

Measurements of antibiotic use in United States dairy systems

by

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B.S., Manchester College, 2002
DVM, Kansas State University, 2006

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

Methods of measuring antibiotic use were explored using data collected from a convenience sample of 29 US dairy farms for the calendar years of 2016 and 2017. Multiple on-farm data sources included handwritten records and four software systems. Data from each source were standardized to a common format for analysis. Antimicrobial use is reported as regimens per cow year with the regimens defined at the product level by route of administration, number of administrations, time frame between first and last administration, grams per regimen, and grams per administration. The primary goal of this study was to identify which measures might be useful for guiding stewardship at the farm level, and to describe the nuances associated with different methods of measurement. For this reason, care was taken to maintain the association between use and the reason for use (detected disease) for all measures reported.

This method of reporting provided enough granularity to compare different methods of antimicrobial use measurement. Measurements of overall use by regimens per cow year indicate that most use occurs as intramammary treatments and cephalosporins are the most commonly used antibiotic class. Use measured by grams per cow year indicates that both Dry Cow therapy and non-intramammary (Other Treatments) contribute significantly to overall use, and use in the penicillin class predominates. Comparisons of measures by regimens, grams, defined daily doses (DDD), defined course doses (DCD) and days of therapy (DOT) are evaluated at the farm level. With the exception of grams, all comparisons of these measures have correlations greater than 0.7 for all categories of use. Potency confounds measures of active substance weight causing use measured as grams of antimicrobial to not correlate as well with other measures (correlations <0.5). This lack of correlation is especially apparent for dry cow therapy where the correlation between grams and other measures is -0.06.

These comparisons raise questions about the relationship between use measures and resistance selection pressure. Further study is needed to identify the exact relationship between use measures and resistance selection pressure, as well as to identify treatment outcome parameters that should be assessed to ensure that animal welfare is appropriately monitored as antimicrobial stewardship is advanced.

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Approved by:

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This report is a collaborative project encompassing the ideas of an entire team of people dedicated to improvement of antimicrobial stewardship. Thank you to everyone who may not have been mentioned above but also contributed.

Dedication

This report is dedicated to the Cows who were productive for subsequent lactations after being appropriately treated with antibiotics, to the veterinarians who prescribed the treatment, and to the herdsman who noticed they were sick and treated them.

Preface

Comparison of Apples and Oranges



This document reports data from the first 2 years of a 5 year project designed to explore options for antimicrobial use monitoring in dairy systems. The long term goal of this project is to inform and support the continued advancement of antimicrobial stewardship within United States dairy production systems.

It is important to recognize that in some ways this report compares apples and oranges. It provides descriptions of antibiotic use where all treatment regimens and all milligrams (mg) of many drugs are added together and presented as a totaled value. Significant care was taken to describe the differences between regimens and drug classes by fully describing the characteristics of the regimens such as number of administrations, total mg per regimen, route of administration, and regimen time frame. Nonetheless, the totals were generated. While the extra details of regimen parameter descriptions are helpful in allowing interpretation, there are times when the totaled values are useful and times when they are not.

These totaled values are not dissimilar from reporting total kilograms or total surface area of apples, oranges, and other fruits in a grocery store. It is an accurate description, but without other information it says nothing about the quality or value of the fruit. It is simply a measure of the amount. It is extremely important to recognize that a total measure, no matter how accurate, cannot be used as an assessment of “appropriateness” (quality) or “judiciousness” (value). Such assessments require detailed understanding of the entire production system including measures taken to prevent disease, methods of disease detection and diagnosis, and continued assessment of treatment outcomes specific to the location. This means that to create a cut point on any measure of total use and define one side as “unacceptable” is entirely unjustified.

Antimicrobial use in dairy systems is aimed at instances of detected, predicted, or historical disease. In order to avoid jeopardizing animal welfare, attempts to reduce use should seek to decrease disease incidence when disease is present, increase the accuracy of disease detection or prediction, and provide tools for validating or invalidating the current existence of historic disease. Perhaps at some point, a sacrifice of animal welfare is necessary for an improvement of human health. However, if priorities for reduction are appropriately implemented, strategic improvement in our ability to manage infectious disease could reduce resistance selection pressure caused by antimicrobial use while preserving animal welfare. It is our hope that this report serves as a springboard for identifying research priorities associated with the management of bacterial disease on dairies, and thus reveals a path forward allowing antimicrobial use reduction that improves both human and animal health outcomes.

Chapter 1 - Overview of Dairy Antimicrobial Use Monitoring

History

The current era of antimicrobial use began after the characteristics of penicillin were first described and promoted for medical use by Alexander Fleming in 1929.¹ Though it was several years before penicillin was commercially available, this discovery and subsequent discoveries of other antimicrobials allowed western medicine to advance rapidly, while relying on antimicrobials to cure both natural infections as well as those iatrogenically introduced during surgery and other procedures.

The potential utility of antimicrobials for livestock production was quickly recognized. Some initial experiments using penicillin for the treatment of mastitis were published in 1944.² By 1946, there were descriptions of intramammary treatment for *Streptococcus agalactiae* and *Staphylococcus aureus*.^{3,4} Penicillin resistance of chronic staphylococcal mastitis was described as early as 1947.⁵ Farmer-applied treatment with penicillin was documented in 1948⁶, and that same year sulfanilamide was used in combination with penicillin for mastitis.⁷ In 1949, a combination of streptomycin and penicillin was used for staphylococcal and streptococcal mastitis.⁸ Additionally, herd eradication of *Strep. agalactiae* was described by treating all cows with 5 doses of 100,000 units of intramammary penicillin. This marked the beginning of herd treatments for the control of streptococcal mastitis.⁹ Today, data from 2014 indicate that antimicrobials requiring a withdrawal period were administered to cattle on 91.3 percent of dairy operations.¹⁰

Increased awareness of antimicrobial resistance has raised questions concerning the appropriateness of antimicrobial use in dairy livestock systems. This is not actually a new issue. Early scientists warned that exposure to antimicrobials can lead to resistance, and Alexander Fleming was among those. When speaking at a dinner party about the potential of oral forms of penicillin, he is quoted by the New York Times to have said: "The greatest possibility of evil in self-medication is the use of too small doses, so that, instead of clearing up the infection, the microbes are educated to resist penicillin and a host of penicillin-fast organisms is bred out which can be passed on to other individuals and perhaps from there to others until they reach someone who gets a septicemia or a pneumonia which penicillin cannot save. In such a case the thoughtless person playing with penicillin treatment is morally responsible for the death of the man who finally

succumbs to infection with the penicillin-resistant organism. I hope this evil can be averted." – Alexander Fleming 1945¹¹

This “possibility of evil” has come in and out of the public policy arena. In 1969, the famous Swann Report¹² was presented to the British Parliament evaluating the potential dangers of antimicrobial use in livestock. In the 1970’s, a task force was created by the Food and Drug Administration (FDA) to examine the safety of use of antimicrobials for growth promotion.¹³ The recommendations of this task force included making veterinary antimicrobial use prescription only and curtailing the use of antibacterials in feeds for growth promotion and disease prevention. In 1977, the director of the Human Resources Division of FDA made a report to the subcommittee on oversight and investigations. In this report, he pointed out that few of the task committee recommendations to the FDA concerning antimicrobial use had been implemented.¹⁴ It was not until April of 2012 when Guidance for Industry (GFI #209) was finalized that a national regulatory policy mentioned resistance concerns associated with certain uses of antimicrobials.¹⁵ Action on that policy came with the complete implementation of FDA GFI #213 in January of 2017.¹⁶ This removed previously approved production claims (e.g., increased feed efficiency, growth promotion) from the labels of medically important antimicrobials administered in the feed and water of food-producing species, and brought remaining uses under veterinary oversight. These antimicrobials must now be used only under veterinary supervision, with a Veterinary Feed Directive for feed-administered antimicrobials and prescription status for antimicrobials administered in water. Consumer marketing has also taken up the issue of antimicrobial use in livestock. National and international marketing chains are now setting policies regarding antimicrobial use. In December of 2018, McDonalds announced a plan to set reduction targets for antimicrobial use within 2 years.¹⁷

Fleming’s concern about moral responsibility is no longer touted simply as a rational extrapolation of science, but occasionally becomes a poster child for agendas seeking to take advantage of fears about this use or to abolish antimicrobial use in livestock altogether. Simply abolishing use could be categorized as thoughtless as well. There is also a moral obligation not to withhold treatment from a diseased individual (human or animal) if that disease could be alleviated by treatment with antimicrobials. But where is that line between possible risk to future human health and current animal suffering or farm economic stability? Is there simultaneously a

responsibility to decrease the disease incidence that is considered normal in today's livestock systems? Or perhaps that is the primary responsibility?

