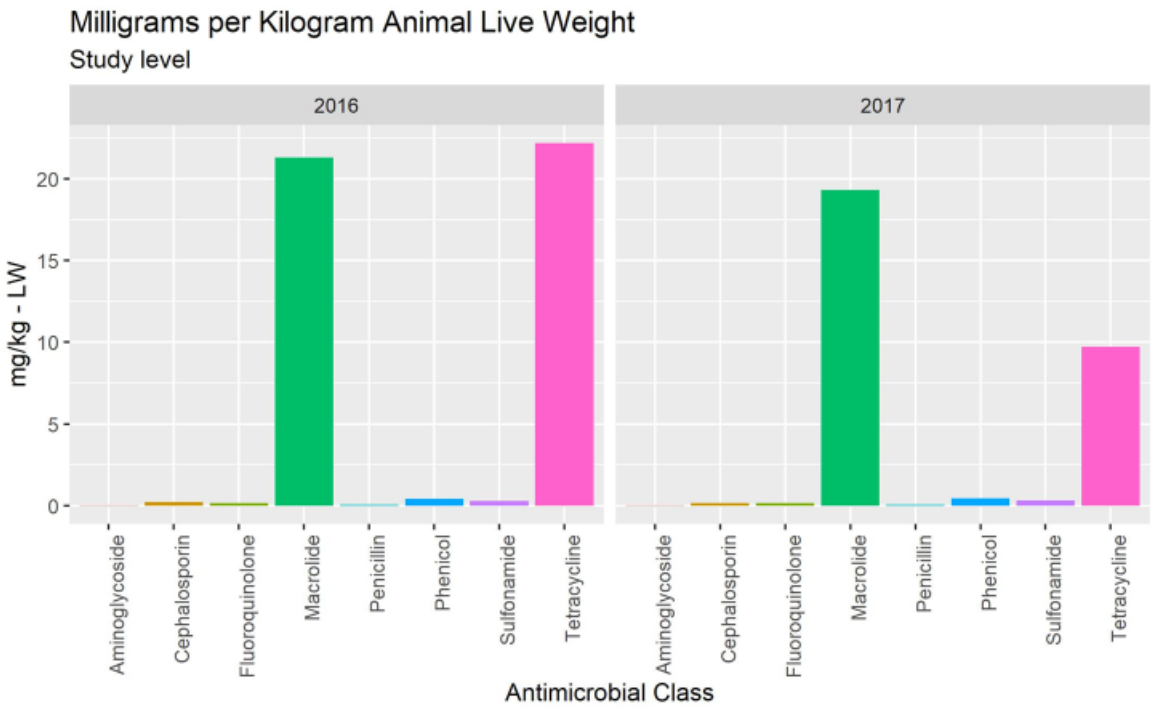

Figure 4 - 2016 and 2017 Milligrams per Kilogram Liveweight Sold Reported by Antimicrobial Class Calculated at the Study Level for Medically Important Antimicrobials

The numerical decrease in total mg/kg-LW is also noticeable in Figure 5 and Tables 9 and 10, where medically important antimicrobial data are reported by antimicrobial class within use category. Table 10 presents the same data as Table 9; Table 9 is presented as values while Table 10 presents the values as percentages of total use for that year for that metric. The feedyard counts reported in Tables 9 and 10 capture any feedyard reporting use of this antimicrobial class within this use category for 1 or both of the study years. The default decimal place setting in Table 9 is 3 decimal places and in Table 10 is 2 decimal places, with decimal places increased in low value cells until a value was displayed.

In-feed medically important antimicrobial use showed the greatest numerical decrease of the three categories, from 42.42 mg/kg-LW in 2016 to 27.98 mg/kg-LW in 2017. Control for BRD

decreased numerically between 2016 and 2017, at 1.11 and 0.81 mg/kg-LW in 2016 and 2017, respectively. Individual animal treatment stayed relatively constant at 1.12 mg/kg-LW in 2016 and 1.39 mg/kg-LW in 2017.

Table 9 - 2016 and 2017 Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold Reported by Use Category and Antimicrobial Class Calculated at the Study Level for Medically Important Antimicrobials

Use Category	Class	Reg/AY		mg/kg-LW		Feedyard Count
		2016	2017	2016	2017	
In-Feed	Macrolide	1.999	2.058	20.859	18.984	18
	Tetracycline	0.907	0.430	21.563	8.999	13
	Total	**2.906**	**2.489**	**42.421**	**27.984**	
Control	Aminoglycoside	0.006	NR	0.025	NR	1
	Cephalosporin	0.112	0.067	0.167	0.099	8
	Fluoroquinolone	0.012	0.00002	0.019	0.0001	3
	Macrolide	0.265	0.239	0.374	0.230	16
	Penicillin	0.008	0.009	0.029	0.045	3
	Phenicol	0.029	0.0001	0.114	0.002	4
	Sulfonamide	0.0003	0.0002	0.010	0.008	1
	Tetracycline	0.067	0.080	0.367	0.421	12
	Total	**0.499**	**0.395**	**1.105**	**0.805**	
Treatment	Aminoglycoside	0.0004	0.004	0.004	0.025	2
	Cephalosporin	0.028	0.029	0.043	0.045	20
	Fluoroquinolone	0.055	0.055	0.138	0.139	20
	Macrolide	0.080	0.089	0.095	0.099	22
	Penicillin	0.003	0.004	0.020	0.022	13
	Phenicol	0.030	0.047	0.286	0.451	21
	Sulfonamide	0.005	0.005	0.259	0.300	9
	Tetracycline	0.045	0.050	0.279	0.306	21
	Total	**0.246**	**0.284**	**1.124**	**1.387**	
All Uses	**Total**	**3.651**	**3.168**	**44.650**	**30.176**	

Table 10 - 2016 and 2017 Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold Reported by Use Category and Antimicrobial Class Calculated at the Study Level for Medically Important Antimicrobials as Percentages. Note that all columns may not appear to sum to 100% due to rounding.

Use Category	Antimicrobial Class	Reg/AY		mg/kg-LW		Feedyard Count
		2016	2017	2016	2017	
In-Feed	Macrolide	54.74%	64.98%	46.72%	62.91%	18
	Tetracycline	24.84%	13.59%	48.29%	29.82%	13
	Total	**79.59%**	**78.57%**	**95.01%**	**92.74%**	
Control	Aminoglycoside	0.17%	NR	0.06%	NR	1
	Cephalosporin	3.07%	2.11%	0.37%	0.33%	8
	Fluoroquinolone	0.32%	0.001%	0.04%	0.0002%	3
	Macrolide	7.26%	7.56%	0.84%	0.76%	16
	Penicillin	0.21%	0.27%	0.07%	0.15%	3
	Phenicol	0.80%	0.004%	0.26%	0.01%	4
	Sulfonamide	0.01%	0.01%	0.02%	0.03%	1
	Tetracycline	1.83%	2.52%	0.82%	1.39%	12
	Total	**13.67%**	**12.47%**	**2.47%**	**2.67%**	
Treatment	Aminoglycoside	0.01%	0.13%	0.01%	0.08%	2
	Cephalosporin	0.77%	0.91%	0.10%	0.15%	20
	Fluoroquinolone	1.50%	1.75%	0.31%	0.46%	20
	Macrolide	2.20%	2.82%	0.21%	0.33%	22
	Penicillin	0.08%	0.13%	0.05%	0.07%	13
	Phenicol	0.82%	1.47%	0.64%	1.49%	21
	Sulfonamide	0.13%	0.16%	0.58%	0.99%	9
	Tetracycline	1.23%	1.59%	0.62%	1.01%	21
	Total	**6.74%**	**8.96%**	**2.52%**	**4.60%**	
All Uses	**Total**	**100.00%**	**100.00%**	**100.00%**	**100.00%**	

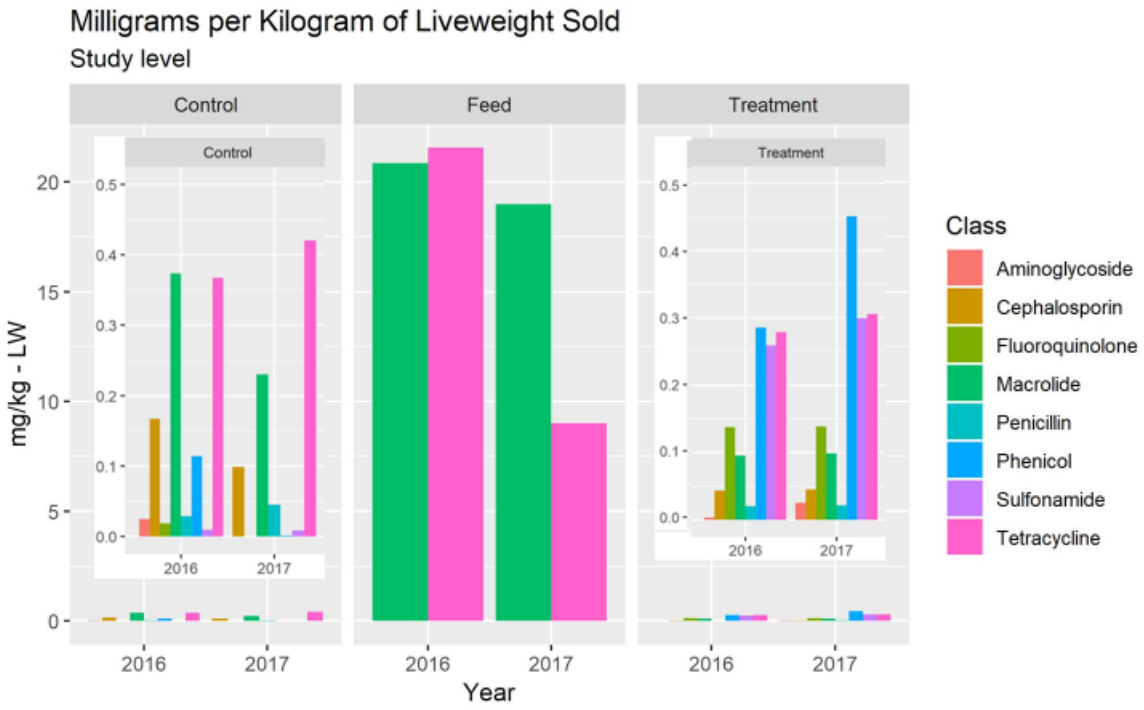

Figure 5 - 2016 and 2017 Milligrams per Kilogram Liveweight Sold reported by Use Category for Medically Important Antimicrobials Calculated at the Study Level. Insets for control and treatment use categories alter the Y axis for increased clarity between antimicrobial classes.

Regimens per Animal Year of Medically Important Antimicrobials

The least granular data for regimens per animal year (Reg/AY) are total regimens reported at the study level; these values are presented in Figure 6 and Table 7. Figure 6 includes illustration of antimicrobial classes within the totals to demonstrate the hidden granularity when only a total regimen value is reported. Study level medically important antimicrobial Reg/AY values demonstrated a numerical decrease from 3.65 in 2016 to 3.17 in 2017.

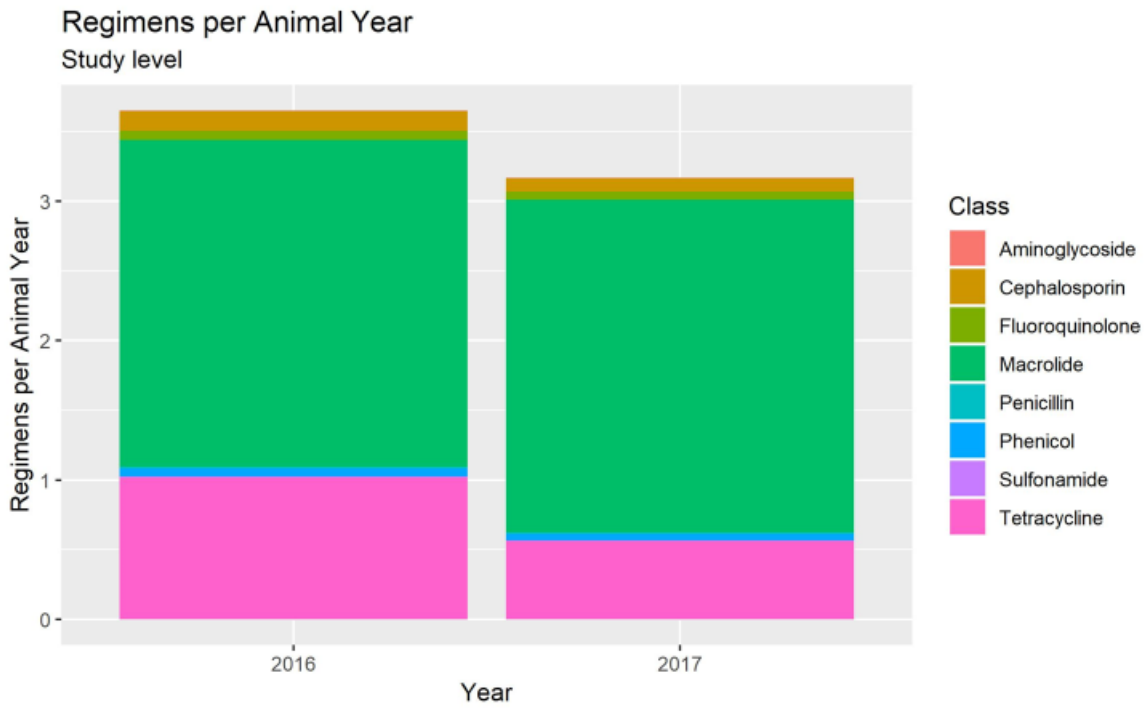

Figure 6 - 2016 and 2017 Total Regimens per Animal Year Calculated at the Study Level for all uses of Medically Important Antimicrobials

Study level medically important antimicrobial regimens per animal year reported by antimicrobial class and year are illustrated in Figure 7 and Table 8. The values ranged from 0.007 and 0.004 Reg/AY for aminoglycosides (2 feedyards reporting) to 2.34 and 2.39 Reg/AY for macrolides (all 22 feedyards reporting, which includes both injectable and in-feed) in 2016 and 2017, respectively. Tetracycline regimens per animal year numerically decreased from 1.02 Reg/AY in 2016 to 0.56 Reg/AY in 2017. The remaining regimens per animal year of medically important antimicrobials changed minimally between 2016 and 2017.

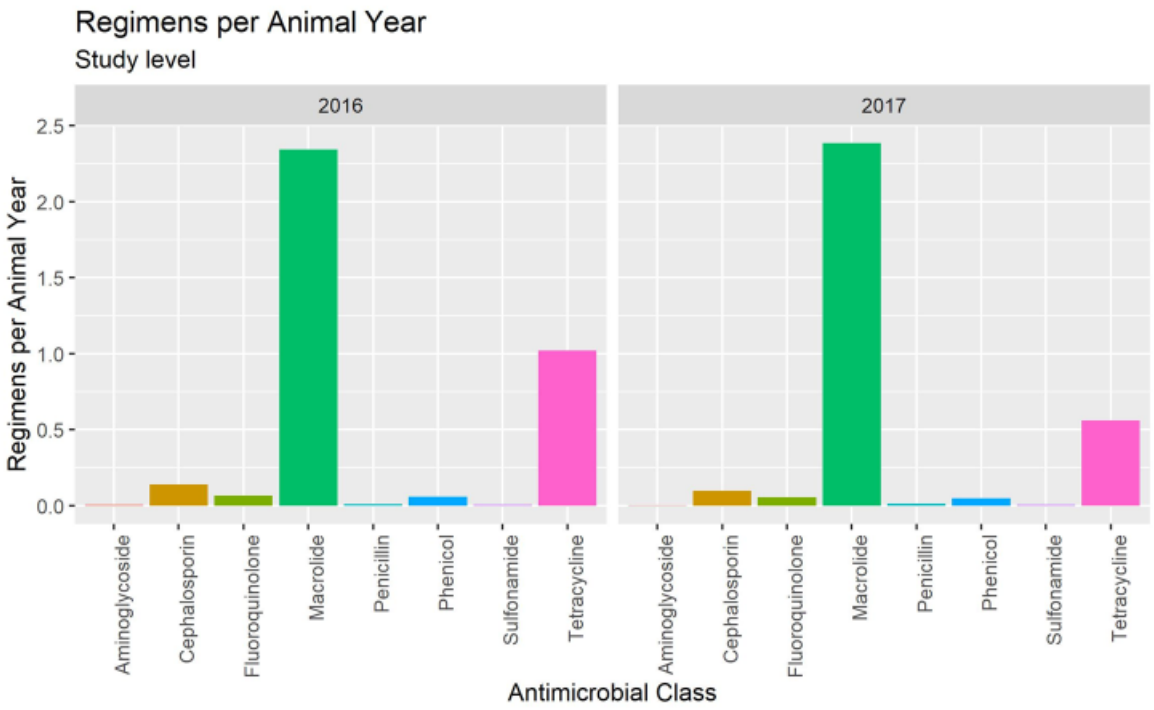

Figure 7 - 2016 and 2017 Regimens per Animal Year Reported by Antimicrobial Class Calculated at the Study Level for Medically Important Antimicrobials

Study level medically important Reg/AY values by project year, and antimicrobial class within use category are displayed in Figure 8 and Tables 9 and 10. Table 10 presents the same data as Table 9; Table 9 is presented as values while Table 10 presents the values as percentages of total use for that year for that metric.

Total Reg/AY for in-feed use were 2.91 and 2.49 for 2016 and 2017, respectively. Use for control of BRD accounted for 0.50 Reg/AY in 2016 and 0.40 Reg/AY in 2017. Individual animal treatment quantification resulted in 0.25 and 0.28 Reg/AY for 2016 and 2017, respectively.

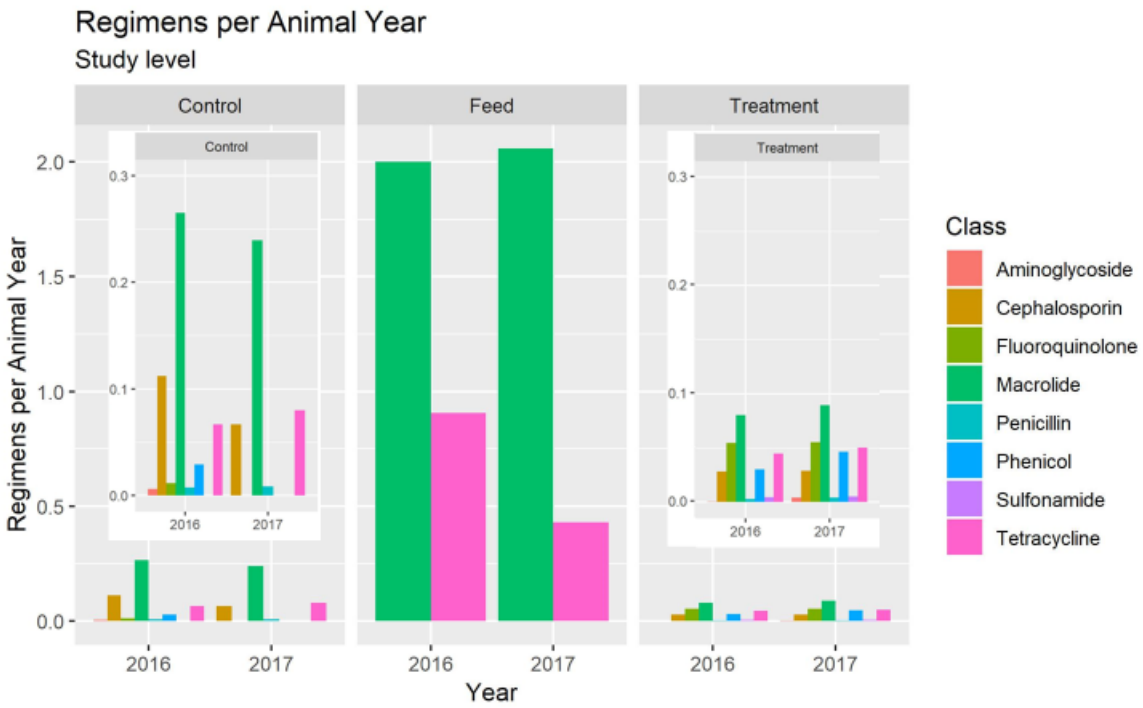

Figure 8 - 2016 and 2017 Total Regimens per Animal Year by Use Category for Medically Important Antimicrobials Calculated at the Study Level. Insets for control and treatment use categories alter the Y axis for increased clarity between antimicrobial classes.