Evaluating antimicrobial use in livestock systems is extremely complex if the goal is to improve antimicrobial stewardship in an effort to decrease resistance selection pressure. It requires examination of the interactions and relationships between numerous bacterial populations, animal populations, drug dynamics, and drivers of human behavior. A basic requirement for applying science to all of these areas is the need for accurate antimicrobial use measures which are useful for describing the interactions mentioned above. Therefore, the main subject of this dissertation is to delve into the depths of antimicrobial use measurement. The goals are to identify useful measures, recognize potential challenges for interpretation, and to help guide future research priorities and policies regarding antimicrobial use in dairy systems. Although this report focuses strictly on measurements of drug use, this should not be interpreted as an indication of importance or priority. It is simply one part of the issue. This dissertation presents only the “drug” side of the “bug-drug” interaction. Particularly, special attention should also be paid to the interaction between the drug and the bacterial populations exposed. Accurate antimicrobial use data are a cornerstone of guiding antimicrobial stewardship and evaluating the interaction of antimicrobial use and resistance selection in a clinical setting.

Current National and International Policies on Antimicrobial use

Due to the human health risk associated with resistant pathogens, antimicrobial resistance is now the subject of national and global policies. Antimicrobial resistance has been on the agenda of the World Organization for Animal Health (OIE) for several decades. More recently, in 2016 antimicrobial resistance was deemed a global health problem and the United Nations (UN) adopted a declaration intending to address the issue using a “One Health” approach.¹⁸ They jointly commissioned the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and OIE to create an action plan.^{18,19} The 4 goals in this action plan are to (1) Improve awareness and understanding; (2) Strengthen knowledge through surveillance and research; (3) Support good governance and capacity building; and (4) Encourage implementation of international standards.¹⁸ The WHO developed a list of veterinary drugs and assigned priority categorizations to each drug according to its importance to human health.¹⁹ Additionally the OIE has a list of antimicrobials categorized by importance to veterinary medicine; FDA uses the list

of medically important antimicrobials (defined by importance to human health) in Appendix A of FDA GFI #152.²⁰

Adopting the strategy of “you can’t manage what you can’t measure” the OIE has encouraged member countries to report antimicrobial use. However, livestock systems differ drastically in the type and quality of data available even within a single nation, let alone between countries where there are drastic differences in production system types, regulations, and disease pressure. In spite of these challenges, the OIE provides a template for reporting yearly antimicrobial use²¹ and received use reports from 73% of the UN member nations in 2017.¹⁸

The OIE is not the only group that has tackled this challenge. Many countries across the globe closely examine antimicrobial use.²²⁻²⁴ There are numerous countries in Europe that have long standing programs documenting antimicrobial use in livestock systems. Funded initially by the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), the Network on quantification of veterinary Antimicrobial usage at herd level and Analysis, CommunicaTION and benchmarkING to improve responsible usage (AACTING) organization provides a summary of world-wide livestock monitoring programs aimed at quantification and benchmarking antimicrobial use at the farm level. Their website allows easy comparison of multiple monitoring programs that exist in European countries.²⁵ Based on this information (Version 1.4 2018-11-07) a total of 16 countries reported monitoring programs. Of these, 11 had programs in the dairy sector. The earliest program described began in Sweden as a pilot program in 1971, but most programs have come into existence within the last 10 years.

While many programs are government run, a few are proprietary. Of the 11 countries reporting dairy monitoring programs in Europe, 2 are voluntary reporting, 5 are required reporting, 1 is a cross-sectional sample, 1 is a convenience sample, and 2 are still under development.

Methods of data collection are diverse. Generally, collection relies on the producer, veterinarian, or a combination of the two for data acquisition and input. However, some programs fund research personnel for this task. Reporting levels and requirements vary for participants from the producer to the pharmacy that provides the drug. Generally, reporting is done by veterinarians, but producers, pharmacies, and feed mills are also sometimes involved. In all but 2 of the dairy programs, the use was reported by the veterinarian with or without involvement from producers.²⁵

In the United States, there are requirements for drug manufacturers to report sales data at the national level²⁶, but there are currently no farm level requirements specifically for antimicrobial use

reporting. Unlike prescriptions in some other countries, if an antimicrobial is purchased directly from a veterinarian this prescription can be in verbal rather than in a written form. There are also requirements for record keeping of prescription antimicrobials at the farm level, but a few antimicrobial products are currently available without prescription. In spite of this, the Pasturized Milk Ordinance (PMO) requires all drug products to be labeled by a veterinarian with the appropriate withdrawal time.²⁷ The necessity to adhere to these withdrawal times in order to maintain a marketable product forces most dairies to record treatments in some form since all antimicrobials have a withdrawal time for meat, milk, or both. These treatment records offer the most granular record of how antimicrobials are used within U.S. dairy systems. Their utility for measuring antimicrobial use is explored in this report.

Some have questioned how antimicrobial use policies may affect the economic viability of the dairy industry by restricting the capability to address infectious disease. A study of the economic effects of antimicrobial use restriction within the dairy industry found that use restriction policies would have a very minor effect at the market level (consumer cost), increasing costs by 2.5 cents per liter of milk.²⁸ Another study specifically evaluated potential costs at the farm level and calculated a \$46-\$73 cost per cow per year if antimicrobials were prohibited entirely.²⁹ These outcomes are extremely dependent on how appropriate the antimicrobial use was prior to removal.

The biggest challenge is relating even the most appropriate and accurate antimicrobial use metrics to the potential for harm from antimicrobial resistance in both human and animal patients. One way to set antimicrobial use metrics up for success is to report “just the facts” and not make unfounded assumptions of the duration of either therapeutic or antimicrobial resistance selection effects. The next level of difficulty entails converting antimicrobial use along with the potential for benefits and detriments, both short term and long term, into economic values which serve to assist in driving change. Regardless, it all starts with understanding what antimicrobial use metrics can do for us when appropriately constructed.

Mechanisms for Antimicrobial Use Reduction

While user fees or sales caps have occasionally been considered as a method of driving change ³⁰, benchmarking has been advocated far more commonly.³¹⁻³⁴ It is useful to establish a version of “normal” that is larger than simply what has happened on an individual farm for the last several years. These benchmarks can also be used to drive questions about why change occurred

or what confounders might be contributing to differences between farms. The idea is that farms will be intrinsically motivated to explore reasons for their usage rank by comparing their use to others. In some countries, the benchmarks are also targets for use reduction. The distribution of farms is established and cut points are identified indicating levels of use that are unacceptable.^{33,35}

The acceptance of policies and monitoring programs by the livestock industry is variable. A survey of farmers and veterinarians in Switzerland found that both groups preferred a program that did not penalize for high use, but rather aimed at simultaneously improving udder health while decreasing use.³⁶

Multiple Methods of Measurement and Their Associated Assumptions

Multiple comprehensive reviews of antimicrobial use metrics exist, and the reader is referred to these for a more comprehensive list of terms and measurements.^{32,37-39} This discussion focuses on the main types of data sources and measures that have previously been applied to dairy systems, their associated assumptions, and challenges for interpretation.

Sources From Which Data Can be Collected

Sales Data

Sales data have frequently been used across the globe to quantify dairy drug use.^{23,40-47} The availability and relative ease of collecting these data are primary drivers of their frequent use. In some countries, required reporting methods indicate species-specific sales. In the U.S. the total substance weight sold is reported, and the species specificity of these sales are estimated by the manufacturers.

A disadvantage of sales data is that they are not particularly useful at the dairy level. Some farms purchase large amounts of drug to be used over 6-12 month time frames. This practice can greatly affect their measured use for a particular year, when actually the drug was not used until the following year. Over time this tends to even out, but can create some short-term challenges for farms who purchase in bulk.

Also, the limited granularity of sales data does not allow for recognizing any specifics about dose administered, time frame of treatments, or disease pressure. This also makes it difficult to interpret the likely selection pressure associated with a particular drug. One study compared sales data in 2 countries with detailed information from actual treatment records.⁴² Significant

discrepancies were found between information based on estimations from sales data vs information derived from treatment record data. In some instances, the estimates from sales data over-estimated use, and in other cases they under-estimated use. However, the study clearly demonstrates that sales data are challenging to use to create readily interpretable data comparisons between countries.⁴²

Empty Drug Containers

Antimicrobial use has been measured in several studies by counting empty containers that are used for treatment.^{48,49} A receptacle specifically for empty drug containers is placed on the farm near treatment areas, and workers are instructed to put all drug container waste in the receptacle. The total drug used is then calculated by counting the empty containers present in the receptacle. While collecting the containers does require some labor, this method solves a few of the challenges associated with other sources. Unlike sales data it measures only drug amounts that were actually used. Due to its simplicity, it is likely that workers cooperate with this request as it requires little extra time for them to use the receptacle.

In a Canadian study, antimicrobial use was evaluated both by treatment records and empty drug containers.⁵⁰ The comparison was made by comparing the number of Animal Daily Doses (ADD) calculated from each data source. This study found a high correlation (0.80) between the two collection methods, with the measurement from empty drug containers consistently being higher than the record. It is notable that this study asked farms to use a record system specific to the study, not the farm record system. This could have contributed to lower quality record keeping as it was not part of the normal routine.

Prescription Data

In some nations, records of prescriptions are mandatory and therefore this can be used as a source of data.^{37,45,51} The advantage of this approach is that the prescription allows use to be tied to species and disease indication. It also provides information about the dosing regimen prescribed, documenting treatment time frames and doses administered. There are 2 disadvantages to prescription data. The first is that not all prescriptions are filled, and not all prescribed treatments are necessarily administered. The main disadvantage is that this information is only available in countries or states with strict regulations about keeping and submitting these prescriptions.

Treatment Records

Treatment records are one of the most granular sources of antimicrobial use data but they are not commonly used as a measure of antimicrobial use. The complexity of gathering and analyzing them often make them an impractical source of data for large monitoring projects. They have occasionally been used in smaller studies primarily to evaluate other measures.^{49,51,52}

The advantage of treatment records is that often the level of detail present allows for the identification of specific opportunities to reduce use either by disease prevention, case definition, or regimen modification. The disadvantage is that they may differ in quality from farm to farm, ranging from non-existent to so complete that they record “no treat” events.

Survey or Interviews

Surveys or interviews have been used to explore public opinion about antimicrobial use⁵³⁻⁵⁶, as well as to attempt to quantify use either quantitatively or qualitatively.^{44,57-59} They have also been used to identify common prescribing practices or drivers of use.⁶⁰⁻⁶⁴ Like any other survey they are prone to recall bias. It can also be challenging to interview the correct person. For example, on some dairies the owner is the one making treatment decisions and administering treatments. On other dairies it may be the herd manager, or other employees. This creates a challenge when deciding who is best suited to answer survey questions, and to obtain consistent results across dairies. However, these methods are very valuable for obtaining basic information that can be used to identify areas where more granular data might be required.

Reporting the Collected Data

As or more important than the method in which the data were collected is the decision related to the numerical approach to reporting the metrics. Not only does the decision as to the numerator (the drug description) and the denominator (the time or population reference) greatly influence the apparent use, but slight changes in either component can make comparisons between countries, species, and production systems very misleading. The most basic component of starting the journey to antimicrobial stewardship is accurately defining the current uses so that they may be evaluated in light of antimicrobial resistance selection, animal welfare, and efficiency of production of animal-derived food products.

Numerators

Weight of Substance

The weight of active substance, often expressed in milligrams (mg) or kilograms (kg), is commonly used as a measure of antimicrobial use.^{23,41,65,66} This measure is relatively straightforward, requiring few formulas for calculations, and relies on very basic definitions. For some compounds such as penicillin, amounts are traditionally expressed in international units (IU) rather than mg. To facilitate use of this measurement these IU are converted to mg.⁶⁵ The guidance document for filling out the OIE template provides all necessary conversion factors.⁶⁷ The weight of substances is often reported by antimicrobial class when reporting sales data. For example, sales data in the United States for 2017 indicate that tetracyclines comprise 70% of medically important antimicrobial use in livestock when measured by kg.²⁶

One of the main reasons for utilizing the weight of active substance is that data about the amount of active substance sold are often easily available and relatively inexpensive to obtain. These data are often available from central sources such as manufacturing companies, creating favorable logistics for data collection. Companies usually already possess electronic databases, making reporting these values even more efficient, although parsing out the data by species and use class may require much more effort and have issues related to accuracy of estimates.

A major challenge in reporting use by weight of substance is that differences in drug potency create a challenge for interpretation. Potency affects the dose used. For example, sulfadimethoxine is a relatively low potency drug and is commonly dosed initially at 55 mg/kg body weight, whereas ceftiofur is a relatively high potency drug and is dosed at 1.1 – 2.2 mg/kg body weight.⁶⁸ This creates 2 problems. The first is that drug choice for therapy might drastically affect the number of mg used even if the rate of disease treatment does not change. Furthermore, the “effect” of each mg of a particular drug on a bacterial population may differ from the “effect” of each mg of another drug. Therefore, when milligrams of multiple drug classes are added together the relationship of this measure to either disease treatment incidence or selection pressure becomes completely confounded by drug potency.

Daily Doses

Due to potency differences causing interpretation challenges associated with summations of drug weight, the concept of quantifying use using a defined daily dose (DDD) with units of $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ was created.⁶⁹ The earliest use of defined doses was not for antimicrobial use specifically, but rather was developed as part of the Anatomic Therapeutic Chemical (ATC)

classification system in the early 70s. This development was motivated by the World Health Organization's desire to "standardize and improve drug use". The system structure was based off of systems previously developed by the European Pharmaceutical Market Research Association.⁷⁰ The adaptation from a marketing system capture approach (drug weight) to one of human health standardization and then to one of veterinary antimicrobial standardization creates a system that has a few constraints for both application and interpretation. First, the standard dose should not be assumed to have any efficacy basis. In fact, for drugs for which there are multiple doses or indications, DDD may be defined as a dose that is never actually administered. This happens when a range of doses is used in the practice of medicine, but a single dose must be agreed upon for standardization purposes.

In 2016, the European Medicines Agency (EMA) published defined doses of veterinary drugs with the guidance of European Surveillance of Veterinary Antimicrobial Consumption (ESVAC).⁷¹ The EMA document provides both defined daily doses (DDDvet) and defined course doses (DCDvet) for veterinary drugs. This document defines a standard amount of active substance in mg/kg units that is administered per day (DDDvet), and another amount as the amount administered during a standard course of therapy (DCDvet). Doses are defined specifically for each species and route of administration. **Table 1.1** shows some examples of commonly used treatments in U.S. dairies and the calculated number of defined daily doses resulting from using the EMA definitions of DDDvet and DCDvet. Comparing the number of days of therapy, with the number of used doses and the number of daily doses reveals that the relationship between these measures is not necessarily consistent over all treatment regimens. One interesting exception to the mg/kg unit is the published dose for intramammary therapy. The dose for these drugs is defined as "the number of tubes" rather than a dose in mg/kg. This is due to the fact that the dose is standard across body weight.

The number of defined daily doses is often interpreted as a proxy for "exposure", as there is a time component to it. For example, if only the number of mg are compared in **Table 1.1**, it appears that there is more oxytetracycline use than ceftiofur use. However, when comparing the number of animals treated, or the number of days treatment was administered, there is apparently more ceftiofur use. If it is believed that the majority of resistance selection pressure is induced by treatment incidence then using a defined course dose rather than weight of active substance has

advantages. If it is believed that selection pressure is best represented by “time exposed”, then the number of defined daily doses might be more relevant.

There are some positive aspects to this standardization. The main advantage is that it allows sales data with units in kg to be converted to a number of defined daily doses (nDDD) or a number of defined course doses (nDCD). This effectively accounts for potency. If the standard defined dose is indeed what was administered to the animal, and the weight at the time of treatment is known, then the relationship between this use measure and the detection of disease or decision to treat is straightforward. However, small discrepancies in either the dose definition or animal weight can become large errors when multiplied over populations of animals.

For the number of daily doses to accurately estimate the days of therapy, or for defined course doses to reflect treatment incidence, the weight of the animals at the time of treatment must be known. In growing animals this can be challenging data to acquire. In adult cows in dairy herds, although there is some variation in body weight (especially Jersey vs Holstein) the magnitude of this variation is not a large percentage of total body weight. This is not true of growing animals where the final weight may be 10 times greater than the initial weight. Therefore the estimation of weight at the time of treatment is simpler in adult dairy cows than in some other livestock production classes.

Table 1.1 Example Calculations

Abbreviation	Measure	Method(s) of Calculation	Cow A	Cow B	Cow C
N/A	Animal Weight (kg)	Scale weight	680	680	680
N/A	Active substance	Product label	Ceftiofur HCL	Ceftiofur HCL	Oxytetracycline
Regimens	Number of disease events treated (Regimens)	Treatment record	1	1	1
mg/Regimen	Total Mg Administered (mg/Regimen)	Treatment record (combined with protocol when necessary)	3600	6000	13454
mg/Administration	Milligrams per Administration (mg/Administration)	Treatment record (combined with protocol when necessary)	1200	1200	13464
Administrations	Number of Administrations	Treatment record (combined with protocol when necessary)	3	5	1
Time Frame	Time Frame (days)	Number of calendar days between the first and last administration	2	4	0
		((Treatment record number of administrations - 1) x (Interval between administrations))			
nDOT	Number of Days of Therapy (days)	Counted directly from treatment record	3	5	1
		Counted indirectly from treatment record using the recorded treatment event combined with the number of administrations indicated in the protocol for that event.	3	5	1
adjDOT	adj Number of days of Therapy (days)	nDOT with an adjustment factor applied to products providing multiple days of therapy with a single administration.	3	5	3
UDD	Used daily dose (mg/kg)	(Treatment record milligrams administered per calendar day) / (Animal weight)	1.8	1.8	19.8
		(Administrations per day x Milligrams per administration) / (Animal weight)			
nUDD	Number of used daily doses (count)	(Substance specific total milligrams) / (UDD x Animal weight)	3	5	1
UCD	Used course dose (mg/kg)	(Total milligrams per regimen) / (Animal weight)	5.3	8.8	19.8
nUCD	Number of used course doses (count)	(Substance Specific Total Milligrams) / (UCDvet x Animal Weight)	1	1	1
N/A	Assumed Animal Weight	Average animal weight treated - defined by available data for a specific geographic location and production system	680	680	680
DDDvet	EMA defined daily dose (mg/kg)	Published	1	1	6.5
nDDD	Number of defined daily doses (count)	(Substance specific total milligrams) / (DDDvet x Assumed animal weight)	5.3	8.8	3.0
DCDvet	EMA defined course dose (mg/kg)	Published	4	4	23
nDCD	Number of defined course doses (count)	(Substance specific total milligrams) / (DCDvet x Assumed animal weight)	1.3	2.2	0.86

Another factor that contributes to the accuracy of the calculated estimation of course doses or daily doses is the amount of variability surrounding the dose of the drug. If the dose is very standard for all indications (e.g., dry cow treatments), then the estimate is quite good. If there is significant dose variability, then the estimate is more difficult to interpret.