Description of Medically Important Antimicrobial Use Data at the Feedyard Level

Metric values at the feedyard level are reported as total Reg/AY and total mg/kg-LW, as well as by antimicrobial class. Due to a wide variety of number of reporting feedyards within each antimicrobial class within each use category, feedyard level data are not reported by antimicrobial class within use category.

Milligrams per Kilogram Liveweight Sold

Table 11 contains the feedyard level medically important antimicrobial mg/kg-LW values by year. Study level values are also presented in Table 11 for comparison to the related mean,

standard deviation, and median values at the feedyard level. The differences in calculations for study level and feedyard level metrics are described at the beginning of this chapter.

The median total medically important mg/kg-LW for 2016 was 43.41, the corresponding value in 2017 was 28.69. Comparison of study level, mean, and median values for each year within each metric allows comprehension of the non-normal distribution of the feedyard level data and also the effect that larger feedyards have on the study level metric values due to closing out more lots per year.

Table 11 - Regimens per Animal year and Total Milligrams of Medically Important Antimicrobials per Kilogram of Liveweight Sold Calculated at the Feedyard Level for 2016 and 2017

Metric	Year	Study Level	Feedyard Level		
			Mean	Std Dev	Median
Reg/AY	2016	3.65	3.53	1.92	3.10
	2017	3.17	3.04	1.24	2.96
mg/kg-LW	2016	44.65	62.13	55.62	43.41
	2017	30.18	43.81	31.26	28.69

Values for medically important antimicrobial mg/kg-LW values by antimicrobial class at the feedyard level are reported in Table 12. Due to only 2 feedyards reporting aminoglycoside use, they are not included in the table. Note that as the relative use of an antimicrobial class decreases, the relationship between study level and feedyard level median and mean values becomes more variable.

Table 12 - Regimens per Animal year and Total Milligrams of Medically Important Antimicrobials per Kilogram of Liveweight Sold at the Feedyard Level for 2016 and 2017 Reported by Antimicrobial Class

Reg/AY						
Class	**Feedyard Count**	**Year**	**Study Level**	**Feedyard Level**		
				Mean	*Std Dev*	*Median*
Cephalosporin	20	2016	**0.140**	0.133	0.206	0.054
		2017	**0.096**	0.084	0.114	0.038
Fluoroquinolone	20	2016	**0.067**	0.077	0.075	0.058
		2017	**0.055**	0.060	0.053	0.040
Macrolide	22	2016	**2.344**	1.965	0.965	2.287
		2017	**2.387**	1.987	1.009	2.315
Penicillin	13	2016	**0.011**	0.027	0.036	0.006
		2017	**0.013**	0.014	0.018	0.003
Phenicol	21	2016	**0.059**	0.069	0.136	0.021
		2017	**0.047**	0.051	0.066	0.023
Sulfonamide	9	2016	**0.005**	0.031	0.034	0.035
		2017	**0.005**	0.027	0.028	0.016
Tetracycline	21	2016	**1.019**	1.332	1.586	0.807
		2017	**0.561**	0.937	0.918	0.534

mg/kg-LW						
Class	**Feedyard Count**	**Year**	**Study Level**	**Feedyard Level**		
				Mean	*Std Dev*	*Median*
Cephalosporin	20	2016	**0.211**	0.201	0.314	0.080
		2017	**0.144**	0.123	0.167	0.060
Fluoroquinolone	20	2016	**0.157**	0.167	0.154	0.112
		2017	**0.139**	0.139	0.111	0.101
Macrolide	22	2016	**21.328**	20.705	12.162	23.304
		2017	**19.313**	19.028	11.355	20.559
Penicillin	13	2016	**0.050**	0.146	0.176	0.065
		2017	**0.067**	0.081	0.091	0.050
Phenicol	21	2016	**0.400**	0.490	0.724	0.143
		2017	**0.453**	0.498	0.662	0.224
Sulfonamide	9	2016	**0.269**	1.751	2.016	0.577
		2017	**0.308**	1.487	1.745	1.017
Tetracycline	21	2016	**22.209**	41.687	53.955	27.147
		2017	**9.725**	25.814	32.584	6.555

Median total mg/kg-LW of medically important antimicrobials ranged from 0.07 for penicillins to 27.15 for tetracyclines for 2016. The range of median total mg/kg-LW of medically important antimicrobials for 2017 was 0.05 for penicillins to 20.56 for macrolides.

Regimens per Animal Year

Total feedyard level medically important antimicrobial values for Reg/AY are reported in Table 11 by year; the median value for 2016 was 3.53, and for 2017 was 3.04. Comparison of study level, mean, and median values for each year within each metric allows comprehension of the non-normal distribution of the feedyard level data and also the effect that larger feedyards have on the study level metric values due to closing out more lots per year. For example, evaluating the feedyard level values for the tetracyclines for both Reg/AY and mg/kg-LW values (Table 12) displays the low median compared to a much higher mean among the feedyard values, and also shows the large standard deviation.

Values for medically important antimicrobial Reg/AY values by antimicrobial class at the feedyard level are reported in Table 12. Median total Reg/AY of medically important antimicrobials ranged from 0.006 for penicillins to 2.29 for macrolides for 2016. The range of median total Reg/AY of medically important antimicrobials for 2017 was 0.003 for penicillins to 2.32 for macrolides.

Description of Medically Important Antimicrobial Regimens Encountered

Regimen descriptions are essential for understanding the pharmacokinetic and pharmacodynamic components of drug exposure. The link of duration of treatment, amount given per dose, amount given per treatment course, or the number of times a drug product is given to the overall pressure exerted relative to antimicrobial resistance selection is unknown at this time.

However, detailed regimen descriptions will be pivotal in research related to components of selection pressure.

This report provides the specific regimen information summarized at a drug class level for 2016 and 2017 data combined. The parameters were first calculated at the individual animal and product level, then the values were combined for overall calculations across all animals in all feedyards. Each regimen is described within the three use categories, which are in-feed, control of BRD, and individual animal treatment as each use category may utilize the same drug product for different indications.

Regimens are described in relation to the following components

- Timeframe
- Number of administrations per regimen
- Milligrams of antimicrobial per administration
- Milligrams of antimicrobial per regimen

Table 13 allocates each of the 2,030,246 total regimens in this study (both years) to each of the antimicrobial class – use category combinations. It is critical that evaluation of regimen parameters be carried out with recognition of the relative number of regimens in each antimicrobial class – use category combination; the tables and figures describing the regimens in this section present the characteristics of the regimens encountered without scaling between the different regimens.

Figures 9-12 are violin plots where the characteristics of the data are presented with height representing range of the population from lowest to highest and the width showing the

distribution of the population along this range. It is important to note that the width is related to distribution within the specific population being described (e.g., macrolide use in feed) and does not represent relative magnitude of the population in relation to other populations. The maximum width is set at a constant as the maximum incidence in a population is used to designate the value for the width; for example in one population the maximum width may be 10 units and in another a value of 3,000 units, but they appear the same width in the figure. A graphical description of the contents of the violin plots is presented in Appendix A.

Table 13 should be consulted to understand the relative contributions of the different regimens to the overall regimen total. For example, while the sulfonamides have high milligrams per administration and per regimen, and appear to dominate some of the figures, they contribute only very small numbers of regimens to overall use.

The range of the populations contain all values and the thin lines illustrating the upper reaches of populations may be, and typically are, due to only a few outliers.

Figures 9-12 also have superimposed box plots where the distribution of the data allows display. The box plot is bounded by the 25^{th} and 75^{th} percentiles with a dot representing the median value for the population. For graphical clarity the lines creating the box and whiskers is white making only the area contained in the boxes (the interquartile range (IQR)) visible within the violin plot.

Table 13 - Number and Percent of Total Regimens by Antimicrobial Class Within Use Class

Use Category	Antimicrobial Class	Total Regimens All Years	Percent of Total Regimens	Percent of Total Regimens by Use Category
In-Feed	Macrolide	1,212,160	59.7%	79.1%
	Tetracycline	393,676	19.4%	
Control of BRD	Aminoglycoside	1,784	0.1%	13.1%
	Cephalosporin	52,866	2.6%	
	Fluoroquinolone	3,378	0.2%	
	Macrolide	150,321	7.4%	
	Penicillin	4,862	0.2%	
	Phenicol	8,390	0.4%	
	Sulfonamide	135	0.0%	
	Tetracycline	43,973	2.2%	
Individual Animal Treatment	Aminoglycoside	1,367	0.1%	7.8%
	Cephalosporin	17,045	0.8%	
	Fluoroquinolone	32,893	1.6%	
	Macrolide	50,792	2.5%	
	Penicillin	2,162	0.1%	
	Phenicol	23,071	1.1%	
	Sulfonamide	2,915	0.1%	
	Tetracycline	28,456	1.4%	
Total		2,030,246	100.0%	100.0%

Regimen timeframe

The regimen timeframe descriptions in Figure 9 and Table 14 illustrate the variation in the number of days between the first and last administration of products in medically important drug classes. The regimen timeframe for control of BRD was always zero as the products were administered as a single injection to each animal, and there were no subsequent administrations which would have a timeframe. Individual animal treatment showed more variation, but most regimens were also a one-time administration of a single injectable antimicrobial. That use is consistent with the products currently on the market labeled for control of BRD, which are also the primary products utilized for individual animal treatment of disease. In-feed regimen

timeframes showed the most variation of the three use categories. Macrolides in the feed had the largest regimen timeframe. Note that the estimated timeframe in Table 14 for macrolides differs from the number of administrations in Table 15. This is due to the nature of administration reporting, including granularity, by different feedyards and illustrates the variation in estimates derived from different types of data structures. Chlortetracycline use was concentrated around a regimen timeframe of 4 days (an initial administration with administration over 4 more days, for a total of 5 days) which is consistent with the label for treatment of bovine respiratory disease. One feedyard utilized the 350 mg chlortetracycline per day regimen without a specified duration on the label. Regimen timeframe descriptions were available for essentially all regimens, as illustrated in Figure 9.

Table 14 - Regimen Timeframe expressed as days between the first and last administration of a regimen.

Regimen Timeframe				
Use Category	**Antimicrobial Class**	**Median**	**Mean**	**Standard Deviation**
Feed	Macrolide	132.00	134.07	70.51
	Tetracycline	4.00	4.21	5.29
Control	Aminoglycoside	0.00	0.00	0.00
	Cephalosporin	0.00	0.00	0.00
	Fluoroquinolone	0.00	0.00	0.00
	Macrolide	0.00	0.00	0.00
	Penicillin	0.00	0.00	0.00
	Phenicol	0.00	0.00	0.00
	Sulfonamide	0.00	0.00	0.00
	Tetracycline	0.00	0.00	0.00
Treatment	Aminoglycoside	0.00	0.00	0.07
	Cephalosporin	0.00	0.02	0.19
	Fluoroquinolone	0.00	0.00	0.05
	Macrolide	0.00	0.00	0.01
	Penicillin	0.00	0.14	0.50
	Phenicol	0.00	0.00	0.01
	Sulfonamide	0.00	0.00	0.02
	Tetracycline	0.00	0.00	0.03

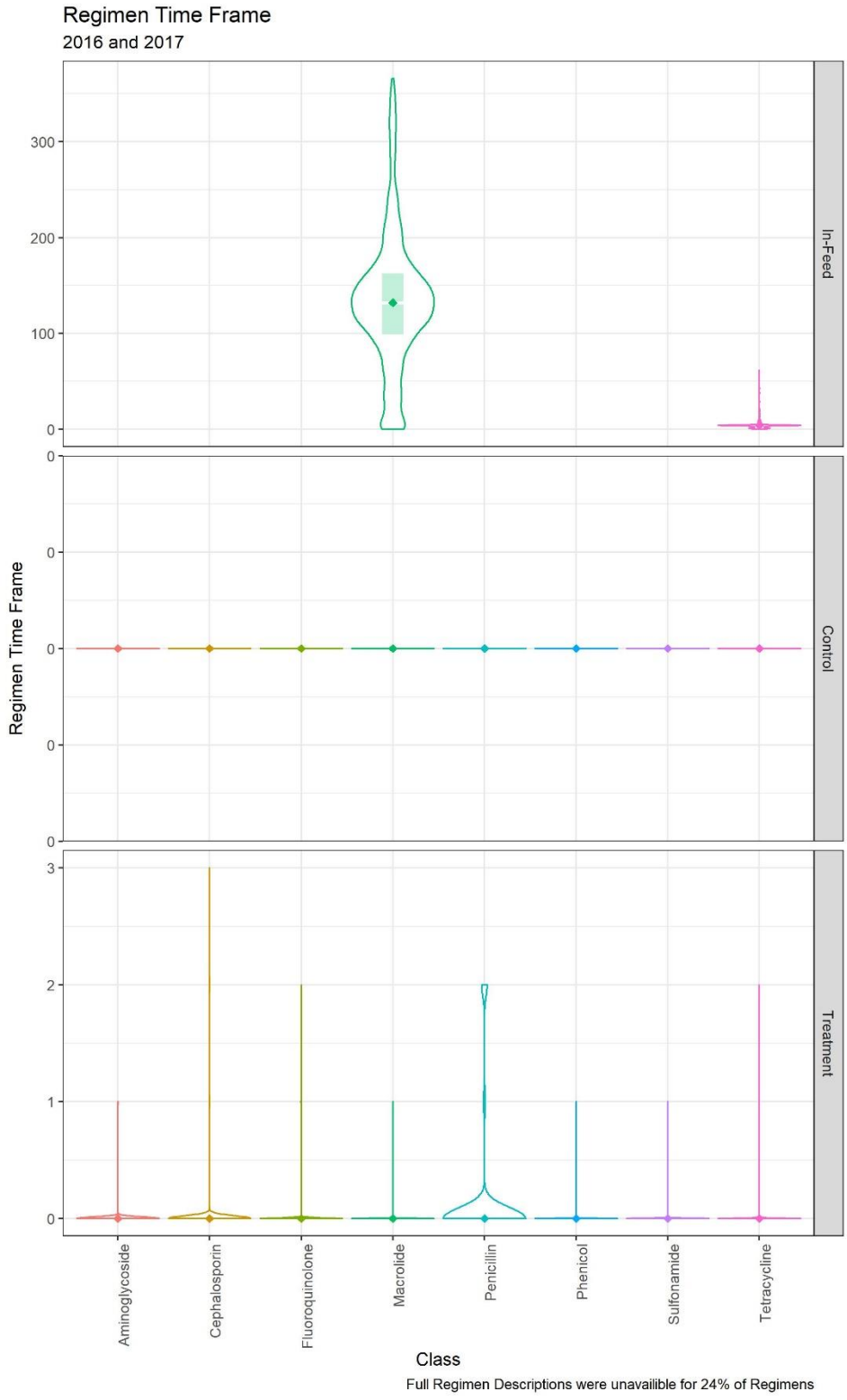

Figure 9 - Regimen Timeframe - Description of days between first and last administration of an antimicrobial regimen by antimicrobial class within use category

Number of Administrations per Regimen

Number of administrations per regimen illustrates the variation in the amount of time a single animal received a dose of an antimicrobial as part of the same regimen as displayed in Figure 10 and Table 15. An administration counted for the same regimen if less than two days had passed before the administration of another dose of the same product. If a different antimicrobial product was administered at a subsequent treatment episode, then it was counted as a new regimen. For control of BRD, this was always one administration per regimen.

The median for all injectable drug classes was one administration per regimen, however, macrolides, cephalosporins, fluoroquinolones, phenicols, and tetracyclines had a few outliers where the records indicated up to 6 administrations per regimen. It is probable that these represent recording errors as administering one of these single administration products multiple times in the same treatment regimen would be highly unusual and very expensive. As expected, in-feed number of administrations per regimen showed the widest range. Macrolides in the feed had the longest mean and median administrations per regimen. Number of administrations per regimen descriptions were available for 79.6% of regimens.

Table 15 - Number of Administrations per Regimen

Administrations per Regimen				
Use Category	**Antimicrobial Class**	**Median**	**Mean**	**Standard Deviation**
Feed	Macrolide	148.97	154.81	85.10
	Tetracycline	5.00	5.02	5.20
Control	Aminoglycoside	1.00	1.00	0.00
	Cephalosporin	1.00	1.00	0.00
	Fluoroquinolone	1.00	1.00	0.00
	Macrolide	1.00	1.00	0.00
	Penicillin	1.00	1.00	0.00
	Phenicol	1.00	1.00	0.00
	Sulfonamide	1.00	1.00	0.00
	Tetracycline	1.00	1.00	0.00
Treatment	Aminoglycoside	1.00	1.004	0.07
	Cephalosporin	1.00	1.022	0.20
	Fluoroquinolone	1.00	1.004	0.06
	Macrolide	1.00	1.002	0.04
	Penicillin	1.00	1.158	0.52
	Phenicol	1.00	1.001	0.03
	Sulfonamide	1.00	1.003	0.06
	Tetracycline	1.00	1.005	0.08

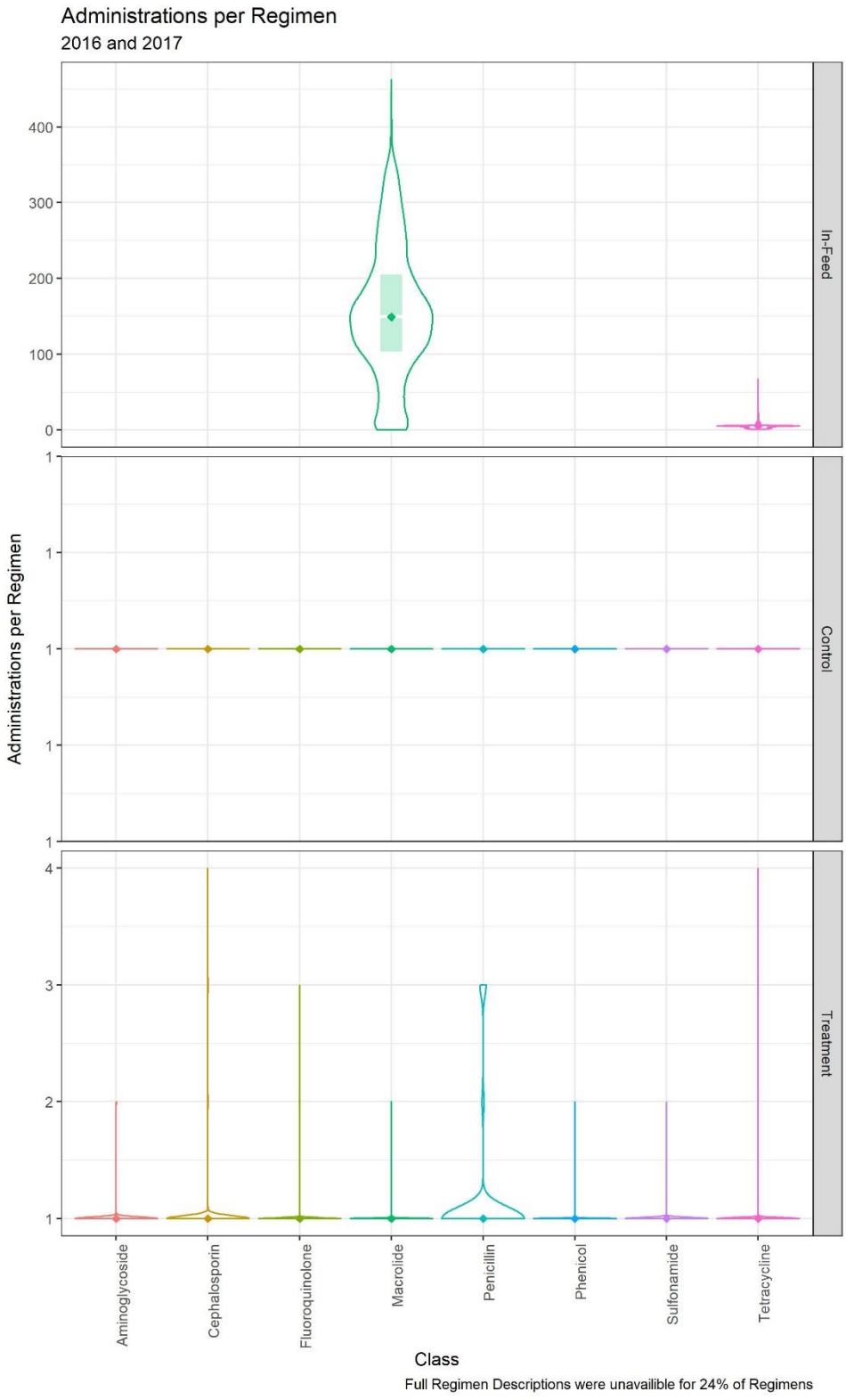

Figure 10 Number of Administrations per Regimen by Antimicrobial Class Within Use Category

Milligrams per Administration

Milligrams per administration describes the total amount of milligrams administered to an animal per administration during the course of a regimen. The milligrams per administration will vary depending on weight of animals treated and drug class. Figure 11 and Table 16 show the disparity in milligrams administered per regimen across the drug classes.