It should also be noted that the number of defined daily doses is usually reported by drug class. Therefore, the details about whether the exposure was a single animal for many days versus a large number of animals for few days is lost. It is unknown which of these would result in the largest selection pressure on bacterial populations. This is important because it is plausible that exposing one population of bacteria multiple times might result in a different rate or magnitude of resistance selection pressure than exposing many populations once.

Variations of defined daily dose exist in the literature. One is the used daily dose (UDD) as calculated in the example Table 1.1. “Used” means that it was actually administered rather than defined. Some studies have compared the UDD calculated from the treatment record to the recommended dose on the product label.^{65,72} Other variations have also been used to identify situations when the DDD does not accurately reflect treatment practices.^{72,73} It should also be noted that small variations in terminology and definitions exist. For example, some countries use their own defined doses rather than those published by EMA. In these cases, the measure is abbreviated as ADD or DDDA to reflect the standardization method.^{48,73}

As described above, the method of dose definition can play a large role in interpretation of the resulting calculated values of antimicrobial use. For example, multiple studies have found that used daily dose (UDD) defined at the farm level is a more accurate estimation of consumption than a prescribed daily dose (PDD), or than a defined daily dose (DDD).^{17,65,71,74} Despite these complicated relationships, nDDD is used frequently as a measure of antimicrobial use due to the relative ease of calculation from readily available data.^{40,45,46,49,59,75-77}

Days of Therapy (DOT)

Days of therapy has been proposed by the Infectious Disease Society of America (IDSA) as an improved alternative to measurements using defined doses in human patients.⁷⁸ Days of therapy is defined as the number of days on which a patient receives an active substance. The specific dose in mg, or the frequency of treatment is not a part of this measure. It is simply a count

of the calendar days for which the patient received treatment with a specific drug. The reason for preferring this to defined doses measures is that the nDOT is not affected by differences between the defined dose and the actual dose administered. This creates a more direct relationship between the use measure and treatment activity. In some studies of livestock systems, the calculation of a UDD results in a very close estimate of the nDOT calculation provided that single-injection drugs with a long duration of activity were not used for treatment.

The main advantage to using nDOTs is that for drugs administered daily they are a relatively accurate reflection of the time the patient was exposed to the drug. For longer duration drugs, such as ceftiofur crystalline free acid, a single dose is administered but therapeutic levels are expected for 3 to 5 days; therefore, counting the calendar days when treatment was administered does not accurately reflect exposure. Estimations of likely treatment durations can be derived from pharmacokinetic and efficacy data. However, the concentrations where the drug was effective would differ by pathogen. For short duration drugs, this amount of uncertainty can be accepted, but for longer duration drugs pharmacokinetic data may indicate a very long half-life. The duration of this half-life and the number of half-lives at which exposure is determined to have ended is not straightforward. In addition, the relationship between the duration and magnitude of therapeutic effects is not necessarily correlated with the duration and magnitude of resistance selection pressure. Beyond the antimicrobial characteristics, both the therapeutic duration and resistance selection pressure of an antimicrobial regimen are highly dependent on the characteristics of both the target pathogen and the other bacterial flora present.

The main disadvantage of nDOTs is that it can only be calculated from treatment records which are generally resource intensive to gather. In human systems where treatments are billed out daily and barcodes are used for point-of-care data capture, this is relatively efficient data to collect. In livestock systems, the availability and quality of treatment records are variable.

Treatment Incidence

Treatment incidence is occasionally used as a measure of antimicrobial use.^{38,52,65} There are two main methods of calculation. The first is to calculate it directly from treatment records.⁵² The second is to calculate the number of course doses and use the course doses to estimate the incidence.⁷⁹ If treatment records and resources for their analysis are available, calculation of treatment incidence from the record is more useful at the farm level. The utility of a treatment

incidence calculated using defined doses is entirely dependent on the assumptions that were necessary for the calculation of nDCD. If the estimation of weight at the time of treatment was accurate, and the dose administered matched very closely to the defined dose, then the estimate will be very accurate. However, variability in either of these 2 parameters can lead to estimates that are very different from the actual number of treatment events.

Treatment incidence calculated from records is very useful at the dairy level as it is well correlated to the level of “detected disease”. When benchmarks of this parameter are received it allows farm management to compare the level of detected disease to other farms and evaluate underlying reasons for higher (or lower) detected disease incidence. It is worth noting that “detected disease” and true disease incidence are not necessarily the same. The amount of detected disease is dependent on case definition, the application of that definition, and the amount of disease pressure. A low incidence of detected disease might represent successful prevention strategies creating a low disease pressure. Alternatively, that same low incidence of detected disease might indicate poor disease detection potentially leading to a decrease in animal welfare. It could also indicate poor quality of treatment records.

Regardless of the fact that it may or may not represent the true incidence of disease, treatment incidence is a useful measure as it is the point at which a decision is made about treatment. In situations where this number is high relative to similar livestock operations, the management team can determine if changes need to be made in disease prevention, retraining needs to happen to improve application of the case definition, or if new diagnostics should be employed to modify the case definition. All these measures lead to opportunities for improved disease management resulting in treatment reduction.

One study evaluated the association between antimicrobial use and the results of systematic clinical exams for metritis and mastitis (by vaginal exam and California mastitis test, respectively).⁸⁰ The detection of more disease in the herd did not correlate well with the amount of antimicrobial use. This study highlights several important points. First, the case definition and its application by those administering treatment is a key factor in antimicrobial use. Second, this could indicate that the measure of use, in this case animal daily dose (ADD), does not actually represent the level of detected disease in the herd due to the assumptions made for calculation or variation in certain regimen specifics.

It should also be noted that there are differences in how the calculation of treatment incidence from records might be performed. For example, a treatment record for a cow indicates that treatment for mastitis began on March 1 for 3 days, and then was treated again on March 6 for 5 more days. Some definitions of treatment incidence would assume that animals immediately recover from disease, and therefore this would count as 2 disease events.⁶⁵ Other definitions would apply a recovery time frame in which the animal was not eligible for a new disease event and any treatments within that time frame would be applied to the initial event. In this example case, if a 7 day recovery period was applied then the same information would count as only 1 disease event.

The primary disadvantage of measuring treatment incidence is that it is entirely dependent on the type and quality of the records available. If there are accurate electronic treatment records then data collection and analysis are relatively efficient. If the farm records are incomplete or non-existent, then measuring treatment incidence is not useful, or is impossible to calculate. The format of records can significantly impact resources needed for collection and analysis as well. Even with complete, electronic records, the degree of standardization of the recording method can significantly alter the time required for analysis due to differences in time necessary for data cleanup.

Denominators

Time or Animals at Risk

In order to make numerator calculations more interpretable they are generally standardized over a denominator measure. Sometimes these measures are typical of other livestock epidemiological studies and consist of animals at risk or time at risk.⁸¹ For example, in dairy herds the average number of adult animals present on the farm might be used as the denominator for benchmarking at the farm level. Alternatively, denominators such as cow days at risk might be used.^{40,48,65}

Biomass

Since many drugs are dosed on a mg/kg basis, total animal body mass (kg) has also been used as a denominator. Occasionally a simple sum of animal body weights is used as a denominator, but more frequently a more specific calculation, “biomass”, is used. Biomass is attractive because it invites comparisons within and across countries and species.^{23,41,47,66}

Although multiple methodologies for calculation exist, in general biomass is calculated by using both the weight of live animals as well as the weight of slaughtered or exported animals within a year time frame.⁸²⁻⁸⁵ The idea is to improve standardization across species by attempting to account for multiple production cycles (poultry, swine) in a year. Biomass helps account for the multiple production cycles and distributes yearly antimicrobial use among all production cycles completed that year rather than just the current one.

Based on use reported in the OIE template²¹ a quantitative report was generated describing use in 2014 using biomass as a denominator.¹⁸ Within the report, it is noted that the granularity of data for calculating biomass varied by country. Biomass calculations in this report were based on point estimates of census data for the number of animals living in the country generated from multiple animal surveillance databases. Sales data were the predominate data source available for quantifying antimicrobial use. The report notes that the final data “are difficult to interpret given that production classes within a species can have very different average weights.”¹⁸

The FDA has proposed a U.S.-specific method for calculation of animal biomass that takes into account domestic animal populations and weights.⁸² In this proposal, U.S.-specific yearly animal population estimates and average weights are suggested, to account for differences in U.S. production systems. This species-specific biomass measure is named “target animal biomass” or “TAB” in order to differentiate it from the European standard biomass measure of a “population correction unit “or “PCU”. The proposed sources of livestock number estimates for the TAB are the existing USDA databases and would include the annual totals of animals slaughtered, single time point inventory of live animals, and annual totals of animals imported or exported. USDA databases will also be used to estimate annual average weights of livestock populations. Average weights at slaughter, weights for animals imported or exported, and annual average weights for livestock kept longer than one calendar year are all sources of information that would be used to estimate this parameter. FDA believes this method of biomass denominator estimation will best fit the circumstances of animal production in the U.S., and will account for annual shifts in population and weights specific to each species.

While it is tempting to “fully standardize” for the sake of comparison, there are also disadvantages. Data granularity differs between countries and between species and therefore the accuracy of calculated biomass varies. When small assumptions are made about an animal weight at slaughter and this number gets multiplied by a relatively large number of animals produced that

year, small errors in initial estimates can result in large errors in final output measurements. This can make results difficult to interpret.

Livestock Units

A measure of Livestock Units (LU) has also been used as a denominator for antimicrobial use.⁷⁷ This measure is essentially the same measure used to quantify livestock for grazing systems. One LU describes 500 kg of liveweight. This unit is straightforward to understand and works well for cattle systems where the production cycles are longer than one year. Interpretation becomes complex if there are multiple production cycles during the time frame used to measure antimicrobial use. However, in countries where this unit is used for census data, it is a number that is easily available.

Combinations of Numerators and Denominators

Clearly, there is a lack of standardization when it comes to measures of antimicrobial use in livestock, but in part this is due to a variety in the underlying reasons for measuring use. In a review of measures used in both human and veterinary species, Collineau, et al. clearly states that the most appropriate measure depends on the reason for measurement.³⁸ This study also suggests that there are generally four reasons antimicrobial use is reported: 1) to measure trends over time, 2) to compare usage between large populations such as countries or species, 3) benchmarking specific populations such as farms or veterinary clinics, and 4) to provide data to study the link between usage and resistance. Each of these focuses has different priority requirements for data availability and granularity.

Studies looking at usage trends over time often are used to support and inform national or international policies or programs.^{18,35,86} The need for comprehensiveness is low and there is little attempt to assess exposure level or duration, or to standardize the population. However, there is a need for consistency in definitions and methods over time. Changes such as re-defining daily doses creates a problem. For these type of studies, sales data are frequently used and are often appropriate.³⁸ However, changes that emerge in these use metrics are heavily influenced by changes in disease patterns within populations such as increased vaccination which decreases disease incidence, or increased antimicrobial use due to disease outbreaks. In some instances, changes in antimicrobial use metrics (e.g., kg of drug weight or days of therapy) are related to

other factors, such as a change in the most common drug used. Since weight of active substance is a commonly chosen numerator for this type of monitoring and is affected by potency, these changes in drug choice vs. amount of use need to be distinguished from one another. Denominators also vary for measuring use over time but are frequently a simple geographic definition of the population, e.g., total mg of medically important drugs sold or distributed in relation to the population of an area of the United States.

The denominator becomes especially critical when usage is to be compared between two populations such as species or countries.³⁸ The numerator does not require much granularity so is often still the weight of active substance, but the denominator becomes very important as there is now a need to standardize for comparison purposes. It is for this purpose that measures like biomass are commonly used in an attempt to provide some common ground for the comparison. Although the results are numerically standardized, the process of standardization distances them substantially from the end user and there may be an incentive to influence the denominator to sway the overall antimicrobial use metric in favor of the party submitting the data. Determining the quality or value of a standardized use number can be extremely challenging. It is tempting to simply assume less is better, but there are many confounders during the standardization process, and this simple view has the potential to risk animal welfare and the conservation of animal resources for the sake of a decreasing metric.

A great deal of granularity is necessary when benchmarking use at the individual dairy level.³⁸ The denominator is extremely important, and can have effects of large magnitude on the final outcomes measure. It is challenging to find a way to standardize farms even within a species or within a specific production system type. For benchmarking, animal days at risk or animals present on farm are frequently used. Because these studies aim at making changes in use at the level of the treatment decision, they often require numerators that are closely linked to disease detection and treatment administration. Measures such as Treatment Incidence, nDCD, or nDDD are common choices.

The most granularity is necessary when trying to link use to resistance development.^{38,79,87} It is here where both measures of use and descriptions of bacterial populations must be at their most granular form. Specifics of dose, timing of administrations, route of administration, and specific drug pharmacokinetics and pharmacodynamic parameters must all be considered in relation to bacterial populations that interact with the drug.

The link between antimicrobial use and selection pressure is difficult to establish. One review published in 2002 indicates very little change in resistance patterns of common mastitis pathogens when evaluated with respect to antimicrobial use.⁸⁸ However, the study evaluated only the time frame between 1994 and 2000. It should be noted that comparisons of phenotypic resistance prevalence over time are heavily dependent on the standardization of both antimicrobial susceptibility testing techniques and interpretive criteria over time. Multiple comparisons of resistance prevalence over time are flawed by failing to recognize changes in these characteristics.

Another study attempted to link antimicrobial use measured as nDDD to methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta-lactamase (ESBL) producing *E. coli* isolated on farms.⁸⁹ The study failed to demonstrate an association between the use measured as nDDD and the likelihood of isolating MRSA and ESBL producing *E. coli* on a farm. Two Canadian studies evaluated the association between farm level antimicrobial use measured as the number of defined daily doses per 1000 cow days and the detection of resistant Gram negative and Gram positive bacteria.^{90,91} Multivariable logistical regression models were built for each drug class. The model building process was extensive and accounted for numerous confounders, but the results were still difficult to interpret. An association was found between ampicillin resistance and use of systemic penicillin as well as penicillin -novobiocin combination at dry off. They also found that florfenicol use was associated with ampicillin resistance. However, resistance to ampicillin and tetracycline was most associated with average herd parity. The authors concluded that the differences they found in antimicrobial resistance could not be fully explained by antimicrobial use alone, raising the question of whether this association with average herd parity is due to duration of exposure to the environment of the herd where antimicrobials are used.

Studies such as this highlight the complexity of identifying drivers of resistance selection. Measuring use simply by the number of defined daily doses leaves out information about timing of administrations, magnitude of dose actually used, route of administration, and the population of bacteria exposed. There is clearly room for further study in this area. The effects of altering administration routes, frequencies, durations, or doses on changing resistance selection pressure have not been fully described. However, as new tools become available and measurements become more granular, perhaps the knowledge base can be expanded to include regimen optimization which reduces resistance selection pressure, thus extending the life of antimicrobials as tools for

disease treatment. It is also possible that drug use is not the only driver of resistance selection pressure within a system.

The choice of measures when deciding to monitor antimicrobial use is a challenging one. Different methods of measurement result in different magnitudes of use relative to baseline.^{92,72} A recent study compared data acquired by on farm treatment records to sales data, and calculated 3 metrics for each data source; mg/PCU, nDDDvet/kg or cow^a, and nDCDvet/kg or cow.⁹² The calculation of 3 different measures for the same farms identifies some challenges for interpretation. While the measures graphically appear correlated, they are certainly not identical. If cut points are applied to use measures, the farms do not always fall in the same category of magnitude of use. The consequences of such a difference could be significant if programs are put in place to curtail use based on one measurement vs another.

Outcome Assessment

Regardless of the numerator-denominator combination chosen, one of the challenges associated with evaluation of antimicrobial use is the moral obligation to balance animal welfare and farm economic stability with the risk of resistance selection pressure. This cannot be done if treatment outcomes and production parameters are not monitored along with antimicrobial use. Several studies have attempted to keep these outcomes in perspective.

The economics of selective dry cow therapy have been studied extensively.⁹³ Additionally, a Belgian study evaluated associations between drug use and udder health, milk quality, milk production, and numerous other herd performance parameters.⁴⁸ This study primarily evaluated the association between use of selective dry cow therapy and overall antimicrobial use with respect to production parameters. They found that selective dry cow therapy alone did not explain differences in antimicrobial consumption.