Table 16 - Milligrams of Medically Important Antimicrobials per Administration

Milligrams per Administration				
Use Category	**Antimicrobial Class**	**Median**	**Mean**	**Standard Deviation**
Feed	Macrolide	89	87	24
	Tetracycline	6,699	6,804	3,359
Control	Aminoglycoside	5,000	5,000	0
	Cephalosporin	2,000	1,965	576
	Fluoroquinolone	2,000	2,173	1,053
	Macrolide	400	625	720
	Penicillin	6,000	4,912	2,070
	Phenicol	5,850	9,340	7,014
	Sulfonamide	58,300	52,227	17,147
	Tetracycline	6,200	6,143	1,050
Treatment	Aminoglycoside	7,600	8,208	2,074
	Cephalosporin	2,200	2,065	944
	Fluoroquinolone	3,100	3,198	1,014
	Macrolide	1,000	1,473	1,261
	Penicillin	5,250	6,638	3,395
	Phenicol	12,000	12,263	3,502
	Sulfonamide	87,450	81,223	39,437
	Tetracycline	8,000	7,967	1,969

The use of sulfonamides for control of BRD is very uncommon as illustrated by the number of regimens in Table 13. The use captured in these data is from a few instances in one feedyard

and may reflect animals that were being processed for other purposes (e.g. vaccine, deworming) and treated for a disease during that time. In-feed use of macrolides has a median of 89 milligrams per administration and tetracylines 6,699 milligrams per administration. The amount of tetracycline (chlortetracycline in these cases) administered is dependent on the weight of the animals for the dominant regimen, while a less commonly used regimen utilizes a standard daily dose. For individual animal treatment, sulfonamides dominated milligrams per administration, but as illustrated in Table 13 the sulfas make up a very small component of the regimens. Milligrams per administration descriptions were available for 74.5% of regimens.

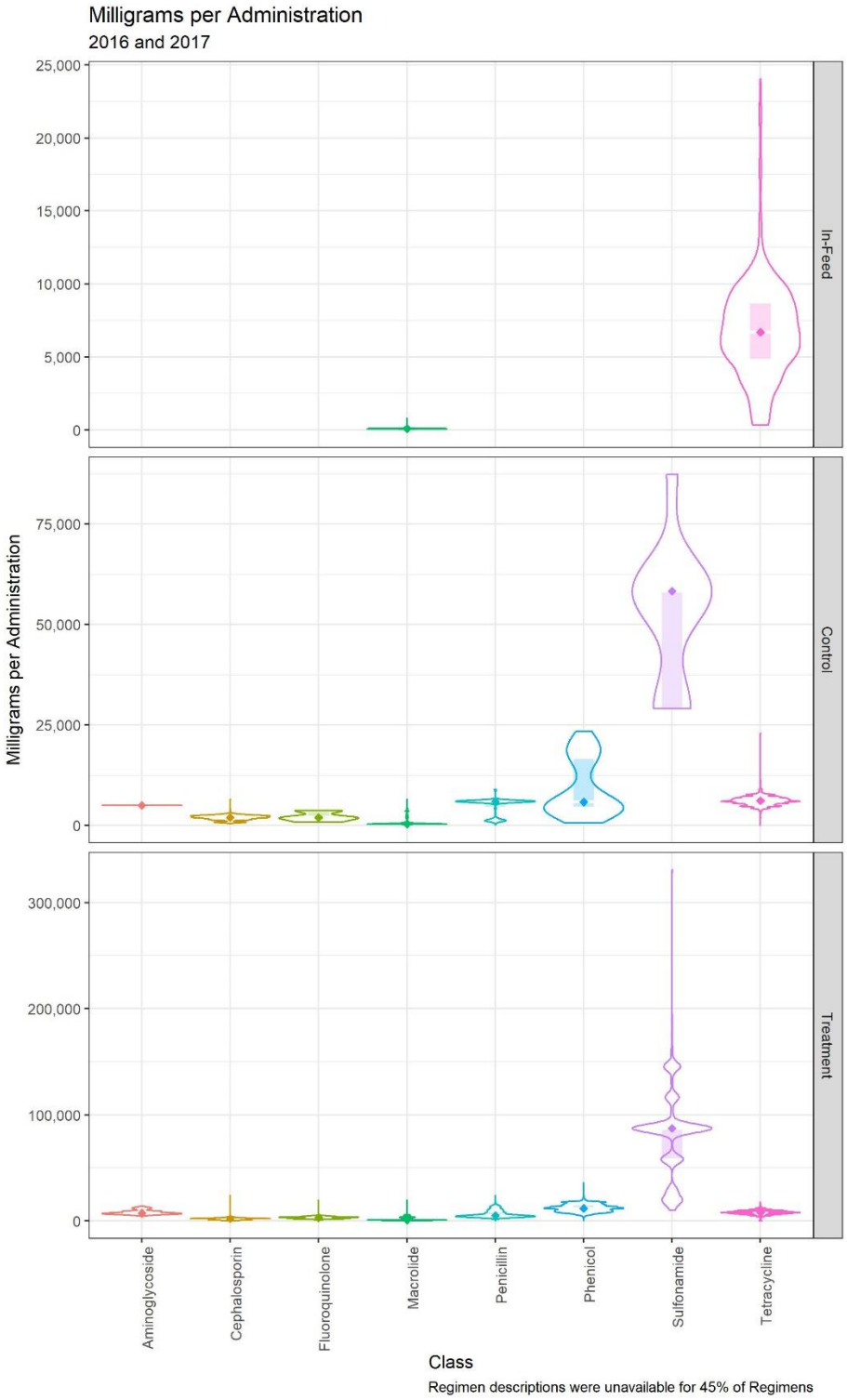

Figure 11 - Milligrams per Administration – reported by antimicrobial class within use category

Milligrams per Regimen

Milligrams per regimen describes the total amount of milligrams administered to an animal during the course of a regimen. Figure 12 and Table 17 show the variation in milligrams administered per regimen across the drug classes. The variation is especially noticeable between use categories as detailed by the y-axis differences.

In-feed use of macrolides has a median of 13,357 milligrams per regimen compared to 29,121 milligrams per regimen for tetracycline in the feed. For individual animal treatment, sulfonamides dominated milligrams per regimen. Milligrams per regimen descriptions were available for 79.6% of regimens.

Table 17 - Milligrams of Medically Important Antimicrobials per Regimen

Milligrams per Regimen				
Use Category	**Antimicrobial Class**	**Median**	**Mean**	**Standard Deviation**
Feed	Macrolide	13,357	13,110	6,251
	Tetracycline	29,121	31,546	22,479
Control	Aminoglycoside	5,000	5,000	0
	Cephalosporin	2,000	1,965	576
	Fluoroquinolone	2,000	2,173	1,053
	Macrolide	400	625	720
	Penicillin	6,000	4,912	2,070
	Phenicol	5,850	9,340	7,014
	Sulfonamide	58,300	52,227	17,147
	Tetracycline	6,200	6,143	1,050
Treatment	Aminoglycoside	7,600	8,239	2,112
	Cephalosporin	2,200	2,070	949
	Fluoroquinolone	3,100	3,204	1,074
	Macrolide	1,000	1,460	1,228
	Penicillin	6,000	7,295	3,665
	Phenicol	12,000	12,243	3,546
	Sulfonamide	87,450	80,119	39,894
	Tetracycline	8,000	7,962	2,142

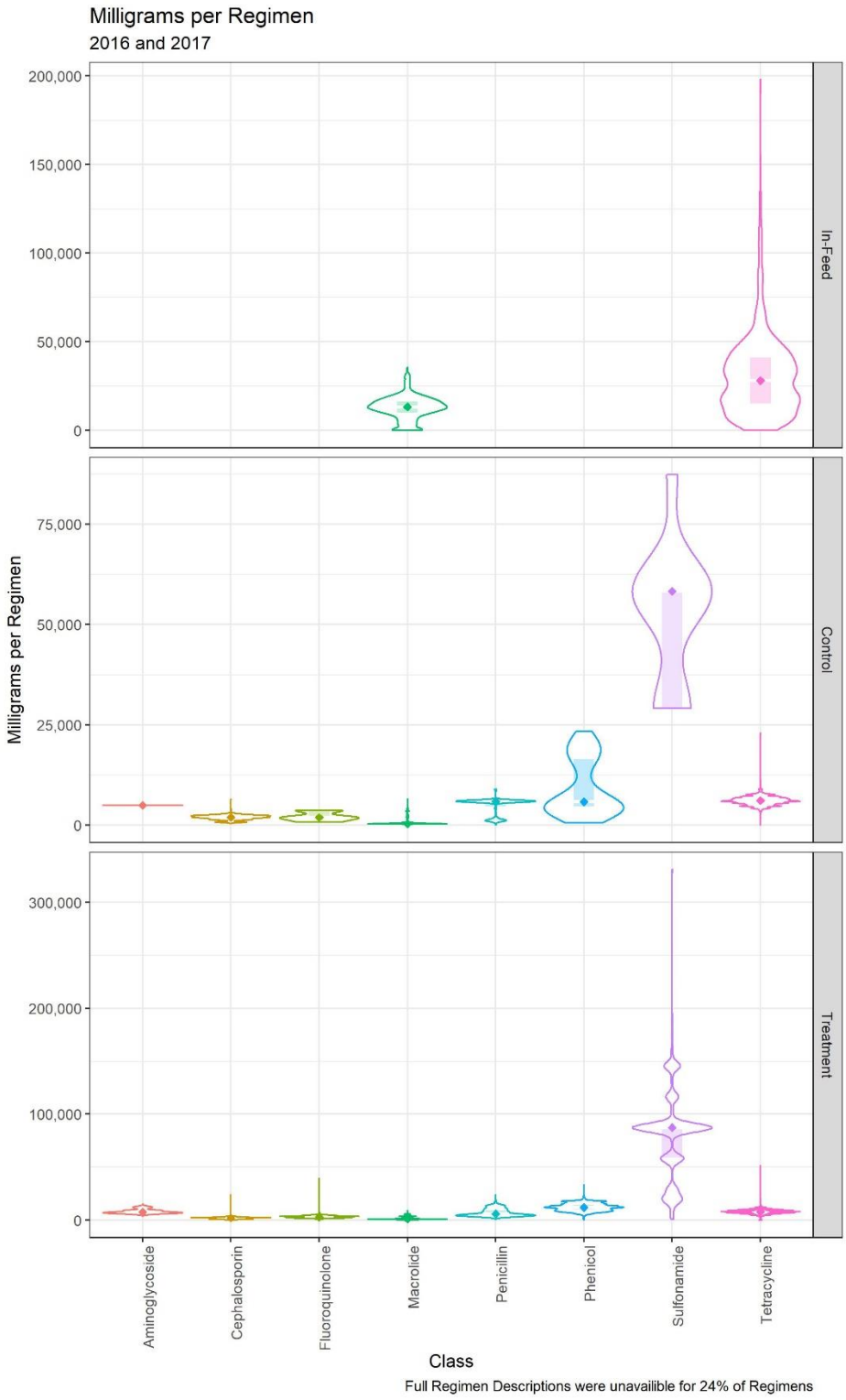

Figure 12 - Milligrams per Regimen - Description of the total number of milligrams given during regimens reported by antimicrobial class within use category

Non-medically Important Antimicrobial Use

Description of Non-medically Important Antimicrobial Use Data at the Study Level

Milligrams of Non-medically Important Antimicrobials per Kilogram Liveweight Sold Reported at the Study Level

Total milligrams of non-medically important antimicrobials per kilogram liveweight sold are reported in Figure 13 and Table 18. Total mg/kg-LW for non-medically important antimicrobials were 93.19 mg/kg for 2016 and 80.76 mg/kg in 2017. Total mg/kg-LW is not described by use category or antimicrobial class as all non-medically important antimicrobial use was in-feed and of the same antimicrobial class, ionophores.

Table 18 - 2016 and 2017 Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold of Non-medically Important Antimicrobials Reported at the Study Level

Year	Reg/AY	mg/kg-LW
2016	2.78	93.19
2017	2.70	80.76

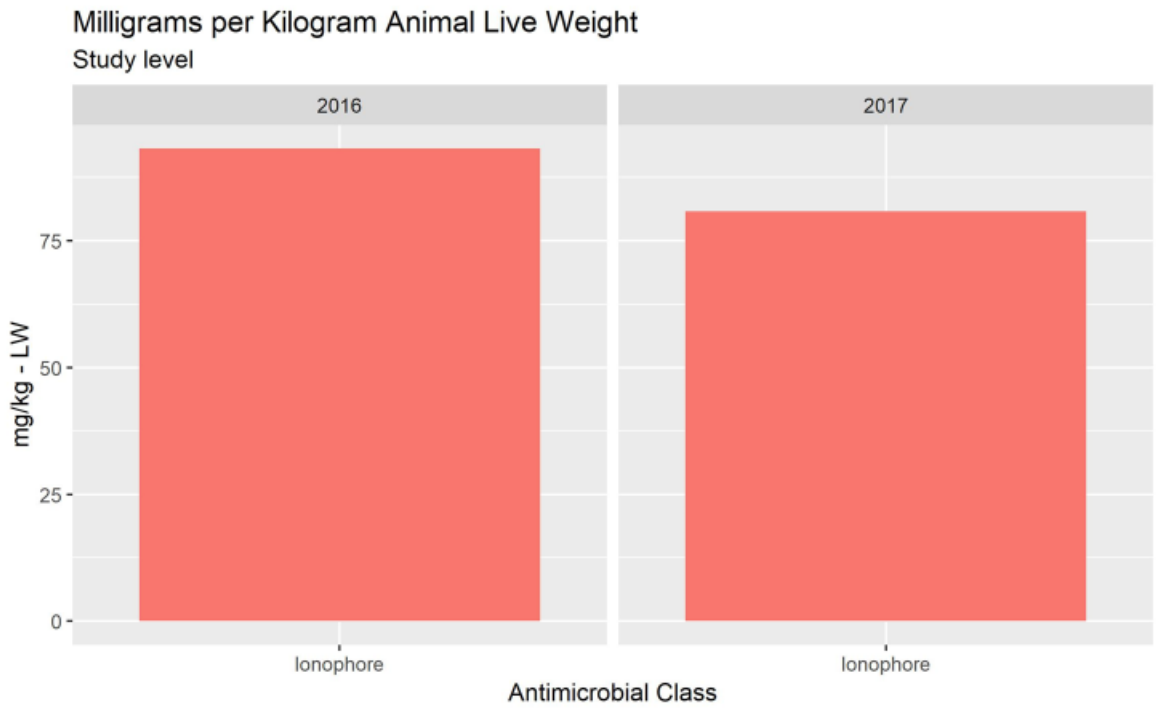

Figure 13 - 2016 and 2017 Milligrams per Kilogram Liveweight Sold of Non-Medically Important Antimicrobials Reported at the Study Level

Regimens per Animal Year of Non-medically Important Antimicrobials Reported at the Study Level

Total regimens of non-medically important antimicrobials are shown in Figure 14 and Table 18. Total regimens per animal year were 2.78 in 2016 and 2.70 in 2017. Total regimens per animal year of in-feed non-medically important antimicrobial use is not described by use category or drug class as all non-medically important antimicrobial uses were ionophores in the feed.

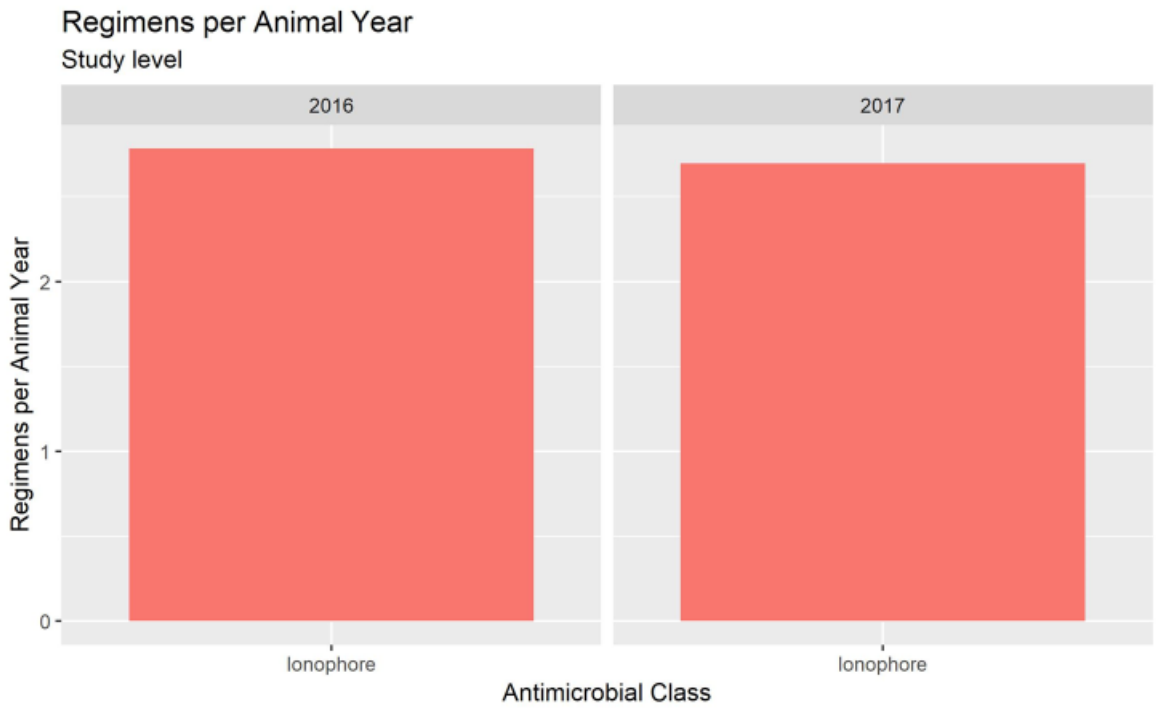

Figure 14 - 2016 and 2017 Regimens per Animal Year of Non-Medically Important Antimicrobials Reported at the Study Level

Description of Non-Medically Important Antimicrobial Use Data at the Feedyard Level

Milligrams of Non-medically Important Antimicrobials per Kilogram Liveweight Sold Reported at the Feedyard Level

Table 19 reports in-feed non-medically important antimicrobial use expressed as mg/kg-LW at the feedyard level, comparing 2016 to 2017. The median non-medically important antimicrobial mg/kg-LW for 2016 was 92.09, and in 2017 was 88.36.