Another study in the United Kingdom examined the herd health and production effects of voluntary participation in a program to eliminate use of drugs identified by the WHO as high priority antimicrobials.⁹⁴ Seven farms and one veterinary clinic participated in the study. Treatment protocol changes included elimination of fluoroquinolone use, fourth-generation

^a EMA standardized IMM and intrauterine doses on a per cow basis, but oral and parenteral doses on a per kg animal weight basis.

cephalosporin intramammary tubes were replaced with products containing penicillin and aminoglycoside preparations, and third-generation cephalosporins were replaced with first-generation cephalosporins or aminopenicillins. They evaluated average 305 day milk production, 100 day calf survival rate, calving index, clinical mastitis case rate, clinical mastitis cure rate, bulk tank somatic cell count, cull rate, and lameness rates. The study found that on average over a 5 year period none of these parameters were negatively affected due to the removal of these high priority antimicrobials from the production systems. Some attention should be paid to the fact that various farms changed their protocols at different time points throughout the period. Therefore, it was only the last year where all farms were consistently not using critically important drugs.

Taken as a whole, the body of work related to antimicrobial use metrics establishes a foundation for further study aimed at optimizing antimicrobial use description in U.S. dairies. The following chapters are an attempt to explore different methods for capturing and describing antimicrobial use within the context of United States dairy production systems. This is not an attempt to identify the “correct” measure, but rather to describe the nuances associated with multiple measures and the challenges and resources necessary to gather the necessary data. The descriptions of various antimicrobial use metrics are intended to be applicable to numerous goals of antimicrobial use measurement including policy development, benchmark interpretation in support of antimicrobial stewardship, and evaluation of resistance selection pressure.

Chapter 2 - Methods of Data Collection and Management

Introduction

Data were initially collected from a convenience sample of 32 dairy farms in the United States, ranging in size from 180 - 6000 adult cows (lactating and dry cows). Dairies were recruited through their veterinarians with whom the investigators had a previously established relationship. The established relationship of the investigators with the veterinarians, and the veterinarians' relationship with their clients were pivotal to the project. Effort was made to include dairies in multiple regions of the United States; however, there were no restrictions for participation other than a willingness to work with the investigators and allowing publication of summarized data.

The original proposal planned to restrict participation to dairies who raised their own replacement calves. This restriction was relaxed in favor of having more participants in the study with data obtainable in a timely manner. Each dairy was visited by the investigators on a yearly basis. Data were collected for the calendar years 2016 and 2017. In 2016, collection was accomplished on all 32 participating dairies. In 2017, one dairy was purchased and the cows were mixed with another herd; this prevented analysis of the 2017 data on that farm. Where comparisons are made between 2016 and 2017 this dairy was excluded from all of the final analysis. On another dairy, data collection was put on hold due to significant management changes complicating the logistics of data collection. Therefore, the single year of data from this dairy is also not included when comparisons between years are made. In another dairy, all collection was accomplished but this dairy was excluded from the final analysis due to lack of retrospectively available intramammary treatment data. This resulted in the inclusion of only 29 farms in the final reported measurements.

Collection of data was accomplished at yearly farm visits. In the first year, data were collected from multiple sources including farm treatment protocols, drug purchase data, survey data, and treatment records. Farm protocols and purchase data were available either electronically as Word®, pdf, or Excel® files or as paper hard copies on farm. Electronic formats were saved to a flash drive and paper formats were captured as digital photos. Survey data were collected on paper at the time the survey was administered and then entered into a spreadsheet for analysis.

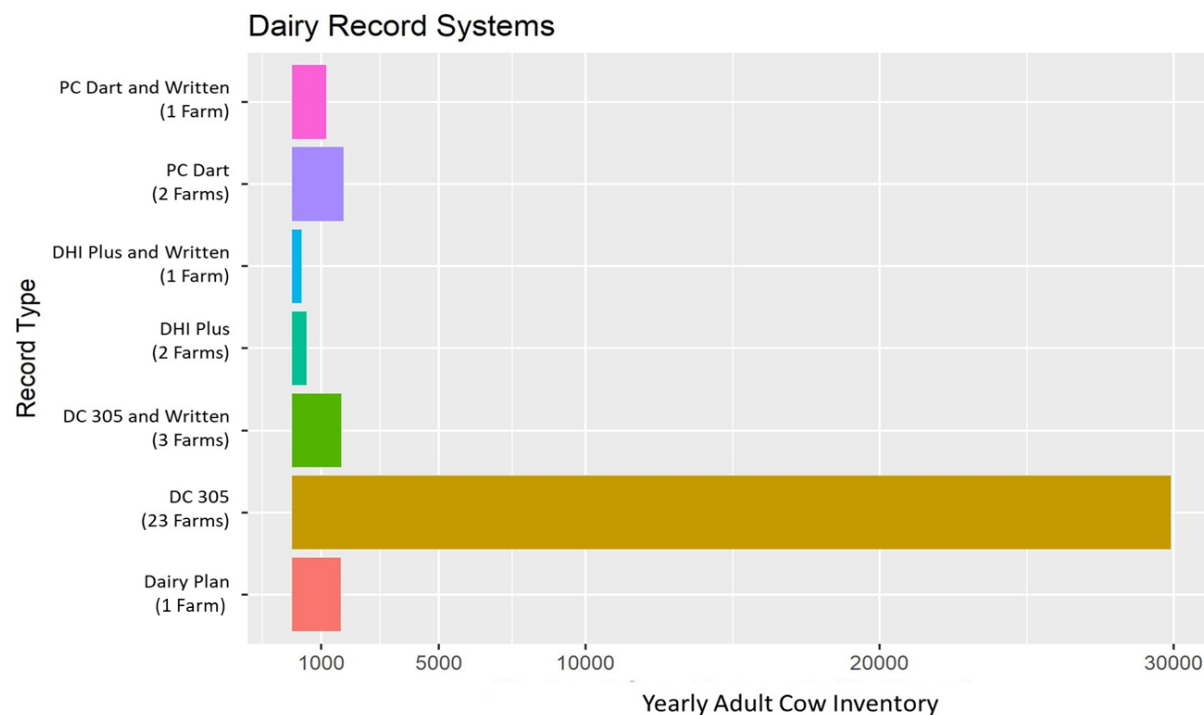
Treatment data included multiple record system types. Within these system types, there was also variation in methods of treatment documentation both between and within farms. A

detailed data standardization process was used to account for the variation encountered. Once standardized, data then passed through rigorous quality assurance procedures and logic checks prior to subjecting the data to analysis. In subsequent years, it was determined that efficiency could be improved if the collection focus was on treatment record data.

Types of Treatment Record Systems

According to the United States Department of Agriculture (USDA) 2014 National Animal Health Monitoring System (NAHMS) survey, individual cow record systems are in place for 95% of operations (99.1% of dairy cows).⁹⁵ Computer based systems were used in 46.5% of operations (82.7% of dairy cows). The 32 dairies initially enrolled in this study utilized one of 4 electronic data record systems; Dairy Comp 305 (26), PC Dart (3), DHI Plus (2), and Dairy Plan (1). **Figure 2.1** shows the number of adult cows in yearly farm inventory according to the record system representing them.

Figure 2.1 Types of Farm Record Systems



All systems allowed tracking of individual animal treatments. For most farms, treatment data were captured by saving a backup copy of the farm record system onto a flash drive. Treatment data and denominator data (cow inventory) were extracted from the farm software as comma separated value (csv) files. For each data system, the commands needed for data extraction were unique. Care was taken to confirm with software providers and other system experts that the extracted data were complete. In several data systems, when cows are sold or die their records become archived. There are generally different commands when extracting these records compared to evaluating the records of cows currently in production. For DC305, the data extraction involved running several commands on the command line, choosing appropriate parameters, and saving 2 csv files for later use, one denominator file and one treatment file. To achieve the necessary level of detail for treatment events in PCDart, a csv was downloaded for each type of event resulting in numerous (10-23) csv files in addition to one denominator file. Treatment data were extracted as a single csv file from DHIPlus, but denominator data required graphing cow numbers by test days, zooming in on the graph to read the value at each test, and then entering these numbers into Excel to be saved as a csv. Retrieval of complete treatment data from Dairy Plan required the creation of 2 custom reports. These reports were then run multiple times over short date ranges to ensure that the data were not truncated during the extraction. These extractions were then appended to a csv file. Two different reports were run to capture both intramammary treatments and other treatments as the comments for each were in separate fields.

Individual dairies varied in their utilization of the electronic systems to record treatment data. Five farms did not use an electronic system for recording treatment information. Instead, these farms utilized hand written treatment records. For these farms, treatment records were captured by digital photograph of the hard copy record during the farm visit. They were later transcribed into an Excel spread sheet and saved as a csv file.

Uncaptured Antimicrobial Use

This study was designed to capture all uses of antimicrobials within dairy systems. However, upon analysis of the data collected it was determined that there were some uses that were unintentionally not captured, or that proved too resource intensive to capture in the initial

years of the project. Examples discussed below include topical hoof treatments, youngstock treatments, non-permanent treatment records, and in-feed use.

Topical Treatment of Hoof Lesions

Treatments applied topically to the hoof are sometimes found in the same record system as systemic treatments, but on some farms these are documented in an entirely different record system. Many of these alternative record systems including topical hoof treatments were captured in year two when it was recognized that they were separate from the main treatment record. However, they have not yet been analyzed. The vast majority of these hoof level treatments are for lesions such as sole ulcers or white line defects. These are usually treated with corrective trimming and no antimicrobials are used. In contrast, treatments aimed at digital dermatitis usually involve direct topical application of an antimicrobial. Records of individual animal topical hoof treatment rarely explicitly listed the drug or amount of topical treatment applied. Discussion with farm managers indicated that treatment for digital dermatitis consists of approximately 500 mg of tetracycline powder applied directly to the lesion. Alternatively, topical lincomycin or lincomycin/spectinomycin may also be used. Since this information was not specifically asked in the official survey, it is not available for all farms at this time. In the use report, there are numerous treatments reported as “Unknown”. The vast majority of these are topical treatments of digital dermatitis.

Treatment Records for Young Stock and Calves

The original intention of this project was to also include records of antimicrobial use in young stock (animal age from weaning until birth of first calf) and calves (age birth to weaning). However, these records were available in a very incomplete format for only 2 of the first 15 farms visited. For the other 13/15 farms no treatment records for calves existed in a format that could be retrieved. On some farms, treatments for young stock were not documented in any format. On other farms they were documented in a temporary format (e.g. chalk marks on hutches), and occasionally the documentation was mixed with records of multiple farms due to their use of a contracted heifer grower. Records from heifer growers encountered in this project did not allow allocation of calves to their respective dairy. The expenditure of time and resources to estimate use in calves was determined to be well beyond the limitations of the current study.

Feed Use Records

All dairies surveyed indicated that they are using ionophores in all lactating cow rations. Records of antimicrobial use in feed are highly variable and range from non-existent to electronic records of each feed batch mixed. These record systems are entirely separate from the treatment record systems for medically important drugs, and thus far time resources have not permitted collection and analysis at a granular level. The most effective method of estimating total ionophore use will be explored in project year three.

Non-Permanent Treatment Records

One dairy recorded all intramammary treatments of clinical mastitis on the whiteboard in the parlor. The combination of this notation and the cow's location in the barn allowed them to effectively manage the treated cows to avoid milk residues, but it did not allow retrospective data capture. Due to this known lack of data, this farm is not reported in the final data analysis. However, it is interesting to note that in spite of having no records of treatments for Clinical Mastitis, the farm was not an outlier in overall use measured by regimens. This was predominantly because the percentage of cows treated in the Other Treatments category was relatively high.

Record Format Standardization Process

All csv files containing data from all systems were uploaded into R (version 3.4.2)^b for standardization. The R packages “tidyverse” and “ggplot2” were used extensively.^{c,d} Due to the extreme variation encountered both in documentation methods and record system types, the original treatment records were standardized to a single format (**Table 2.1**). **Table 2.2** shows the same original data, but instead of the standardized record value it shows the data source used to indicate the standardized value. These 2 tables should be interpreted as a pair; the row numbers and original data are identical between the 2 tables. In **Table 2.1**, examples of original data records as collected are presented on the left. The standardized format is displayed in the middle

^b 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/>.

^c Hadley Wickham (2017). tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. <https://CRAN.R-project.org/package=tidyverse>

^d H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

of the table. Calculated values as discussed in Chapter 3 are displayed on the right. The standardized format includes disease, drug product, route of administration, number of administrations, interval between administrations, dose in mg, and detected disease incidence.

Standardization of Disease Syndrome

The steps necessary to achieve standardization were iterative and differed somewhat depending on the original data format. Under the heading Original Treatment Record, **Table 2.1** represents several typical examples of data in its original form. Generally, the first step was to identify the disease associated with the treatment. This was partly due to a desire for disease information to remain connected to treatment, but it was also so that farm-specific protocol information could be applied if necessary. Disease information was most often included in the “Event” or similar column in the treatment record. The standardization process can be observed by using table 2.1 to compare the “Event column” in the original treatment record to the “Disease Syndrome” column under the standardized record heading. Sometimes it was simply a completion of an abbreviation such as converting MAST to Mastitis (rows 6-10). The event “DRY” is commonly used to indicate dry cow treatment and is therefore also associated with Mastitis (rows 1-4). The process continued by categorizing abbreviated diagnoses into their associated disease syndrome; “RP” and “RETAINP” indicate a retained placenta and are therefore associated with Metritis (rows 5 and 12), and “LAME” is associated with Lameness (row10). “BIG CALF” is indicative of calving trauma and so it is also associated with Metritis (row 11). “TRAUMA” is associated with injury and therefore is categorized as “Other”. This type of association differed from farm to farm. For example, some farms indicated traumatic calving as “trauma” so on those farms it was associated with Metritis. “FRESH2” (rows 13-16) required information from the survey (see **Table 2.2** rows 13-16) where they had indicated common practices providing enough information to associate it with calving difficulties. However, sometimes there was simply not enough information available to associate a particular treatment with a disease condition (row 19). In these cases, the disease syndrome was identified as “Unknown”. More descriptions of disease categories along with examples of original record formats associated with them are shown in **Table 2.3**.

Standardization of Treatment Product Administered

The next step in the process was to identify the drug product used for treatment. Although there are no examples of this shown, there were numerous non-antimicrobial treatments (i.e. flunixin, calcium, dextrose, dexamethasone, magnesium hydroxide, etc) listed in the treatment record. These were identified as “Non Antimicrobial” treatments. They were included in the benchmark reports provided to the farms as they added valuable information about detected disease incidence. However, they are not included in this report. In a similar manner to identifying disease syndrome from the Event column, the drug product was generally specified in the Remark column, but it often required information from the Event column as well. The abbreviated indicator of a drug was standardized to the brand name used in the database. Sometimes this was a simple process, “SPEC-DC” = Spectramast DC, or “EXCENEL” = Excenel. But other times it required some creativity combined with later confirmation by farm personnel that the codes had been interpreted correctly.

Record customization options are important for adequate adaptation to individual farm management needs. However, this variability can create challenges for standardization of reporting and/or benchmarking. Some software systems limit the number of characters that can be used to describe each treatment, but allow any input of that character length. Row 6 shows an example of this using “POLSLCRF” to indicate treatment with Polyflex and Spectramast LC in the right front quarter. Other treatment records include items such as “RRLRSPOL” or “BAEXDTO”. These correspond to RR=Right Rear, LR=Left Rear, S=Spectramast, POL=Polyflex, BA=Banamine, EXD=Excede, TO=Today. As with standardization of disease, farms vary in the information present in the treatment record. Sometimes all the information was present in the record itself, but other times alternate sources had to be consulted in order to confirm the treatment administered (see column “Drug Product” table 2.2).

Determining Dose and Frequency of Administration

During the process of identifying the product, the amount and frequency of product administration were also determined. Farms also vary in the level of treatment detail that is compressed into the short character strings. Some records are very detailed in spite of the character restriction and include descriptions such as “POLY5#25” or “25POLY.5”, which correspond to 25 ml of Polyflex (ampicillin trihydrate) for 5 days. In this case of Polyflex, the concentration at

which the ampicillin trihydrate was reconstituted must also be determined in order to calculate the milligrams used. This information was gathered as an additional question added to the survey after the first 2 farms once it was realized that this information was not captured in another manner. Very frequently the protocol was necessary to determine dose amounts administered (Table 2.2 column “Administration Dose”).

Data Source Prioritization

Information relevant to the standardized record was often present in multiple data sources. When this occurred, sources were prioritized in the following sequence: treatment record, survey, purchases, protocols, and finally the product label. This sequence of prioritization gives preference to information that required more active recording effort compared to information generated from more passive record systems. Occasionally sources conflicted, and this method of prioritization was used to maintain consistency in the standardization process.