Table 19 - Regimens per Animal Year and Milligrams per Kilogram Liveweight of Non-medically Important Antimicrobials by Year Calculated at the Study and Feedyard Level

Metric	Year	Study Level	Feedyard Level		
			Mean	Std Dev	Median
Reg/AY	2016	2.78	2.49	0.97	2.13
	2017	2.70	2.49	0.85	2.15
mg/kg-LW	2016	93.19	101.93	34.04	92.09
	2017	80.76	92.28	22.47	88.36

Regimens per Animal Year of Non-medically Important Antimicrobials Reported at the Feedyard Level

Table 19 reports in-feed non-medically important antimicrobial use expressed as Reg/AY at the feedyard level, comparing 2016 to 2017. The median non-medically important antimicrobial Reg/AY for 2016 was 2.13, and in 2017 was 2.15.

Discussion

Metrics reported

Total mg/total kilogram

The total milligrams of antimicrobial use were calculated by drug class for medically important antimicrobials for three different use categories: in-feed use, control of BRD, and individual animal treatment. Use of non-medically important antimicrobials was calculated for in-feed use only, as this was the only use.

Total milligrams of antimicrobial use is heavily influenced by drug class as well as the regimen. Reporting antimicrobial use by total milligrams may approximate use trends over a period of time, but is not helpful for making feedyard level antimicrobial comparisons, nor for

enabling benchmarking for the purposes of supporting evaluation of antimicrobial stewardship. Presenting the total milligrams at a drug class level can show a potentially more useful picture as it helps remove some of the inaccuracy of combining different potencies of drug products. Even within an antimicrobial class, different products have very different potencies. For example, the dose for tilmicosin for the treatment of BRD is 10–20 mg/kg of bodyweight while the regimen of tulathromycin for the same indication is 2.5 mg/kg. Switching from the high end of the flexible label regimen for tilmicosin to the label regimen for tulathromycin would result in an apparent reduction in macrolide use of 87.5% on a milligram basis, with potentially minimal difference in effective antimicrobial exposure.

Regimens/animal year

Regimens are defined as a specific drug product, dose, route, duration, and interval of a course of treatment in an individual animal. Regimens per animal year can provide either an overall exposure estimate or may be used as a more granular metric when reported by use category, antimicrobial class, and even specific disease being treated. Regimens emulate a similar pattern as treatment incidence depending on the information used for calculation.[1,2]

In this study, the authors' intent was to remove as many assumptions as possible related to duration and magnitude of therapeutic and resistance selection effects. By using regimen descriptions, no assumptions are made relative to length of time the drug is present in any of the multiple pharmacological compartments in the body, nor are assumptions made on duration or magnitude of exposure for bacterial populations. These assumptions would have been necessary to assign exposure durations to single-injection therapies. In the future, additional information

may be available to better inform decisions as to what components of a regimen contribute the most to antimicrobial resistance propagation.

Macrolides and tetracyclines as examples of the effect of granularity in data

One of the difficulties in presenting summarized antimicrobial use in total mg/kg-LW is the tendency for the data to be reported as a single value as shown in Figure 3. The lack of data granularity at this level provides little context for interpretation of the values reported. This is especially noticeable for tetracyclines and macrolides. Displaying total mg/kg-LW by antimicrobial class contributions as in Figure 4 illustrates tetracyclines and macrolides exceeding the other drug classes in proportion of total mg/kg-LW they contribute, with the same trend displayed for Reg/AY in Figure 7. As the data increases in granularity with total values displayed by antimicrobial class within use category (Figures 5 and 8), the impact of in-feed use on overall use is noticeable.

Reasons for Variations in Outcomes

Chapter 2 discussed the difference in record systems that were in place in the participating feedyards. As a result of this disparity, there were challenges in availability of a cohesive dataset requiring significant efforts in data standardization. Potential sources of inaccuracy could come from multiple areas, although multiple approaches were taken to control these errors.

1. Data entry
    a. Entry errors could be related to non-recorded instances of individual animal therapy, control of BRD or in-feed use.
    b. Entry errors during data entry from hard-copy records

c. Missing information from on-feedyard documentation

2. Incomplete transfer of records to investigators

Another reason for variations in outcome is related to variability within the denominators. Denominator variability can come from multiple factors. The variation in number of regimens per animal year is heavily influenced by in-weight. In-weight is directly connected with risk classification of cattle and has effects on the management of those animals. In-weight is also connected to the amount of days a group of cattle remains in the feedyard which corresponds to the number of animals represented in an animal year and the number of times an animal is at risk for disease or for use of antimicrobials for control of disease during the year.

An example to illustrate instances of variation is shown in Table 20. The feedyard in this example has one pen which may be used in one of 3 scenarios. All scenarios incorporate the same rate of gain, 3 pounds (1.37 kg) per day, with tylosin fed in the ration every day at 90 mg/head per day and identical weight-out values of 680 kg. In scenario one, the cattle come in weighing 182 kg, are in the pen as one lot for the entire year and then are sold. In scenario 2, one lot comes in on day 1 weighing 430 kg, stays for 183 days, and is sold. Another identical lot immediately replaces these cattle and are also fed 183 days before being sold. The pen has cattle in it all year long, being sold at the same weight, but there are two lots coming in heavier and being sold in a shorter period of time. Scenario three takes this progression to the next step; three lots of cattle occupy the pen during the year, each coming in weighing 514 kg and only being fed for 122 days before being sold. As in scenarios 1 and 2, there are cattle in the pen every day of the year, but they are in 3 different lots; each lot is still sold at 680 kg bodyweight. For purposes of calculation, the lot days on feed for each scenario is adjusted to 365.

The total number of tylosin regimens for scenarios 1, 2, and 3 are 100, 200, and 300, respectively. The animal years are the same for each scenario; the space was occupied by 100 cattle every day for a year, resulting in 36,500 head days. This amount of head days divided by 365 results in 100 animal years for each scenario. As metric values in the bottom two rows of the table move from left to right, the mg/kg-LW appears "better" (less use) and the Reg/AY appears "worse" (more use).

Table 20 - Variation in Outcomes Example

Scenario	1	2	3
Number of Head per lot	100	100	100
Number of lots/year	1	2	3
Days on feed/lot	365	183	122
Total days-on-feed	36,500	36,500	36,500
Tylosin dose mg/hd·day	90	90	90
Total tylosin (mg)	3,285,000	3,285,000	3,285,000
Weight-in (kg)	182	430	514
Weight-out (kg)	680	680	680
Regimens	100	200	300
Animal years	100	100	100
Total kilograms liveweight sold	68,000	136,000	204,000
Milligrams antimicrobial per kg live weight sold (mg/kg-LW)	48.31	24.15	16.10
Regimens per animal year (Reg/AY)	1	2	3

The same amount of antimicrobial is fed in the pen every day in all three scenarios, yet the scenarios appear quite different according to the metric values because of the effect of time in residence in the feedyard for each group, the turnover rate of the feedyard, and the cumulative sale weight for cattle closed out in that year. Now consider other variations due to times of near capacity populations vs. low populations, different weights-in and weights-out, different rates of gain, different waiting periods before starting tylosin in the ration, different inclusion rates, and multiple other variations.

This example illustrates how the in-weight and days on feed can affect antimicrobial use outcome measurements at a very basic level and a few of the distinctions that are associated with interpreting the outcome measurements.

Conclusion

In light of the information presented in Chapters 1 and 2 along with the results presented in this chapter, the processes of accessing, aggregating, and combining multiple record-keeping systems requires the understanding of the complex series of procedures required to produce a single outcome measure. The danger lies within using that single number without understanding the nuances and assumptions throughout the process.

Making sweeping conclusions across feedyards is not advisable due to high variability in types of cattle fed, nutrition differences, geography, disease pressure, and industry economics. All of these factors must be considered in an overall interpretation of antimicrobial use in feedyards.

References

1. Catry B, Laevens H, Devriese LA, et al. Antimicrobial resistance in livestock. *Journal of Veterinary Pharmacology and Therapeutics* 2003;26:81-93.

2. Pardon B, Catry B, Dewulf J, et al. Prospective study on quantitative and qualitative antimicrobial and anti-inflammatory drug use in white veal calves. *Journal of Antimicrobial Chemotherapy* 2012;67:1027-1038.

Chapter 4 - Antimicrobial Use Metrics for 18 Beef Feedyards Reflecting Outcomes from Surveys and Use Data

Methods of Survey Data Collection

Introduction

Collecting data by survey requires less resources for each participant than are required for collection of actual use data. A concern is how closely survey data reflect the actual antimicrobial use for each participant as well as for an animal production industry.

To address the question of agreement between antimicrobial use metrics determined from survey and use data, a feedyard survey instrument was reverse engineered from the desired outcome metrics which would match the metrics derived from use records as reported in Chapter 3.

All surveys were administered and recorded in person by a single experienced feedyard veterinarian who recorded all responses and could ask clarification questions and guide the respondent in providing the appropriate data. Surveys were administered within 6 months of the end of the year in question and survey outcomes were then compared to the reference year.

General survey information regarding the designated year

The survey respondent was given the opportunity to refer to the cattle closed out in the designated year or to refer to antimicrobial use during a calendar year as a surrogate. Three respondents chose the latter option; in these instances, the closed-out cattle in that year were still

a reference point for cattle numbers, but the number of cattle receiving an antimicrobial for control of BRD or individual animal therapy were referenced to the calendar year.

Head-in for lots closed out in the given year – This number was the basis of all calculations for feed consumption and also for the number of animals receiving treatments when a percent of the population was provided as a morbidity estimate. Adjustments for death loss during the feeding period were not made when the head-in number was combined with reported average days on feed to estimate days of ration consumption for in-feed drug consumption calculations.

Average days-on-feed (DOF) – This value was captured either for all cattle in general, or in some cases, values were provided as average DOF for different classifications (e.g., steers, heifers, calves, yearlings, Holsteins). When DOF estimates were provided for different classifications, a weighted average value was calculated as an average DOF for the feedyard.

Total animal head days (head days) – This value was obtained by multiplying head-in (either total or for each subcategory estimate) by the appropriate DOF estimate. If subcategory estimates were calculated, they were summed to derive the overall total animal head days.

Total weight sold – This value was obtained from use data collected from each feedyard. Some of the respondents could easily access this value, and it is reasonable that this would be done during a survey administration period. Therefore, total weight sold was calculated by using actual records as weighed at the feedyard immediately prior to shipment. The denominator of total live weight sold is the same value for both surveys and use data; therefore, the only variable between survey and use data in the metric "milligrams antimicrobial/kg liveweight sold" is the estimation of the milligrams of antimicrobials used.

Survey Administration Methods

Survey information for in-feed antimicrobial use: macrolides (medically important) and ionophores (non-medically important)

Questions were designed to capture data enabling the calculation of the total milligrams of antimicrobials used, the number of times animals were exposed to each drug, and the number of administrations in an animal regimen. Depending on the preference of the respondent, one of two methods were utilized to estimate in-feed antimicrobial regimens.

Method 1: An average daily feed consumption value was estimated by the respondent along with the ration inclusion rate of the antimicrobial. Both consumption and inclusion rate were specified as to whether they were on an as-fed or dry matter basis. The respondent also indicated the number of days during the feeding period when cattle were not fed an antimicrobial which otherwise was fed for the entire feeding period (e.g., tylosin was not fed during an initial ration adjustment period in some feedyards). In some cases, estimated consumption was matched with antimicrobial inclusion rates and durations of feeding for multiple rations in a ration step-up program. Average daily feed consumption and ration antimicrobial inclusion rates could be reported from memory by the respondent, or by accessing ration formulation sheets and feed records. In some cases, the consulting nutritionist assisted in providing these data.

Method 2: A targeted daily dose (mg/head·day) was reported by the respondent along with an estimate of proportion of the feeding period during which the antimicrobial was fed. Periods of non-inclusion of an antimicrobial were recorded and then subtracted from the estimated average DOF for the feedyard or as reported for the specific class of cattle in question. The inclusion rate could be reported from memory or determined from ration formulation sheets or feed records. In

one case, records were reviewed, and the targeted daily doses were reported the next day. In other cases, the consulting nutritionist assisted in providing these data.

Survey information for in-feed antimicrobial use: chlortetracycline (medically important)

Chlortetracycline was administered through the feed by two different regimens.

1. The dose of 10 mg/lb of bodyweight per day for up to 5 days is labeled for treatment of bacterial enteritis caused by *Escherichia coli* and bacterial pneumonia caused by *Pasteurella multocida* organisms susceptible to chlortetracycline. The estimated number of cattle, the days of administration, and estimated weight at the time of administration were recorded.
2. A daily standard dose of 350 mg per day is labeled for continuous use to control bacterial pneumonia associated with the shipping fever complex caused by *Pasteurella* spp. susceptible to chlortetracycline. When administered, the number of cattle receiving this regimen and duration were recorded.

Survey information for control of bovine respiratory disease (BRD)

Respondents were asked if antimicrobials for control of BRD were administered to any cattle during the designated period. If an affirmative response was received, the number of cattle, weight at time of administration for control, and the drug regimen were captured. A response of "label dose" was most commonly reported for the regimen, in which case the label dose was entered and combined with the reported average weight of the cattle to derive the amount of antimicrobial used. When a dose range was available for an antimicrobial and a specific dose was not reported, a constant value was selected and used. For the two most common occurrences of

this reporting method, the middle of the dose range was used for the single- injection enrofloxacin regimen and the low end of the dose range was used for tilmicosin.

Some respondents reported multiple groups of cattle with defining characteristics which received an antimicrobial for control of BRD, e.g. differing sources or in-weights. In these cases, separate calculations were conducted based on the reported average weight of these groups, the number of cattle, and the drug regimen.

Survey information for individual animal therapy

Respondents were asked to describe treatment regimens, estimate number of cattle treated, and estimate average weight at the time of treatment for the following diseases.

- Bovine respiratory disease
    - Arrival weight ≤ 700 lbs with a high risk of BRD
    - Arrival weight ≤ 700 lbs with a low risk of BRD
    - Arrival weight > 700 lbs
- Musculoskeletal disease
- Central nervous system disease
- Enteric disease
- Other

Regimens were captured in relation to initial treatments and subsequent treatments. Numbers of cattle receiving initial treatment, their average weight at the time of treatment, and regimens were recorded. Then, the respondent could estimate percentages receiving subsequent treatment (e.g. second, third regimens) or could provide number estimates for each subsequent treatment. Weights at the time of treatment for subsequent treatments were considered to be the same as initial treatments. Doses were captured by the respondent providing specific dosing regimens, or they could specify “label dose”, in which case the same approach as for control of BRD was taken.

Only antimicrobials were recorded with no quantification of ancillary therapy such as non-steroidal anti-inflammatory drugs or steroids.

Data Management

Survey Administration Details

Table 21 outlines the characteristics of survey administration and management. The mean (± SD) time to complete the survey was 31 ± 19 minutes with a range of 13 to 93 minutes. Survey data entry from the paper form into an Excel spreadsheet required a mean (± SD) of 67 ± 31 minutes with a range of 27 to 120 minutes. This time included checking entered data back against the hard copy record.

Survey respondents were the cattle manager for 3 surveys, the owner for 3 surveys, and the general manager for 11 surveys. One survey was answered by the owner and cattle manager together. Sixteen survey responses were based on the cattle closed-out in the designated year and two respondents utilized the calendar year instead.

Feedyard nutritionists were consulted for ration inclusion information by four of the survey respondents while the remaining 14 elected to answer on their own. Eight respondents based the in-feed use on estimated average daily consumption combined with ration inclusion rates. Responses for nine feedyards were based on targeted daily doses. One feedyard provided both consumption with inclusion rate and targeted daily doses for the in-feed antimicrobial uses.

Table 21 - Survey Administration Characteristics

Time for survey (minutes)	
High	93
Low	13
Mean	30
Stdev	19
Time to enter survey (Minutes)	
High	120
Low	27
Mean	67
Stdev	31
Respondent	
Cattle Manager	3
Owner	3
General Manager	11
Cattle Manager and Owner	1
In-feed reporting method	
Consumption with inclusions	8
Targeted daily dose	9
Combination	1
Nutritionist consulted for in-feed estimate assistance	
Yes	4
No	14
Time period for survey responses	
Cattle closed out in designated year	16
Cattle present during designated year	2

Output Descriptions

For the antimicrobial use records from the feedyards (record) comparison to the survey use data (survey), 18 of the 22 participating feedyards were included. Three feedyards were excluded as they did not complete a survey. The fourth feedyard excluded was due to extreme similarities between the feedyard data provided and the survey data; the feedyard data were heavily informed by the survey responses due to antimicrobial use data consisting only of purchase receipts.

In this report, two output metrics are presented, milligrams of medically important antimicrobial per total kilogram of animal liveweight sold (mg/kg-LW) and regimens of medically important antimicrobials per animal year (Reg/AY). The combination metrics were calculated in the same manner for both survey data and use data.

Regimens/animal year

Regimens can be used as a representation of disease pressure due to being defined as a specific drug product, dose, route, duration, and interval of a course of treatment in an individual animal. The intent was to remove any assumptions in regimen description based on route of delivery, or the magnitude and duration for which antimicrobials are present in multiple locations, including the microbiota. Regimens per animal year provide a view of the use on a specific feedyard related to a time denominator with the capability to be reported by antimicrobial class and by the use categories of in-feed, control of BRD, and individual animal treatment. Regimens have been demonstrated to exhibit a similar use pattern to treatment incidence depending on the information used for calculation.[1,2]

Total milligrams/ kilogram liveweight sold

The total milligrams of medically important antimicrobials were calculated from the antimicrobial use data provided by all feedyards for three different areas: individual animal therapy, control of BRD, and in-feed use. Total milligrams of antimicrobial use is heavily influenced by drug class potency, therefore, reporting by total milligrams of antimicrobial/total kilograms liveweight sold may give insight of use trends over a period of time, but is not helpful for making granular feedyard level antimicrobial use observations.

Results

Total Medically Important Antimicrobial Regimens per Animal Year at the Study Level

Regimens of Medically Important Antimicrobials per Animal Year

Figure 15 and Table 22 display the total regimens of medically important antimicrobials per animal year summarized by use data (record) and survey. For additional transparency, the relative contribution of each antimicrobial class is illustrated within each total in Figure 15. When these data are summarized in this manner at the study level, total regimens of medically important antimicrobials per animal year appear to be very close. Total regimens per animal year of all medically important antimicrobials are 2.98 for feedyard data and 3.75 for survey data. However, the lack of granularity in this depiction reduces the ability to visualize the individual feedyard variability present as well as contributions of different antimicrobial classes.

Table 22 - Record and Survey Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold of Medically Important Antimicrobials Calculated at the Study Level

Data Source	Reg/AY	mg/kg-LW
Record	2.98	31.85
Survey	3.75	41.67

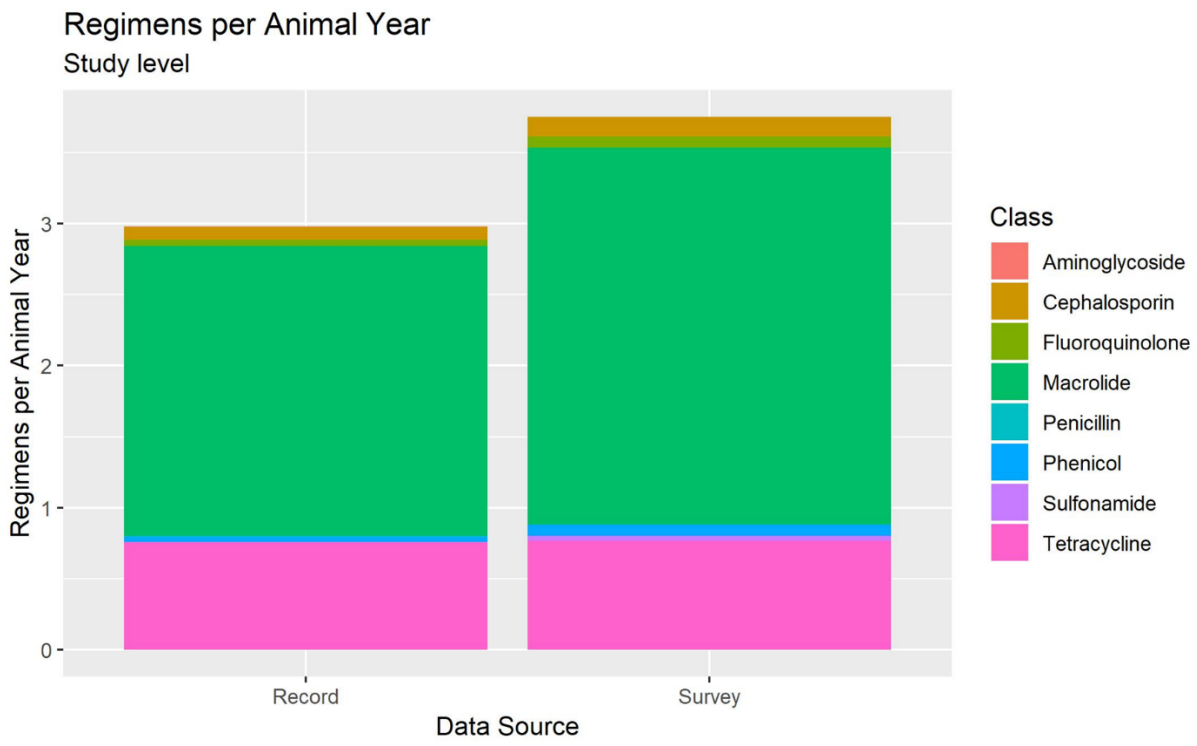

Figure 15 - Total Regimens per Animal Year of Medically Important Antimicrobials Comparing Record to Survey at the Study Level

Medically important antimicrobial drug class regimens per animal year as shown in Figure 16 and Table 23 ranged from 0.01 for aminoglycosides to 2.04 for macrolides when calculated from feedyard record data. Survey data analysis resulted in a range from 0.0003 regimens per animal year for penicillins to 2.65 regimens per animal year for macrolides. Survey data responses did not include any aminoglycoside use.

Table 23 – Medically Important Antimicrobial Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold Reported by Drug Class Calculated at the Study Level for both Record and Survey Sources

Metric	Class	Record	Survey
Reg/AY	Aminoglycoside	0.01	NR
	Cephalosporin	0.09	0.14
	Fluoroquinolone	0.05	0.08
	Macrolide	2.04	2.65
	Penicillin	0.01	0.0003
	Phenicol	0.03	0.08
	Sulfonamide	0.00	0.03
	Tetracycline	0.76	0.77
	Reg/AY Sum	**2.98**	**3.75**
mg/kg-LW	Aminoglycoside	0.02	NR
	Cephalosporin	0.14	0.19
	Fluoroquinolone	0.12	0.17
	Macrolide	17.10	21.13
	Penicillin	0.05	0.001
	Phenicol	0.32	0.68
	Sulfonamide	0.24	3.32
	Tetracycline	13.86	16.19
	mg/kg-LW Sum	**31.85**	**41.67**

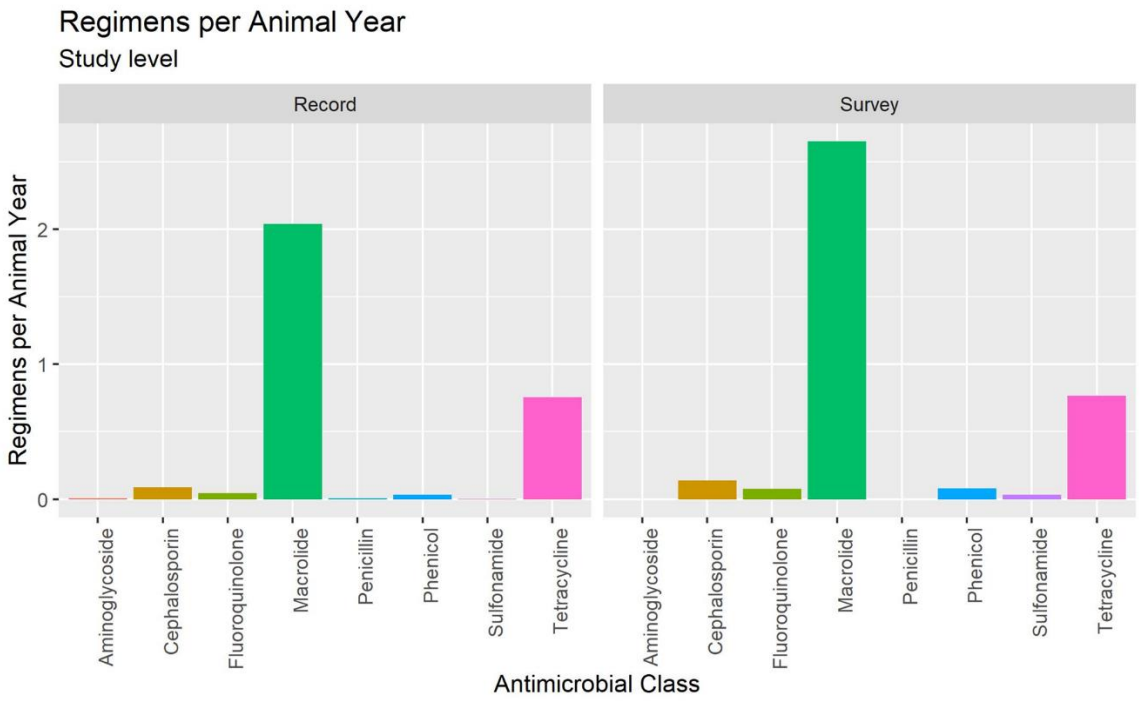

Figure 16 - Total Regimens per Animal Year of Medically Important Antimicrobials Comparing Record to Survey by Antimicrobial Class Calculated at the Study Level

Total regimens of medically important antimicrobials per animal year by antimicrobial class within use category, as calculated at the study level, are reported in Figure 17 and Tables 24 and 25. Tables 24 and 25 present the same data, Table 24 as values and Table 25 as percentages of totals for each primary cell in a column. The default setting for decimal places in Table 24 is 3 places and in Table 25 is 2 places, with expansion of decimal places as required to illustrate a value in low value cells. Figure 17 presents the total amount of medically important antimicrobial regimens within each use category by antimicrobial class.

Calculations for in-feed use of medically important antimicrobials at the study level resulted in values of 2.44 regimens per animal year from feedyard data and 2.90 regimens per animal year from survey results. Control for BRD was calculated as 0.32 regimens per animal year from feedyard data and 0.36 regimens per animal year from survey data. Individual animal

treatment showed the largest proportional difference of the three use categories with feedyard record data estimating a smaller value than survey data, 0.23 and 0.49 regimens per animal year, respectively. At the study level, the survey Reg/AY values expressed as a percent of feedyard record data Reg/AY values were 119.2%, 113.3%, and 215.3% for in-feed, control of BRD, and individual animal treatment, respectively.

Table 24 - Medically Important Antimicrobial Record and Survey Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold by Use Category and Antimicrobial Class Calculated at the Study Level

Use Category and Antimicrobial Class	Reg/AY		mg/kg-LW		Feedyard Count	
	Record	Survey	Record	Survey	Record	Survey
In-feed						
Macrolide	1.765	2.237	16.815	20.823	15	16
Tetracycline	0.669	0.664	13.337	15.558	10	11
In-feed total	**2.435**	**2.901**	**30.152**	**36.381**		
Control of BRD						
Aminoglycoside	0.006	NR	0.025	NR	1	NR
Cephalosporin	0.064	0.104	0.096	0.130	6	2
Fluoroquinolone	0.00001	NR	0.00001	NR	1	NR
Macrolide	0.204	0.247	0.201	0.172	13	13
Penicillin	0.006	NR	0.038	NR	2	NR
Phenicol	0.0001	0.002	0.001	0.017	2	1
Sulfonamide	0.0003	NR	0.010	NR	1	NR
Tetracycline	0.040	0.010	0.230	0.058	7	1
Control of BRD total	**0.320**	**0.363**	**0.601**	**0.377**		
Treatment						
Aminoglycoside	0.000003	NR	0.00001	NR	1	NR
Cephalosporin	0.025	0.036	0.039	0.055	17	13
Fluoroquinolone	0.046	0.078	0.120	0.168	17	15
Macrolide	0.070	0.167	0.083	0.132	18	16
Penicillin	0.002	0.0003	0.008	0.001	8	1
Phenicol	0.033	0.078	0.323	0.662	17	17
Sulfonamide	0.004	0.034	0.227	3.324	7	5
Tetracycline	0.047	0.094	0.293	0.571	18	14
Treatment Total	**0.227**	**0.488**	**1.093**	**4.913**		
Total all uses	**2.982**	**3.752**	**31.847**	**41.671**		

NR = Not Reported

Table 25 - Medically Important Antimicrobial Record and Survey Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold by Use Category and Antimicrobial Class Calculated at the Study Level as Percentages. Note that all columns may not appear to sum to 100% due to rounding.

Use Category and Antimicrobial Class	Reg/AY		mg/kg-LW		Feedyard Count	
	Record	Survey	Record	Survey	Record	Survey
In-feed						
Macrolide	59.21%	59.62%	52.80%	49.97%	15	16
Tetracycline	22.45%	17.70%	41.88%	37.34%	10	11
In-feed total	**81.66%**	**77.33%**	**94.68%**	**87.31%**		
Control of BRD						
Aminoglycoside	0.21%	NR	0.08%	NR	1	NR
Cephalosporin	2.14%	2.77%	0.30%	0.31%	6	2
Fluoroquinolone	0.0002%	NR	0.00002%	NR	1	NR
Macrolide	6.84%	6.60%	0.63%	0.41%	13	13
Penicillin	0.20%	NR	0.12%	NR	2	NR
Phenicol	0.004%	0.06%	0.002%	0.04%	2	1
Sulfonamide	0.01%	NR	0.03%	NR	1	NR
Tetracycline	1.34%	0.26%	0.72%	0.14%	7	1
Control of BRD total	**10.75%**	**9.67%**	**1.89%**	**0.90%**		
Treatment						
Aminoglycosides	0.0001%	NR	0.00004%	NR	1	NR
Cephalosporin	0.83%	0.96%	0.12%	0.13%	17	13
Fluoroquinolone	1.54%	2.08%	0.38%	0.40%	17	15
Macrolide	2.36%	4.46%	0.26%	0.32%	18	16
Penicillin	0.05%	0.01%	0.03%	0.003%	8	1
Phenicol	1.11%	2.07%	1.02%	1.59%	17	17
Sulfonamide	0.14%	0.90%	0.71%	7.98%	7	5
Tetracycline	1.57%	2.52%	0.92%	1.37%	18	14
Treatment Total	**7.60%**	**13.00%**	**3.43%**	**11.79%**		
Total all uses	**100.00%**	**100.00%**	**100.00%**	**100.00%**		

NR = Not Reported

Figure 17 - Total Regimens per Animal Year of Medically Important Antimicrobials Comparing Record to Survey by Antimicrobial Class Within Use Categories at the Study Level. Insets for control and treatment use categories alter the Y axis for increased clarity between antimicrobial classes.

Total Milligrams of Medically Important Antimicrobials per Kilogram Liveweight Sold at the Study Level

Milligrams per Kilogram Liveweight Sold

Figure 18 and Table 22 display the total milligrams of medically important antimicrobials per kilogram liveweight sold summarized between reported feedyard use data (record) and survey. Figure 18 adds the granularity of illustrating medically important antimicrobial class contribution to the overall total. When the data are summarized in this manner, milligrams per kilogram liveweight appear to be numerically higher for survey estimates. Total mg/kg-LW for medically important antimicrobials at the study level are 31.85 for feedyard record data and 41.67 for survey

data. However, the lack of granularity in this level of depiction reduces the ability to visualize the individual feedyard variability present.

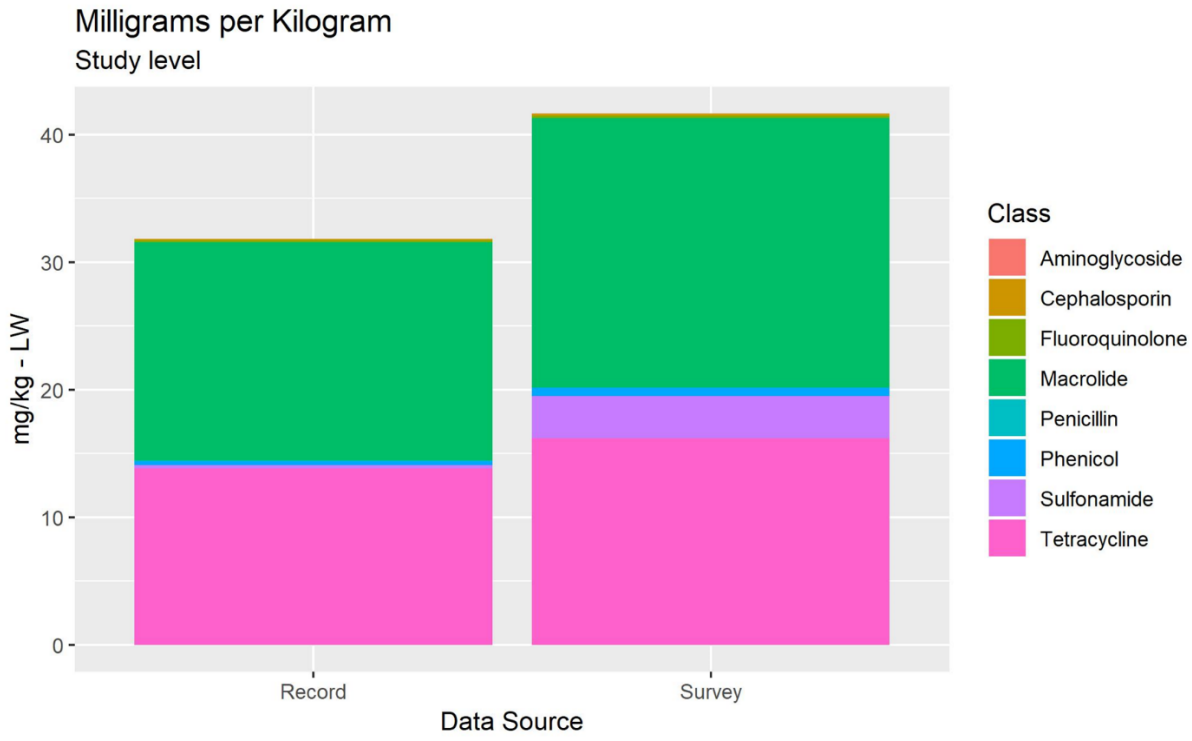

Figure 18 - Total Milligrams of Medically Important Antimicrobials per Kilogram Liveweight Sold Comparing Record to Survey at the Study Level

Figure 19 and Table 23 contain values for milligrams of medically important antimicrobials per kilogram liveweight sold by antimicrobial drug class. Feedyard record data values for mg/kg-LW ranged from 0.02 for aminoglycosides to 17.10 for macrolides. Survey mg/kg-LW values ranged from 0.001 for penicillins to 21.13 for macrolides. Survey data responses did not include any aminoglycoside use.

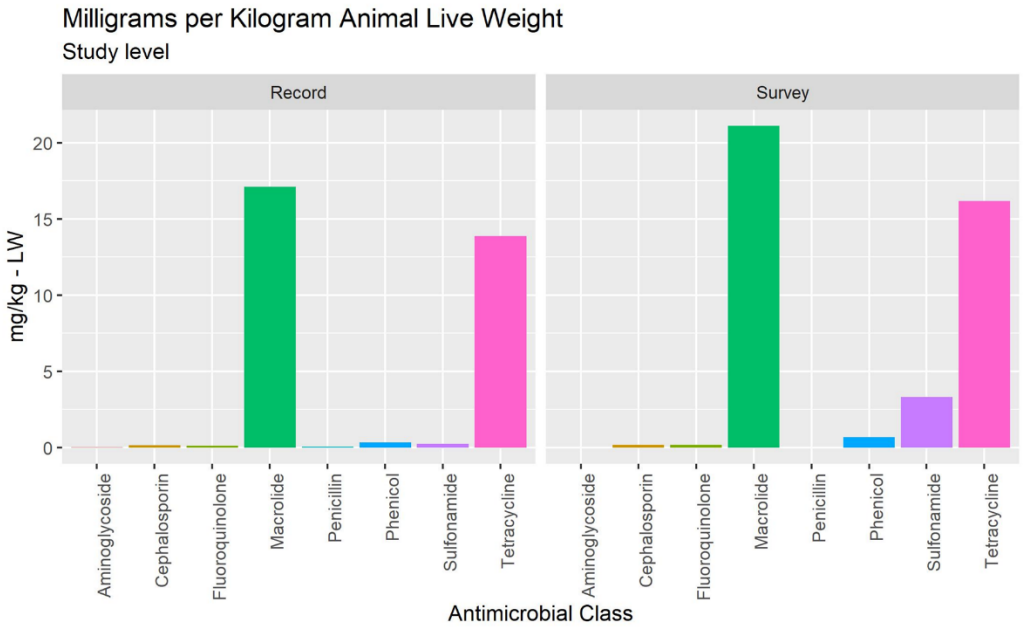

Figure 19 - Total Milligrams of Medically Important Antimicrobials per Kilogram Liveweight Sold Comparing Record to Survey by Drug Class at the Study Level

Total milligrams of medically important antimicrobials per kilogram liveweight sold by antimicrobial class within use category are shown at the study level in Figure 20 and Tables 24 and 25. Table 24 reports values while Table 25 reports the values as percentages of the total use for that column.

In-feed use showed the largest numerical difference of the three use categories with feedyard record data having a smaller value than survey data, 30.15 and 36.38 mg/kg-LW, respectively. Control of BRD values were 0.60 mg/kg-LW for feedyard record data and 0.38 mg/kg-LW for survey results. Individual animal treatment values were 1.10 and 4.91 mg/kg-LW from feedyard record data and survey results, respectively. For study level data, the survey data values expressed as a percent of feedyard record data values are 120.7%, 62.7%, and 449.4% for in-feed, control of BRD, and individual animal treatment use categories, respectively. A small

over-estimate of sulfonamide regimens in the survey is multiplied to a much larger difference in mg/kg-LW due to the high milligram regimens of the sulfonamides.

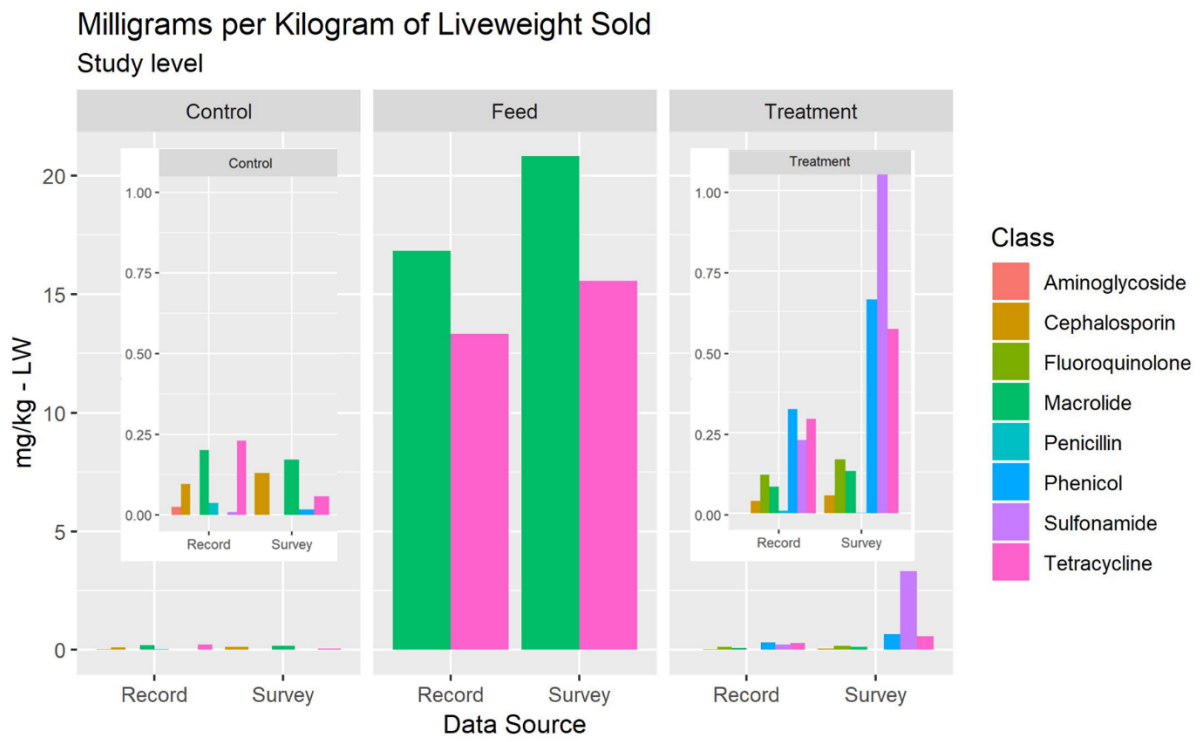

Figure 20 - Total Milligrams of Medically Important Antimicrobials per Kilogram Liveweight Sold Comparing Record to Survey by Use Categories at the Study Level. Insets for control and treatment use categories alter the Y axis for increased clarity between antimicrobial classes.

Feedlot Record Data and Survey Data Regimens per Animal Year of Medically Important Antimicrobials at the Feedyard Level

Regimens per Animal Year

Table 26 presents regimens of medically important antimicrobials comparing feedyard data to survey data at the feedyard level. The median for Reg/AY of medically important antimicrobials at the feedyard level as determined from feedyard record data is 3.2 as compared to 3.3 determined from survey data.

Table 26 - Comparison of Record and Survey Estimates of Regimens per Animal Year and Milligrams per Kilogram Liveweight Sold of Medically Important Antimicrobials at the Feedyard Level

Metric	Source	Study Level	Feedyard Level		
			Mean	Std Dev	Median
Reg/AY	Record	2.98	3.39	1.56	3.23
	Survey	3.75	3.69	1.80	3.34
mg/kg-LW	Record	31.85	47.70	32.08	41.80
	Survey	41.67	50.85	42.58	28.57

The differences in Reg/AY of medically important antimicrobial use by antimicrobial class, as calculated from feedyard record data and survey data, are shown in Table 27. The feedyard record data included the use of aminoglycosides by one feedyard and there were no survey reports of aminoglycoside use; these data are not included in Table 27. Penicillin use was not reported in enough surveys to allow calculation of estimates of variation to be presented in contrast to the feedyard record data; penicillin class values are also not included in Table 27.

Table 27 – Comparison of Record and Survey Estimates of Regimens per Animal year and Milligrams per Kilogram Live Weight of Medically Important Antimicrobials at the Feedyard level Reported by Antimicrobial Class

Metric	Class	Source	Study Level	Feedyard Level		
				Mean	*Std Dev*	*Median*
Reg/AY	Cephalosporin	Record	0.09	0.08	0.11	0.05
		Survey	0.14	0.12	0.16	0.04
	Fluoroquinolone	Record	0.05	0.06	0.05	0.05
		Survey	0.08	0.09	0.14	0.03
	Macrolide	Record	2.04	2.14	1.02	2.63
		Survey	2.65	2.37	1.06	2.57
	Phenicol	Record	0.03	0.05	0.08	0.02
		Survey	0.08	0.11	0.11	0.06
	Sulfonamide	Record	0.004	0.04	0.04	0.02
		Survey	0.03	0.14	0.12	0.13
	Tetracycline	Record	0.76	1.03	1.12	0.65
		Survey	0.77	1.08	1.19	0.44
mg/kg-LW	Cephalosporin	Record	0.14	0.12	0.16	0.07
		Survey	0.19	0.19	0.21	0.09
	Fluoroquinolone	Record	0.12	0.14	0.10	0.11
		Survey	0.17	0.20	0.31	0.09
	Macrolide	Record	17.10	20.17	11.57	20.98
		Survey	21.13	22.66	12.05	23.32
	Phenicol	Record	0.32	0.49	0.71	0.17
		Survey	0.68	1.24	1.81	0.66
	Sulfonamide	Record	0.24	1.75	1.95	0.77
		Survey	3.32	12.95	10.19	12.20
	Tetracycline	Record	13.86	26.04	29.68	15.44
		Survey	16.19	24.46	33.53	9.38

Milligrams of Medically Important Antimicrobials per Kilogram Liveweight Sold at the Feedyard Level

Table 26 illustrates the medians and means for milligrams of medically important antimicrobials per kilogram liveweight sold at the feedyard level comparing feedyard record data to survey data. The medians for total milligrams of medically important antimicrobials per kilogram liveweight sold for feedyard use were 41.8 and 28.6 for data derived (record) and survey derived values, respectively.

The values as determined by both feedyard data (record) and surveys at a feedyard level for total milligrams of medically important antimicrobials per kilogram liveweight sold characterized by antimicrobial class are shown in Table 27. The sulfonamide values are dramatically different between survey and feedlot record data values, with the survey respondents overestimating the amount of sulfa use which would be captured in their records for that year.

Comparison of Survey and Record Results

Methods

Evaluation of the relationship between the outcome metrics for feedyard and survey data was by utilizing basic quantitative assessments.

To calculate the correlation coefficients for feedyard use and survey response data, the total regimens per animal year and total milligrams per kilogram liveweight sold were summarized for the respective data sources by feedyard as matrices in R (version 3.5.1).[5] The correlation command

[5] R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

was then used to calculate correlation coefficients for the variables of survey and feedyard data sources for the values for regimens per animal year (Reg/AY) and milligrams per kilogram liveweight sold (mg/kg-LW) at the feedyard level.

A non-parametric two-sample rank sum test was also performed in R comparing feedyard use and survey response data for Reg/AY and mg/kg-LW. A non-parametric test was chosen due to the non-normal data distribution and related use of median versus mean.

Results

The correlation coefficient comparing feedyard use to survey results for Reg/AY is 0.605 which indicates a moderately strong positive correlation. When describing antimicrobial Reg/AY by use category, the reported correlation coefficients show more variation as compared to mg/kg-LW. The coefficient for control of BRD Reg/AY of medically important antimicrobials was 0.442. In-feed and individual animal treatment Reg/AY for medically important antimicrobials displayed correlation coefficients of 0.573 and 0.575, respectively.

Total medically important mg/kg-LW appears to have a similar positive relationship with a correlation coefficient of 0.780. Control of BRD and in-feed use mg/kg-LW correlations at the feedyard level yielded results of 0.634 and 0.770, respectively. Individual animal treatment mg/kg-LW had a correlation coefficient of 0.433.

Comparing Reg/AY values for feedyard use and survey response data using a Wilcoxon rank sum test resulted in a p-value of 0.3247 and a 95% confidence interval for the difference between the median values of -1.06 to 0.25. The comparison of mg/kg-LW yielded a p-value of 0.7019 and a 95% confidence interval of -14.49 to 10.387. The non-significant p-values with confidence intervals that include zero indicate that the null hypotheses that use metrics derived from feedyard data are equal to surveys has failed to be rejected for Reg/AY and mg/kg-LW.

Discussion

The interpretation of the relationship between feedyard use data and survey response data should be done with caution. The survey was administered to all responders by the same individual with good working knowledge of beef feedyard production. This experience allowed the survey administrator to ask appropriate follow-up questions when administering the survey instrument if the respondent's answer did not completely provide the needed information. Production system knowledge was instrumental in designing the survey instrument itself to allow for calculation of outcome parameters from a variety of combinations. However, this flexibility may not be possible if the survey administration is not versed in beef feedyard systems.

Some variation was removed for the denominator of the metric mg/kg-LW due to the total kg of liveweight sold for each year coming from the record data for that feedyard for both feedyard data and survey calculations. In contrast, both the numerator and denominator for Reg/AY came from their respective source, either feedyard data or the survey.

Recall bias should be acknowledged as well. The authors attempted to reduce recall bias by limiting the time period for survey administration to no more than six months past the study period in question.

The survey administration was designed to be completed in a reasonable time frame during a visit with the option for the respondent to ask other personnel or consultants for input on an estimated number. This approach represents a reasonable field approach to survey conduct and could be accurately replicated in the future.

Median values at the study and feedyard level may be interpreted as suggesting surveys are reasonably representative of medically important antimicrobial use. However, the correlation

results suggest that using survey data to rank feedyards according to extent of antimicrobial use, and especially to implement regulatory or mandatory reduction programs, could result in different classification as to rank.

The failure to reject the null hypothesis of no difference between survey and use data does not indicate that the two sources are in fact the same; rather, from this analysis, it is possible to say that this analysis failed to show that survey and record (data) results are different.

As this report has demonstrated, survey collected data can be a potentially useful tool. However, the collection and interpretation of antimicrobial use information from survey instruments are only as useful as the query behind them. As a tool for ranking beef feedyards in terms of antimicrobial use for potential sanctions or restrictions, survey collected antimicrobial data is not appropriate. However, for providing insight on antimicrobial use trends and antimicrobial use practices in beef feedyards, survey information may be a viable tool provided the administration and analysis are done appropriately.

References

1. Catry B, Laevens H, Devriese LA, et al. Antimicrobial resistance in livestock. *Journal of Veterinary Pharmacology and Therapeutics* 2003;26:81-93.

2. Pardon B, Catry B, Dewulf J, et al. Prospective study on quantitative and qualitative antimicrobial and anti-inflammatory drug use in white veal calves. *Journal of Antimicrobial Chemotherapy* 2012;67:1027-1038.

Chapter 5 - Data Collection Costs, Logistics, Time Commitments, and Scale-up Estimates

Introduction

Records of time commitments and financial resources were maintained by the study investigators to describe logistical requirements, financial investment, and the possibility of expansion of a program to quantify antimicrobial use in beef feedyards. In addition to the data collection and description portion of the FDA cooperative agreement, the authors tracked time and expenses related to feedyard recruitment, data handling, and analysis. Travel time and lodging expenses are associated with feedyard recruitment while time for data collection and entry were relevant once a participant had been enrolled in the project.

Feedyard recruitment began in 2017 and continued through 2018. Data collection occurred over the two year period of 2017 – 2018 with analysis and report preparation extending into 2019. The data collection and reporting efforts were focused on antimicrobial use in cattle which were sold in 2016 and 2017. Activities, procedures, and outcomes for the efforts involved are presented in chapters 2-4.

Resources Expended

Non-Participants

A total of 10 visits were made to nine feedyards which are not part of the final data set. Other time expenditures related to non-participants included initial inquiries through state cattle organizations and veterinarians, which included interactions through phone contacts and follow-up on potential participant status.

Participants

Twenty-eight visits to participating feedyards were conducted during the first 2 years of the study. Twenty-one of the 22 feedyards had a minimum of one visit.

- The survey was conducted during the only visit for 15 of the feedyards; this visit may have been a recruitment visit, or in some cases the data agreement had previously been signed.
- One feedyard had one visit for data collection and survey administration with a second visit for data collection for the second year.
- One feedyard had an initial visit for recruitment and survey administration with two additional visits for data collection.
- Two feedyards had an initial recruitment visit followed by a subsequent visit to conduct the survey.
- Two feedyards had an initial recruitment visit but schedules for conducting a subsequent visit for a survey were not successfully coordinated, and therefore a survey was not completed. The feedyards were still included in the data collection, but were not utilized in the comparison of data and surveys in Chapter 4.
- One feedyard was recruited, signed the data sharing agreement via electronic transfer, and provided records in a spreadsheet format via electronic transfer with no site visit required.

Expenses and time were tracked for traveling and data collection. All travel for the 40 visits (including participants and non-participants) was conducted by ground vehicle over a total

of 26.5 days; these days are calendar days and typically included substantially more than 8 hours. A total of 11,376 miles were recorded related to study travel, an average of 429 miles per visit day, which at an average speed (with stops) of 50 mph equates to approximately 8.5 hours of driving per day. Per diem and lodging costs associated with the visits were $4,011; some travel was billed to or split with veterinary meetings due to feedyard visits being conducted in route to or from the meeting. Expenses for these trips included per diem and room expenses for one person for approximately 50% of the visits and two people for the other visits.

Data collected from six of the enrolled feedyards were manually entered and aggregated due to a lack of electronic download capability. Time required for data entry ranged from one hour for a small feedyard with only purchase data available up to 60 hours for a larger feedyard with extensive individual animal treatment data. On average, approximately 30 minutes of data entry were required for each lot within a feedyard when manual entry was required. Lot-level information included individual animal antimicrobial treatment data, lot data, and processing data. Lots in this report are referring to an economic group of cattle that come in, are fed together, and leave the feedyard on the same timeframe. A total of approximately 155 person-hours were required over both years to enter data for the six feedyards requiring manual entry. All of the feedyards that required manual data entry and aggregation were on the smaller end of the size spectrum for the participating feedyards (<6000 hd per year).

Approximately 14 person-hours were used for entering ration inclusion data in relation to feed consumption across all feedyards. The procedure for ration entry can be made more efficient in subsequent years by including a ration inclusion information entry in the data input process.

Management of data in the database, creation of the database entry files, standardization of data, preparing output tables and charts, and report preparation used approximately 828 hours for

both years. Breaking out the hours between the categories is possible, but in reality much of the data management, standardization, output preparation, and generation of the report occurred at the same time. Creation of specific code and output formats would not need to be repeated unless a feedyard changes to a different record system type or moved from record systems that required manual entry to one with the capability of electronic data transfer. These hours also reflect a learning curve in the programs and methods used; more experienced personnel and/or an established system would likely reduce this time commitment. Time spent learning different software systems are not included in these estimates.

Overall estimate of resources utilized

An overall estimate of 842 hours of data entry, quality assurance, analysis, and report preparation time occurred for the 22 feedyards. The overall estimate of hours will continue to go up as each additional year of data is collected and aggregated.

Travel time, miles, and lodging/per diem expenses for the 22 participating feedyards averaged 1.4 days, 474 miles, and $167, respectively, for each feedyard. These values include resources required for visiting feedyards which did not participate in the study or elected to not continue participation after enrollment, as it is anticipated that expansion of the monitoring footprint would have similar proportions of non-participants among contacts.

Projections

To expand this program to include more feedyards, data management time estimates vary by the data structure available for each feedyard. Table 28 lists the variation between record systems types for the time needed to add a feedyard for each system; electronic system one, system

one combined with system two, system one combined with a custom system, and purchase records would require similar time for additional feedyards. The similarities across these types are due to the standardization and quality control necessary to ensure data quality. Custom record systems and manual entry yards require more time for data input. Custom systems can also have specific formatting issues that require time to alter to a format cohesive with the master dataset. Manual entry yards require additional time for data entry, but, alternatively, this also allows for information to be entered into a pre-set spreadsheet which can reduce manipulation of the data once in the dataset.

Table 28 - Time needed for additional feedyards by record system type

Record System Type	Time/Feedyard (hours)
Electronic system #1	8
Electronic system #2 combined with system #1	8
Electronic system #1 combined with custom system	8
Custom record system	12
Electronic system #3 - manual entry from provided records	15
Records for purchase or number treated - Manual entry	8

Conclusion

The information on the time and expenses spent on feedyard recruitment and data handling was requested to assist in potentially developing future feedyard data projects. The first two years of data collection at the level of individual animal use within feedyards required a substantial time

and financial investment. Any future scale-up efforts to increase participation would have a reasonably predictable cost.

It is estimated that the addition of new feedyards would on average require approximately 1.4 days of travel time covering 474 miles and requiring $167 in lodging and per diem per new participating feedyard enrolled. Travel time and cost may increase depending on recruitment success. The time needed for data management including data import, standardization, and quality control varies between eight and fifteen hours per feedyard. The estimate of time, distance, and cost can vary significantly from the given estimates due to feedyard location, size, record type, and consistency of data available. Continued collection of data at such a granular level requires a solid understanding of the industry and how the data should be interpreted once they are collected.

Chapter 6 - Microfluidic qPCR on Fecal Samples from Adult Beef Cows Fed Chlortetracycline Mineral

Tetracycline resistance genes in fecal samples from beef cattle administered chlortetracycline as part of a mineral formulation

Katie J. Hope

Randall Singer

Nora F. Schrag

Michael D. Apley

Introduction

The link between antimicrobial use in food-producing animals and antimicrobial resistant bacterial infections in human health is of great concern as food-producing animals are seen as a potential origin and disseminator of organisms of concern.[1] To address these concerns, on January 1, 2017, changes initiated with the release of The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals Guidance for Industry Document #209 took effect. One of the changes brought forth by the implementation of GFI 209 was to move the use of medically important antimicrobials in feed and water from over-the-counter (OTC) to under veterinary oversight in the form of veterinary feed directives (VFDs) and prescriptions, respectively.[2] A core responsibility of the veterinarian in authorizing the use of these products is the commitment to antimicrobial stewardship.

One of the medications that moved from OTC to VFD and prescription status was chlortetracycline (CTC). Tetracyclines make up approximately 64% of the weight of medically

important antimicrobials sold with food animal labels in the United States[3]. Chlortetracycline (CTC) is approved for use in feed for beef cattle for multiple diseases including bovine respiratory disease and control of active infection of anaplasmosis (Aureomycin®, Zoetis). When fed as a hand-fed feed for control of an active anaplasmosis infection in cattle over 700 pounds bodyweight, CTC is fed to provide 1.1 mg/kg (0.5 mg/lb) of body weight per day for the duration of treatment. A hand-fed feed is one that is given to an animal or a group of animals in a set amount each day.

A part of the practice of antimicrobial stewardship is the consideration by the veterinarian of whether the antimicrobial use may contribute to the selection of antimicrobial resistance genes. Resistance to tetracyclines can be attributed to the acquisition of one or more tetracycline (*tet*) resistance genes. Acquiring *tet* resistance genes provides at least one of several principal resistance mechanisms to the bacterium, those being ribosomal protection, efflux pumps, or enzyme inactivation.[4] The challenge is in evaluating the contribution of antimicrobial use to resistance selection pressure for these mechanisms in light of other factors such as diet and environment.

The animals in this study were part of a separate, concurrent study evaluating plasma pharmacokinetics of chlortetracycline administered as part of a mineral formulation appropriate for daily hand feeding. The aim of this study was to evaluate changes in quantity of resistance genes present in fecal samples collected during the administration of chlortetracycline in mineral in both a dry lot and pasture environment, with an emphasis on selected *tet* genes.

Materials and Methods

This protocol was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) as protocol #3912.

Cattle and Husbandry

This study was performed in two phases. In phase 1, cattle were placed in a dry lot setting. After completion of phase 1, the cattle were housed in a pasture setting to complete phase 2. The cattle included in this study were not administered any antimicrobials for a minimum of 45 days prior to initiation of treatments

Phase 1

A total of 15 adult cows, a subset of a larger group for a concurrent study, were housed in a dry lot with shared waterer, *ad libitum* grass hay, and daily supplementation with 6 pounds/animal of a mixture of 50% dry distillers grains and 50% ground corn. There was a six-week acclimation period prior to the study. The cows varied in age from 3 - 8 years and were bred for less than 30 days at the start of the study. Body weights ranged from 474 – 715 kg. To achieve accurate dosing, weights were recorded on study days 1, 7, 10, and 14 and the daily dose of CTC was adjusted to achieve 1.1 mg/kg (0.5 mg/lb) bodyweight per day.

Animals were randomized to three treatment groups of five animals each (Table 1).

1. Treatment C (control) - administered non-medicated mineral supplement.
2. Treatment B (bolus) - administered boluses containing medicated mineral at a dose of 1.1 mg/kg chlortetracycline per pound of bodyweight. Animals were rewarded with a small amount of grain mix post-dosing to ensure all boluses were successfully ingested.
3. Treatment D (dried distillers grains) - administered medicated mineral at a dose of 1.1 mg/kg chlortetracycline by mixing mineral with a minimal amount (<0.35 kg) of grain/distillers grains mix and feeding while the animal was in an individual pen and with an individual feed pan. Intake was confirmed before releasing the animal from the feeding pen.

Treatments were administered to each animal once daily. When treatments were complete, the animals were returned to group housing.

Treatments were administered from day 11 (beginning after sample collection) to day 18 followed by a two-week washout period. For the second set of treatment administrations, treatment B and D animals were assigned to the alternative CTC administration route as part of a crossover pharmacokinetic study. Schrag, et al. demonstrated no significant differences in plasma concentrations between the animals in this study given CTC mineral as bolus or in a grain mix.[5] Treatment administration for the second part of the crossover study began on day 41 (beginning after sample collection) and ended on day 55 for phase 1. (Table 29)

Phase 2

The 15 cows from phase one were moved to a native grass pasture setting with a period of no mineral access for approximately 45 days. The cows were then acclimated to free choice non-medicated mineral per product label for two weeks. After this acclimation period, mineral containing CTC at 6000 g/ton was hand fed in a mineral feeder daily beginning on study day 144 (beginning after sample collection) to all cows, including control group from phase 1. Once per day, the feeder was refilled so that it held enough mineral to treat the entire group at 1.1 mg CTC/kg bodyweight for that day. The cattle had the appropriate amount of medicated mineral provided daily for 21 days. (Table 29)

Table 29 - Treatment and dosing for phases 1 and 2

Treatment	Product Formulation	Chlortetracycline Daily Dose, mg/lb	Number of Animals	Dosage method
D	Medicated mineral mix containing 6000 g/ton chlortetracycline	0.5	5	Individually administered by mixing in 0.34 kg grain supplement.
B	Medicated mineral mix containing 6000 g/ton chlortetracycline	0.5	5	Individually administered by oral bolus.
C	Non-medicated mineral mix	0	5	Individually administered by mixing in 0.34 kg grain supplement
Mineral Feeder	Non- medicated mineral mix	0.5	15	Ad lib access to mineral feeder containing dose sufficient for treatment of entire group for one day. Mineral was dispensed to feeder daily.

Sample Collection

Fecal samples were collected for phase 1 on days 0 and 11 (pre administration of the first CTC dose), then days 18, 25, 32, 41 (pre administration of start of second CTC regimen), 48, and 55. In phase 2, fecal samples were collected on days 85, 144 (prior to CTC mineral made available), 151, 158, and 165. Table 30 describes the sampling days and treatments for both phases. Samples were placed in individual bags labeled by an animal ID number.

Table 30 - Sampling days for phase 1 and 2

Collection	Day	Treatments – from previous sample point to sample time	Number of Animals Sampled
Phase 1			
1	0	No Mineral	15
2	11	No Mineral	15
3	18	Treatment B, C, and D	15
4	25	Treatment B, C, and D	15
5	32	Non Medicated	15
6	41	Non Medicated	15
7	48	Treatment B, C, and D	15
8	55	Treatment B, C, and D	15
Phase 2			
9	85	No Mineral	15
10	144	Mineral Feeder - Non-medicated	15
11	151	Mineral Feeder -Medicated	15
12	158	Mineral Feeder -Medicated	15
13	165	Mineral Feeder -Medicated	15

Sample Handling

All samples were immediately placed on ice post-collection. After transportation back to the laboratory, the fecal samples were recorded in a collection log with the sample date and animal identification, and assigned a unique lab identification number. Once the sample information was recorded, approximately one gram of feces in duplicate was placed into a 5 mL collection tube. All fecal samples were then stored at -80° C until further laboratory analysis was performed.

Laboratory Methods

DNA extraction

DNA was isolated from 200 mg of each fecal sample using the QIAamp FastDNA® Stool Mini Kit for Stool and the FastPrep® Instrument (MP Biomedicals, Santa Ana, CA, USA)

following manufacturer instructions. The final DNA elution volume was 100 µL and was stored at -20°C until further analysis.

Primer selection and validation

A total of 44 antimicrobial resistance genes (ARG) representing different molecular mechanisms of resistance and different antimicrobial classes were targeted for this study. The 16S rRNA gene and integrons (*intl1*, *intl2*, and *intl3*) were also included. Primer sets and sequences for the gene standards were sourced from published literature [6] or designed from all known gene allele sequences downloaded from GenBank® [7] and CARD [8]. Primer sets and standards were chosen based on universal gene specificity, similar annealing temperature at or near 60°C, amplicon size of less than 300 base pairs, and less than 60% GC content. The primer sets and standard sequences designed for this study were created from alignment of gene allele sequences using the Basic Local Alignment Search Tool (BLAST, NCBI) with the conserved sequence used as input into the Integrated DNA Technologies PrimerQuest Tool (Integrated DNA Technologies, Coralville, IA, USA).

For each primer set, the resulting *in silico* amplicon plus 20 base pairs outward from the primer annealing sites at both 5' and 3' ends of the amplicon were used as the standard sequence. All standard sequences were synthesized using the gBlocks® Gene Fragments technology (Integrated DNA Technologies, Coralville, IA, USA). The 48 gBlock standards were individually quantified and pooled to prepare a 10-fold serial dilution of the qPCR standard mixture, with the final concentration of each gene ranging from $2x10^0$ to $2x10^6$ copies per µL. The complete list of ARG, primers, and standard sequences can be found in **Table S1.**

Microfluidic Quantitative PCR (MF-qPCR)

To simultaneously quantify the entire ARG array of each sample, microfluidic quantitative PCR (MF-qPCR) with the GE 96.96 Dynamic Array™ (Fluidigm, South San Francisco, CA, USA) was used. This is a high-throughput, highly sensitive method that relies on a microfluidic platform to run a greater number of reactions simultaneously than is possible in conventional qPCR. The MF-qPCR was performed on two technical replicates of DNA from each sample after pre-amplifying the target genes with a specific target amplification (STA). A more detailed explanation of this method can be found elsewhere. [6,9-11]

Data Analysis

Fluidigm Real-Time PCR Analysis software version 4.1.3 was used to extract and analyze the raw data from the MF-qPCR under default settings with the quality threshold set to 0.65 and Ct threshold to 0.1ΔRN. Using a customized Microsoft Access database (Microsoft Office 2016, Redmond, WA, USA), standard curves were generated for each gene using the original copy number before STA and the Ct values of the standard dilution reactions. The goodness-of-fit (adjusted R^2) and the amplification efficiency were calculated for each standard curve, resulting in an R^2 of ≥ 0.90 and amplification efficiencies ranging from 90 to 110% for all genes.

The Limit of Detection (LOD) of the assay was 2 copies per µL of DNA. Back-calculation to copies per gram of feces was performed by multiplying the number of copies in each sample by the DNA elution volume from the DNA extraction (100 µL) and multiplying that result by the amount of feces that was used for DNA extraction (0.2 g). The final quantitative value for each ARG in each sample was the arithmetic mean of the two technical replicates.

Statistical Methods

To evaluate the effect of the CTC treatment as well as the changes in gene quantity over time, a GLM procedure was utilized that included a between-subjects factor (treatment group) and a within-subjects factor (sampling date). Differences between consecutive sampling dates were assessed with difference contrasts. Assumptions of the models were evaluated with Box's M and Mauchly's test of sphericity. Significance levels were set at $\alpha = 0.05$ for all analyses. Standard statistical software was used (SPSS version 25.0, IBM Corp., Armonk, NY).

Results

A total of 180 samples were collected across all time points. Analyses were only performed for *tet*(A), *tet*(L), *tet*(M), *tet*(Q), *tet*(W), and *tet*(X) of the 44 ARGs at this time. There was 100% detection in all samples for the six *tet* ARGs analyzed. Timepoint values for each of the *tet* ARGs are illustrated in Figure 21.

There were no differences in the log10 ARG copies per gram noted between treatment and control groups at any of the sampling time points for *tet*(M), *tet*(Q), *tet*(W), or *tet*(X). There were no changes across study sampling times for *tet*(Q), *tet*(W), and *tet*(X) log10 copies per gram of feces.

Differences in the number of copies of *tet*(A) were statistically significant when treatment B was compared to treatment D ($p = 0.023$) and *tet*(L) had statistically significant different copies for treatment C compared to treatment D ($p = 0.042$).

Tet(M) had a statistically significant difference between all time points ($p < 0.05$) but did not show any significant differences in time by treatment group interaction. *Tet*(L) had significant time differences at sampling point 1 compared to 2 ($p=0.000$), sampling point 5 compared to 6

(p=0.002), and sampling points 9 to 10, 10 to 11, 11 to 12, and 12 to 13 (p = 0.000, 0.037, 0.000, 0.001, and 0.000, respectively).

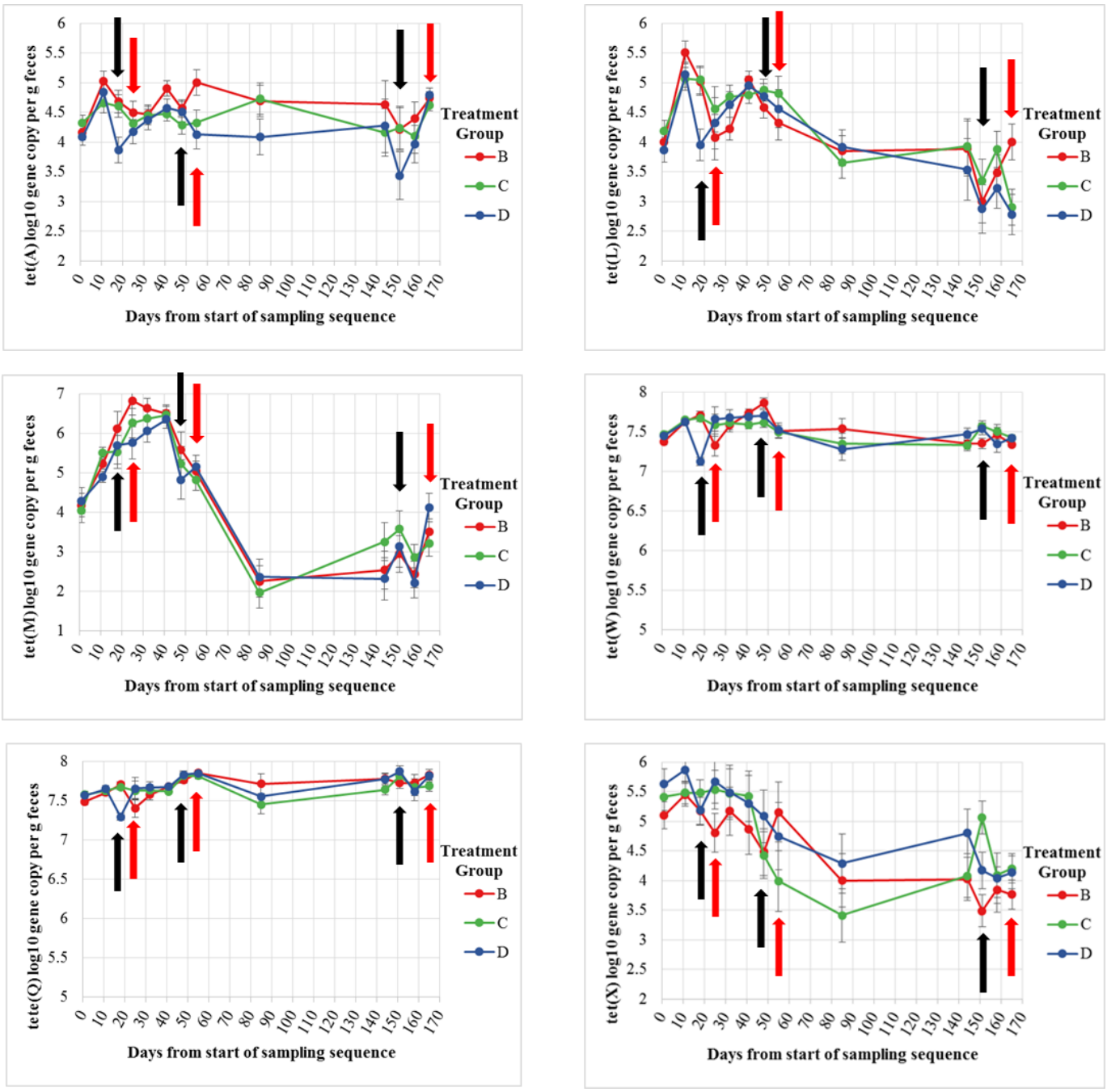

Figure 21 - log 10 copy per gram of feces for tet(A), tet(L), tet(M), tet(Q), tet(W), and tet(X) over time from beginning of sampling period through phase 1 and 2. The black arrow indicates the initiation of CTC administration and the red arrow indicates the cessation.

Discussion

This study investigated the changes in specific tetracycline resistance genes in the feces of adult beef cows following individual administration of chlortetracycline at 0.5 mg/lb in confinement, followed by group administration of chlortetracycline at the same daily target dose to the same cattle on pasture.

For *tet*(M), *tet*(Q), *tet*(W), and *tet*(X), the lack of a statistically significant difference between treatment and control groups reflects reports of variability and domination of the environment in resistance gene changes. Miller, et al. reported no difference in abundances of *tet*(A), *tet*(B), and *tet*(M) between fecal swabs and pen floor samples, and *tet*(B) and *tet*(M) abundances did not differ between occupied pens (control and treatment) and empty pens.[12] This is supportive of *tet* resistance genes in animals being heavily influenced by an environmental effect. Similarities in changes in *Enterococci* resistance to tetracyclines in samples from control and treatment groups in a study by Müller, et al. resulted in a comparable conclusion. In that study, feedyard calves fed tylosin with a high concentrate diet housed in separate pens at the same facility were evaluated for changes in the incidence of liver abscesses and presence of total antimicrobial resistance in isolated *Enterococcus* species. Resistance in the *Enterococci* isolates increased in the control and treatment group as time progressed. The change in both groups supports the hypothesis that the environment has a potential effect on the gastrointestinal flora.[13] It was also suggested that *tet*(Q), *tet*(M), *tet*(A), *tet*(Y), and *tet*(X) abundances found for cattle on a conventional dairy farm varied based on the individual animal. Certain resistance genes could also be found in soil samples on the dairy, but the authors did not report a list of genes found.[14]

The six tetracycline resistance genes analyzed in this study encode resistance in one of three ways: efflux pump, ribosomal protection, or enzymes. Two genes, *tet*(A) and *tet*(L), encode resistance by efflux pumps. Three genes, *tet*(M), *tet*(Q), and *tet*(W) convey resistance with ribosomal protection. One gene, *tet*(X), encodes enzymatic protection in a bacterium.[15] The enzymatic protection is achieved by oxidation of the tetracycline to various degradation products as described by Markley, et al.[16]

A preference for Gram positive or Gram negative bacterial species by specific *tet* genes could also contribute to the decline seen for *tet*(M) and *tet*(L) as study time points 8 and 9 coincided with the animals moving from dry lot housing to pasture along with a temporally associated change in ration. *Tet*(M) and *tet*(L) are potentially carried by a larger number of Gram positive bacterial genera than Gram negative.[15] The resistance genes *tet*(A) and *tet*(X) are only carried by Gram negative bacterial genera. *Tet*(Q) and *tet*(W) are carried by both Gram negative and Gram positive; however, they are attributed to a larger number of Gram negative genera. (Table 31)

Table 31 - Tetracycline resistance genes, tet(A), tet(L), tet(M), tet(Q), tet(W), and tet(X), and the associated Gram positive and Gram negative Genera [15]

Tetracycline resistance gene	**Gram Positive Genera (including *Mycobacterium, Mycoplasma, Nocardia, Streptomyces,* and *Ureaplasma*)**	**Gram Negative Genera**
tet(A)	None identified	*Laribacter, Bordetella, Ochrobactrum, Plesiomonas, Variovorax, Acinetobacter, Aeromonas, Alcaligenes, Chryseobacterium, Citrobacter, Edwardsiella, Enterobacter, Escherichia, Flavobacterium, Klebsiella, Morganella, Proteus, Pseudomonas, Rahnella, Rhizobium, Riemerella, Salmonella, Serratia, Shigella, Veillonella, Vibrio*
tet(L)	*Geobacillus, Oceanobacillus, Pediococcus, Ronbinsoniella, Vagococcus, Virgibacillus, Savagea, Actinomyces, Bacillus, Bifidobacterium, Bhargavaea, Clostridium, Enterococcus, Lactobacillus, Listeria, Mycobacterium, Nocardia, Paenibacillus,*	*Mannheimia, Orchrobactrum, Variovorax, Acinetobacter, Actinobacillus, Aeromonas, Citrobacter, Enterobacter, Escherichia, Flavobacterium, Fusobacterium, Gallibacterium, Klebsiella, Kurthia, Morganella, Myroides, Pasteurella,*

	*Peptostreptococcus, Sporosarcina, Staphylococcus, Streptococcus, Streomyces, Trueperell*a	*Proteus, Rahnella, Salmonella, Veillonella, Vibrio*
tet(M)	*Abiotrophia, Afipia, Amycolatopsis, Anaerococcus, Bacterionema, Brachybacterium, Catenibacterium, Cottaibacterium, Erysipelothrix, Finegoldia, Helcococcus, Mycoplasma, Aerococcus, Arthrobacter, Gardnerella, Gemella, Granulicatella, Lactococcus, Savagea, Actinomyces, Bacillus, Bifidobacterium, Bhargavaea, Clostridium, Corynebacterium, Enterococcus, Eubacterium, Lactobacillus, Listeria, Microbacterium, Mycobacterium, Nocardia, Paenibacillus, Peptostreptococcus, Sporosarcina, Staphylococcus, Streptococcus, Streptomyces, Trueperella*	*Eikenella, Hafnia, Kingella, Ralstonia, Dialister, Acinetobacter, Aeromonas, Alcaligenes, Bacteroides, Citrobacter, Edwardsiella, Enterobacter, Escherichia, Flavobacterium, Fusobacterium, Haemophilus, Klebsiella, Kurthia, Lawsonia, Morganella, Neisseria, Pantoea, Pasteurella, Photobacterium, Proteus, Providencia, Pseudoalteromonas, Pseudomonas, Psychrobacter, Rahnella, Rhizobium, Riemerella, Salmonella, Selenomonas, Serratia, Shewanella, Shigella, Stenotrophomonas, Veillonella, Vibrio*
tet(Q)	*Ruminococcus, Gardenella, Mobiluncus, Clostridium, Eubacterium, Lactobacillus, Streptococcus*	*Capnocytophaga, Anaerovibrio, Bacteroides, Fusobacterium, Mitsuokella, Neisseria, Porphyromonas, Prevotella, Reimerella, Selenomonas, Subdolgranulum, Veillonella*
tet(W)	*Collinsella, Roseburia, Purvimonas, Actinomyces, Bacillus, Bifidobacterium, Clostridium, Corynebacterium, Lactobacillus, Staphylococcus, Streptococcus, Streptomyces, Trueperella*	*Acidaminococcus, Acinetobacter, Bacteroides, Brevendimonas, Butyrivibrio, Chryseobacterium, Citrobacter, Escherichia, Fusobacterium, Klebsiella, Lawsonia, Megasphaera, Mitsuokella, Neisseria, Porphyromonas, Prevotella, Pseudomonas, Rhizobium, Selenomonas, Shewanella, Subdolgranulum, Veillonella*
tet(X)	None identified	*Comamonas, Delftia, Epilithonimonas, Springobacterium, Wautersiella, Acinetobacter, Bacteroides, Enterobacter, Escherichia, Klebsiella, Myroides, Pseydomonas, Riemerella, Salmonella, Serratia, Vibrio*

The effects of the feedyard production system on ARG pressure have been evaluated. A longitudinal study by Noyes, et al. followed feedyard cattle through the beef production system.[17] The samples collected provided information on several hundred ARGs with diversity of the resistome decreasing significantly through the feeding period. The reduction in diversity was noted by the loss of certain ARGs, especially those that conveyed resistance to antimicrobials not utilized in the study animals. However, ARGs that encoded resistance to the tetracyclines and macrolides, both of which were used in the study, remained prevalent. An interesting observation

of the ARGs was the lack of difference across the samples derived from 2 non-adjacent pens in each of 4 different feedyards. The feedyards were all conventionally managed and employed similar in-feed and parenteral treatment practices which could have contributed to similar background environment effects across the feedyards. All pens enrolled in the study were given in-feed tylosin, but individual parenteral use was minimal and varied between pens. The authors emphasized the possibility of environmental factors based on historical antimicrobial exposure contributing to similarities across pens and feedyards.

The effects of changes in diet, environment, and method of CTC administration between phase 1 and phase 2 are important to consider. Jacob, et al. looked at the effects of feeding wet corn distillers grains on antimicrobial susceptibilities in feedyard cattle.[18] The authors looked at antimicrobial resistance patterns in *Escherichia coli* O157, *Salmonella* spp, generic *Escherichia coli*, and *Enterococcus* spp and found no difference between treatment groups. Two resistance genes, *tet*(M) [tetracycline] and *erm*(B) [macrolide] were also quantified from fecal samples; their concentrations were not different between 0% wet corn distillers grains or 25% wet corn distillers grain.

Another study conducted by Edrington, et al looked at the effect of distillers grains on prevalence of *E. coli* O157 and *Salmonella*.[19] A decrease in the prevalence of *Salmonella* in the feces was noted in cattle fed distillers grains compared to corn only after 132 days on feed when fed with dry rolled corn, with no treatment difference for *E. coli* O157. The authors did not perform any antimicrobial susceptibility testing on the *Salmonella*. The results presented in these two studies supports the hypothesis that while a dietary change may have contributed to the decrease in number of copies of *tet*(M) and *tet*(L) when moved from a dry lot to pasture, an environmental component should not be ruled out. The amount of distillers grains and corn fed to

the 15 cows during the study was less than a typical feedyard concentrate diet. The changes in the number of copies per gram of *tet*(M) are potentially related to environment and diet as the number of copies began increasing prior to CTC administration (day11) and decreased significantly once the cattle were moved to pasture.

Interpretation of changes in abundances of *tet* ARGs in this study should be done with caution as there are limitations in the study design. The treatment and control groups were housed together throughout the study period. This could contribute to a non-detectable difference of ARGs between groups due to the shared environment, including feed and water. During the transition from dry lot housing to pasture, the dosing manner of CTC was also changed in addition to the discontinuation of the distillers grains/corn concentration supplement. The cows were individually dosed during phase 1 but were offered free choice consumption of the CTC in mineral once on pasture. Schrag, et al. showed that during this time period, the CTC intake was sporadic with timepoints that some animals had undetectable serum CTC levels.[5] The effects of environment, diet, and change in administration of CTC are intertwined and cannot be attributed separately in this study. However, the information presented provides a basis for future research. There is information available on chlortetracycline use in feedyard cattle in relation to ARGs, but there are limited data available on adult beef cows and resistance selection pressure.

The results presented here suggest potential effects of environment, ration, and method of CTC administration on the concentration of select *tet* resistance genes in the feces of cattle. The tetracycline resistance gene *tet*(M) appears to have responded to CTC exposure in conjunction with concentrate feeding in a dry lot environment with an expansion of prevalence, followed by rapid decline after removal of concentrate supplementation and movement to pasture with group administration of CTC. These changes could be associated with a high physiologic cost of

maintaining function of the *tet*(M) resistance mechanism and could also be associated with a bacterial population change in which the organisms harboring the *tet*(M) gene declined in population.

These results suggest that resistance gene selection in confinement and on pasture should be evaluated in separate studies and that extrapolation between the two environments should be avoided. This study illustrates the difficulties in assigning resistance selection pressure to specific antimicrobial use regimens, as defined by antimicrobial use metrics, as diet and environmental changes likely contributed as well. The biologic significance of these changes related to both animal and human health have yet to be determined.

References

1. Department of Health and Human Services Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013, Accessed 8-15-2019 at, https://www.cdc.gov/narms/resources/threats.html
2. Administration FaD. Guidance for Industry—Veterinary Feed Directive Regulation Questions and Answers (revised). 2015. *FDA guidance for industry* 2015.
3. Food and Drug Administration, Center for Veterinary Medicine. Antimicrobials sold or distributed for use in food-producing animals in 2017, 2018, Accessed 8-15-2019 at, https://www.fda.gov/animal-veterinary/cvm-updates/fda-releases-annual-summary-report-antimicrobials-sold-or-distributed-2017-use-food-producing
4. Koo HJ, Woo GJ. Distribution and transferability of tetracycline resistance determinants in Escherichia coli isolated from meat and meat products. *Int J Food Microbiol* 2011;145:407-413.
5. Schrag NH, K.; Apley, M.D.; Coetzee, J.F.; Magin-Bissel, G.; Olson, K.C. Plasma concentrations of chlortetracycline achieved by administration as part of a mineral formulation for beef cattle. *in preparation* 2019.
6. Sandberg KD, Ishii S, LaPara TM. A microfluidic quantitative polymerase chain reaction method for the simultaneous analysis of dozens of antibiotic resistance and heavy metal resistance genes. *Environmental Science & Technology Letters* 2017;5:20-25.
7. Benson DA, Karsch-Mizrachi I, Lipman DJ, et al. GenBank. *Nucleic acids research* 2008;37:D26-D31.
8. McArthur AG, Waglechner N, Nizam F, et al. The comprehensive antibiotic resistance database. *Antimicrobial agents and chemotherapy* 2013;57:3348-3357.

9. Ahmed W, Zhang Q, Lobos A, et al. Precipitation influences pathogenic bacteria and antibiotic resistance gene abundance in storm drain outfalls in coastal sub-tropical waters. *Environment international* 2018;116:308-318.
10. Ishii S, Segawa T, Okabe S. Simultaneous quantification of multiple food-and waterborne pathogens by use of microfluidic quantitative PCR. *Appl Environ Microbiol* 2013;79:2891-2898.
11. Zhang Q, Ishii S. Improved simultaneous quantification of multiple waterborne pathogens and fecal indicator bacteria with the use of a sample process control. *Water research* 2018;137:193-200.
12. Miller E, Vikram A, Agga GE, et al. Effects of In-Feed Chlortetracycline Prophylaxis in Beef Cattle on Antimicrobial Resistance Genes. *Foodborne Pathog Dis* 2018;15:689-697.
13. Muller HC. Effects of Intermittent Feeding of Tylosin Phosphate during the Finishing Period on Feedlot Performance, Carcass Characteristics, Antimicrobial Resistance, and Incidence and Severity of Liver Abscesses in Steers. *Journal of Animal Science* 2018;96:2877.
14. Kyselkova M, Jirout J, Vrchotova N, et al. Spread of tetracycline resistance genes at a conventional dairy farm. *Front Microbiol* 2015;6:536.
15. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* 2001;65:232-260.
16. Markley JL, Wencewicz TA. Tetracycline-inactivating enzymes. *Frontiers in microbiology* 2018;9.
17. Noyes NR, Yang X, Linke LM, et al. Resistome diversity in cattle and the environment decreases during beef production. *Elife* 2016;5:e13195.
18. Jacob ME, Fox JT, Narayanan SK, et al. Effects of feeding wet corn distillers grains with solubles with or without monensin and tylosin on the prevalence and antimicrobial susceptibilities of fecal foodborne pathogenic and commensal bacteria in feedlot cattle. *J Anim Sci* 2008;86:1182-1190.
19. Edrington TS, MacDonald JC, Farrow RL, et al. Influence of wet distiller's grains on prevalence of Escherichia coli O157:H7 and Salmonella in feedlot cattle and antimicrobial susceptibility of generic Escherichia coli isolates. *Foodborne Pathog Dis* 2010;7:605-608.

Chapter 7 - Conclusion

If current trends hold true, our ability to treat infectious diseases in both human and veterinary medicine will be severely restricted as more pathogens emerge with resistance to multiple antimicrobial drug products. The purpose of this dissertation was to provide a beginning for more in-depth research into the correlation between antimicrobial use and resistance selection pressure. Defining and describing regimens provides additional information that is not gathered in sales or defined dose metrics. Regimens allow for more detailed description of the treatment route, duration of treatment, number of animals exposed to a specific product and amount, and formulation of product. The information provided in regimens allows for exploration of what process or processes potentially contribute more to selection pressure.

Data collection should begin with a goal. If the goal is to monitor antimicrobial use trends over time, then less granular data may be required such as national sales data over a population denominator. For programs that would aim to reduce antimicrobial use by applying punitive measures, antimicrobial use should be tracked at the individual farm level with health data included. Including health data would allow animal welfare to be tracked as well since reduction of antimicrobial use at the cost of welfare is unacceptable.

Relating antimicrobial use to antimicrobial resistance is difficult. To develop a data collection program that would provide the necessary information is an ambitious process, but may be done with more information on antimicrobial resistance development and propagation in an established microbiome.

To acquire more granular data than national sales data requires a time intensive effort due to the widespread types of production systems, geography, and record systems. The time needed to gather and aggregate antimicrobial use data from a statistical sample is possible, but would

require a solid financial backing. In the United States, there is currently no government mandated reporting at the veterinarian or producer level on antimicrobial use. However, in countries that have very active antimicrobial collection programs, that reporting is mandatory. Denmark is an example of government-supported antimicrobial use collection due to longevity of the current program. Without antimicrobial use reporting being mandatory, 100% participation may not be achievable. An alternative to government mandated reporting that is more realistic would be when reporting is required as the basis for gaining access to certain markets. As consumer groups become more active in requesting transparency about antimicrobial use practices in food-animal production, large corporations are providing antimicrobial use policy requirements in order to be allowed to be a supplier. The trend is progressing towards greater availability of antimicrobial use information for the consumer; this means animal production systems must be able to justify why antimicrobials are utilized in their facilities. The impact on animal welfare should not be forgotten in the midst of consumer and supply chain demands. Evaluating antimicrobial use to determine if it is necessary for animal health or if the use is performed as a habit can promote better practices for all involved. This opens the door for informative discussions throughout production systems and offers the chance to share knowledge. Confidential benchmarking may be the key to discussions.

Further research in this area should focus on determining the impact of antimicrobial use on antimicrobial resistance selection. Selection pressure could be evaluated in relation to duration of treatment, route of antimicrobial administration, or environmental impact on the animal microbiome with and without additional antimicrobial use. Properly administered surveys could also be a useful tool for acquiring antimicrobial use estimates as long as the interpretation limitations are completely understood.

In light of the information presented in Chapter 1 through 6, the process of accessing, aggregating, and combining multiple record-keeping systems requires the understanding of the complex undertaking required to produce a single outcome measure. The danger lies in using that single number without understanding the nuances and assumptions throughout the process.

As dire as the predictions sound, the problem of antimicrobial use and resistance selection has provided a unique opportunity to unite specialists from all areas to work together. By combining data scientists, human and veterinary clinicians, and researchers, the ability to provide answers increases substantially.

Table S1. Complete list of the array with the antibiotic resistance genes (ARG), primer sequences, and references.

Gene Name	Forward (5'→3')	Reverse (3'→5')	Reference
16S rRNA	CCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGG	Muyzer et al., 1993
aacA	GTGTAACACGCAAGCACGAT	AGCCTCCGCGATTTCATAC	Szczepanowski et al., 2009
aadA5	ATCTTGCGATTTTGCTGACC	TGTACCAAATGCGAGCAAGA	Szczepanowski et al., 2009
ampC	CCTCTTGCTCCACATTTGCT	ACAACGTTTGCTGTGTGACG	Szczepanowski et al., 2009
bla$_{KPC}$	GATACCACGTTCCGTCTGG	GCAGGTTCCGGTTTTGTCTC	Hindiyeh et al., 2008
bla$_{NPS}$	GGACCATCGTCATCGAGTCT	ATTCGCAATCGAATACTGGG	Szczepanowski et al., 2009
bla$_{OXA}$	TGATGATTGTCGAAGCCAAA	GCCTGTAGGCCACTCTACCC	Ross et al., 2015
bla$_{SHV}$	AACGGAACTGAATGAGGCGCT	TCCACCATCCACTGCAGCAGCT	Chia et al., 2005
bla$_{VIM}$	CGCAGCTTTCTGGTTGGTAT	CGTGTCACCGAGTTTCTGAG	Szczepanowski et al., 2009
bla$_{CMY}$	ACTCCGGGCGCTAAGCGACTTTAC	CGCCAATACGCCAGTAGCGAGAC	Johnson et al., 2011
bla$_{CTX}$	AGCGGCAGTCGGGAGGCAGAC	GCCCGGAATGGCGGTGTTTA	Johnson et al., 2011
bla$_{IMP}$	AAGTTAGTCAMTTGGTTTGTGGAGC	CAAACCACTACGTTATCTKGAGTGTG	Calderaro et al., 2017
bla$_{NDM-1}$	TGACGCGGCGTAGTGCTCAGTGT	GCGGCGGGGATTGCGACTTAT	Johnson et al., 2011
bla$_{PER-2}$	CCGTGGTAGCAAATGAAGCG	ACCGGTTTTATGCGCCACTA	Johnson et al., 2011
bla$_{TEM}$	CCGTGTCGCCCTTATTCCCTTTTT	GCTCTTGCCCGGCGTCAACAC	Johnson et al., 2011

Gene Name	Forward (5'→3')	Reverse (3'→5')	Reference
dfr13	AATCGGTCCGCATTTATCTG	TTGGTAAGGGCTTGCCTATG	Szczepanowski et al., 2009
ermB	GATACCGTTTACGAAATTGG	GAATCGAGACTTGAGTGTGC	Chen et al., 2017
ermF	CGACACAGCTTTGGTTGAAC	GGACCTACCTCATAGACAAG	Ma et al., 2011
floR	TCGTCATCTACGGCCTTTTC	CTTGACTTGATCCAGAGGGC	Szczepanowski et al., 2009
intI1	CCTCCCGCACGATGATC	TCCACGCATCGTCAGGC	Goldstein et al., 2001
intI2	GACGGCTACCCTCTGTTATCTC	TGCTTTTCCCACCCTTACC	Barraud et al., 2010
intI3	GGATGTCTGTGCCTGCTTG	GCCACCACTTGTTTGAGGA	Barraud et al., 2010
mcr-1	ACACTTATGGCACGGTCTATG	GCACACCCAAACCAATGATAC	Bocanegra-Ibarias et al., 2017
mecA	AAAAAGATGGCAAAGATATTCAA	TTCTTCGTTACTCATGCCATACA	Szczepanowski et al., 2009
mecC	GCAAGCAATAGAATCATCAGACAA	CGATTCCCAAATCTTGCATACC	This study
qacG	TGGTTATTTCTGGCTACGGC	TTTGAGTGTCAGCGACAGGA	Cummings et al., 2010
qnrB	AAATATGGCTCTGGCACTCG	CTTTCAGCATCGCACGACTA	Szczepanowski et al., 2009
qnrS	GACGTGCTAACTTGCGTGAT	TGGCATTGTTGGAAACTTG	Marti et al., 2013
strB	CGCAGTTCATCAGCAATGTC	GCCTGTTTTTCCTGCTCATT	Szczepanowski et al., 2009
sul1	CCGTTGGCCTTCCTGTAAAG	TTGCCGATCGCGTGAAGT	Heuer et al., 2007
sul2	GACAGTTATCAACCCGCGAC	GTCTTGCACCGAATGCATAA	Szczepanowski et al., 2009

Gene Name	Forward (5'→3')	Reverse (3'→5')	Reference
sul3	TCCGTTCAGCGAATTGGTGCAG	TTCGTTCACGCCTTACACCAGC	Pei et al., 2006
tet(A)	GCTACATCCTGCTTGCCTTC	CATAGATCGCCGTGAAGAGG	Ng et al., 2001
tet(B)	AGTGCGCTTTGGATGCTGTA	AGCCCCAGTAGCTCCTGTGA	Looft et al., 2012
tet(C)	TGTTTCGGCGTGGGTATG	CATTAGGAAGCAGCCCAGTAG	This study
tet(L)	TCGTTAGCGTGCTGTCATTC	GTATCCCACCAATGTAGCCG	Ng et al., 2001
tet(M)	GTGGACAAAGGTACAACGAG	CGGTAAAGTTCGTCACACAC	Ng et al., 2001
tet(Q)	CGCCTCAGAAGTAAGTTCATACACTAAG	TCGTTCATGCGGATATTATCAGAAT	Looft et al., 2012
tet(S)	CAAGGATTGTACGGTTGGAAA	TTTCGAAGCTAAGATATGGCTC	Szczepanowski et al., 2009
tet(W)	GAGAGCCTGCTATATGCCAGC	GGGCGTATCCACAATGTTAAC	Aminov et al., 2001
tet(X)	AGCCTTACCAATGGGTGTAAA	TTCTTACCTTGGACATCCCG	Ghosh et al., 2009
vanA	GTAGGCTGCGATATTCAAAGC	CGATTCAATTGCGTAGTCCAA	Bell et al., 1998
vanB	TTGCATGGACAAATCACTGG	GCTCGTTTTCCTGATGGATG	Graham et al., 2008
vatB	GGAAAAAGCAACTCCATCTCTTGA	TCCTGGCATAACAGTAACATTCTGA	Looft et al., 2012
vatC	CGGAAATTGGGAACGATGTT	GCAATAATAGCCCCGTTTCCTA	Looft et al., 2012
vatE	GACCGTCCTACCAGGCGTAA	TTGGATTGCCACCGACAATT	Looft et al., 2012
vgbB	CAGCCGGATTCTGGTCCTT	TACGATCTCCATTCAATTGGGTAAA	Looft et al., 2012