Table 2.1 Record Format Examples

Original Treatment Record							Standardized Treatment Record							Calculated Values		
Row Number	Dairy	Cow ID	Date	Event	Remark	Protocols	Disease Syndrome	Drug Product	Route of Administration	Number of Administrations	Interval between Administrations (hrs)	Administration Dose (mg)	Detected Disease Incidence	Regimen Dose (mg)	Number of Regimens	Regimen Time Frame (days)
1	A	1531	1/13/2016	DRY			Mastitis	Quartermaster P	IMM	1	0	4000	0	4000	1	0
2	A	298	1/13/2016	DRY	SPEC-DC	SpectraDry	Mastitis	Quartermaster D	IMM	1	0	4000	0	4000	1	0
3	B	Lucy	9/22/2017	DRY			Mastitis	Spectramast DC	IMM	1	0	2000	0	2000	1	0
4	B	6789	9/22/2017	DRY	tomorrow		Mastitis	ToMORROW	IMM	1	0	1200	0	1200	1	0
5	B	1531	12/12/2017	RP	Excede 22		Metritis	Excede	Parenteral	2	72	4400	1	8800	1	3
6	A	243	4/8/2016	MAST	POLSLCRF		Mastitis	Polyflex	Parenteral	5	24	5000	0.5	25000	1	4
7	A	875	10/11/2016	MAST	TODA/3Q	Today	Mastitis	Spectramast LC	IMM	3	24	125	0.5	375	1	2
8	B	4325	11/8/2016	MAST	RF	Today	Mastitis	ToDAY	IMM	2	12	600	1	1200	1	0.5
9	B	9874	12/21/2016	MAST	TOD LFRF	Today	Mastitis	ToDAY	IMM	2	12	200	1	400	1	0.5
10	B	2346	1/4/2017	LAME	EXN2SIM3	EXENEL	Lameness	Excenel	Parenteral	3	24	400	1	800	1	0.5
11	A	879	3/4/2017	BIG CALF	EXN5.25	EXENEL.SQ	Metritis	Excenel	Parenteral	5	24	1250	1	6250	1	4
12	A	43	3/31/2017	RETAINP	EXC\CAL	Excenel\Cal	Metritis	Excenel	Parenteral	5	24	1500	1	7500	1	4
13	B	23	9/25/2017	FRESH2	EXCENEL/	Excenel	Metritis	Excenel	Parenteral	1	24	1500	0.25	6000	1	3
14	B	23	9/26/2017	FRESH2	EXCENEL/	Excenel	Metritis	Excenel	Parenteral	1	24	1500	0.25			
15	B	23	9/27/2017	FRESH2	EXCENEL/	Excenel	Metritis	Excenel	Parenteral	1	24	1500	0.25			
16	B	23	9/28/2017	FRESH2	EXCENEL/	Excenel	Metritis	Excenel	Parenteral	1	24	1500	0.25			
17	B	3245	11/29/2017	TRAUMA	EXEN3D28	EXENEL3.25	Other	Excenel	Parenteral	3	24	1400	1	4200	1	2
18	B	22	12/13/2017	SICK	EXC		Unknown	Excenel	Parenteral	1	24	1250	0.5	2500	1	1
19	B	22	12/15/2017	SICK	EXC		Unknown	Excenel	Parenteral	1	24	1250	0.5			

Table 2.2 Sources of Information for Standardization

Original Treatment Record							Source of Standardized Information					
Row Number	Dairy	Cow ID	Date	Event	Remark	Protocols	Disease	Drug Product	Route of Administration	Number of Administrations	Interval between Administrations (hrs)	Administration Dose (mg)
1	A	1531	1/13/2016	DRY			Tx Record	Survey	Label	Label	Label	Label
2	A	298	1/13/2016	DRY	SPEC-DC	SpectraDry	Tx Record	Survey	Label	Label	Label	Label
3	B	Lucy	9/22/2017	DRY			Tx Record	Survey	Protocol	Label	Protocol	Protocol
4	B	6789	9/22/2017	DRY	tomorrow		Tx Record	Tx Record	Protocol	Label	Protocol	Protocol
5	B	1531	12/12/2017	RP	Excede 22		Tx Record	Tx Record	Protocol	Protocol	Protocol	Protocol
6	A	243	4/8/2016	MAST	POLSLCRF		Tx Record	Tx Record	Protocol	Protocol	Protocol	Protocol
7	A	875	10/11/2016	MAST	TODA/3Q	Today	Tx Record	Tx Record	Protocol	Protocol	Protocol	Tx Record
8	B	4325	11/8/2016	MAST	RF	Today	Tx Record	Tx Record	Tx Record	Protocol	Protocol	Tx Record
9	B	9874	12/21/2016	MAST	TOD LFRF	Today	Tx Record	Tx Record	Tx Record	Protocol	Protocol	Tx Record
10	B	2346	1/4/2017	LAME	EXN25IM3	EXENEL	Tx Record	Tx Record	Tx Record	Tx Record	Tx Record	Tx Record
11	A	879	3/4/2017	BIG CALF	EXN5.25	EXENEL.SQ	Tx Record	Tx Record	Tx Record	Tx Record	Tx Record	Tx Record
12	A	43	3/31/2017	RETAINP	EXC\CAL	Excenel\Cal	Tx Record	Tx Record	Protocol	Protocol	Protocol	Protocol
13	B	23	9/25/2017	FRESH2	EXCENEL/	Excenel	Survey	Tx Record	Protocol	Tx Record	Tx Record	Protocol
14	B	23	9/26/2017	FRESH2	EXCENEL/	Excenel	Survey	Tx Record	Protocol	Tx Record	Tx Record	Protocol
15	B	23	9/27/2017	FRESH2	EXCENEL/	Excenel	Survey	Tx Record	Protocol	Tx Record	Tx Record	Protocol
16	B	23	9/28/2017	FRESH2	EXCENEL/	Excenel	Survey	Tx Record	Protocol	Tx Record	Tx Record	Protocol
17	B	3245	11/29/2017	TRAUMA	EXEN3D28	EXENEL3.25	Tx Record	Tx Record	Protocol	Tx Record	Tx Record	Tx Record
18	B	22	12/13/2017	SICK	EXC		No Info	Purchase	Protocol	Tx Record	Tx Record	Protocol
19	B	22	12/14/2017	SICK	EXC		No Info	Purchase	Protocol	Tx Record	Tx Record	Protocol

*Note: The column named “Protocols” in the original farm record is from the electronic software system. This column creates functions in the farm management database that create lists of cows that need to be treated. “Protocol” listed as a cell value is the “farm treatment protocol”. These are related, but not necessarily identical.

Tx Record = Treatment Record, Purchase = Purchase Records, Protocol = Farm Protocol,
Label = Drug Product Label, Survey = On Site Survey, No Info = no information available

Table 2.3 Disease Syndrome Definitions and Examples

Disease Syndrome	Description	Examples and Explanations
Digestive	Disease involving the gastrointestinal system.	“off feed”, “bloat”, non-antimicrobial treatments such as mineral oil, magnesium oxide boluses, or other products labeled for digestive upset.
Displaced Abomasum (DA)	Displacement of the abomasum	“DA”, “LDA”, “RDA” - Use of antimicrobials in this category is primarily surgical prophylaxis. This category was added at the request of farm veterinarians due to its alignment with other on farm disease management strategies.
Lameness	Clinical lameness	“LAME”, occasionally events such as “hoof trim” were also included if they indicated therapy of hoof pathology. Routine hoof trims are not included.
Mastitis	Clinical or Subclinical Mastitis	“MAST”, “MASTITIS” or other descriptions identifying treatment of specific quarters of the mammary gland.
Metabolic	Non-infectious metabolic disease	“Milk Fever”, “MF”, “Ketosis” – the majority of these treatments involved non antimicrobials.
Metritis	Uterine pathology of any type	“Metritis”, “METR”, “RP”, “Big Calf”, “Dystocia”, “Hard Pull” – any event involving therapy for uterine pathology.
Respiratory	Respiratory tract disease	“RESP”, “Respiratory”, “Pneumonia” – other abbreviations of these words
Other	Disease not included in other categories	“hardware” (traumatic reticulopericarditis), “pink eye” (bovine keratoconjunctivitis), “injury”, and a variety of other miscellaneous diseases.
Unknown	No information available	“fever”, “sick” - No information about underlying disease was present in the record to indicate what disease system was affected. Sometimes this is due to a lack of consistent identifiable clinical signs, other times is due to a lack of recording.

Variations in Treatment Documentation Methods

In addition to different formats and data sources, a variety of methods were encountered for recording animals who receive multiple treatments for the same disease event. These differences existed both within and across record system types. The variability between farms was much greater than the variability within a farm, though some variation in recording method was also noted within farms.

There were two predominant methods for recording repeated treatments of the same drug. The first method documented all treatments to an individual cow in a single entry (see **Table 2.1**

row 12). The second method listed each individual dose in the record as a separate entry (see **Table 2.1** rows 13-16). Regardless of original documentation method, all records were converted to the standard format that described all disease treatments as regimens (see **Table 2.1** “Number of Regimens” column). While these rows are still separate in the table example, the next step was to collapse them for each individual cow, disease, event, and drug so that all treatments included in a regimen are combined into a single data row.

For an example of the first method, imagine a case where a sick cow receives a dose of ampicillin trihydrate once daily for 5 days. Many electronic systems are designed in a way that requires only a single entry to enroll a cow in an electronic treatment protocol of multiple administrations. If the electronic system is being used to its full capacity, this cow will be “enrolled” in a 5-day ampicillin protocol (note: this “protocol” is an electronic protocol, related to but separate from the “farm protocol”). This means the electronic record system will cause her number to be listed with other animals needing a dose of ampicillin trihydrate each day for the next 4 days. This aids in both efficiency of treatment data input, as well as the logistics of identifying and gathering the appropriate list of cows to be treated each day. During survey data collection, farm workers and management indicated that deviations from automated lists sometimes occurred if the cow “looked great and was milking well” early in the treatment time frame. For example, if a cow showed clinical improvement on day 3, she was not treated on days 4 and 5; the fact that the last 2 doses were never administered does not get recorded. Therefore, retrospective evaluation of these records would lead to an over-estimation in the treatments administered.

For an example of the second method, envision the previous example of a cow receiving 5 days of ampicillin. Each daily administration would appear as a separate entry in the treatment record. If she was considered to have recovered on day 3 and does not receive treatment on day 4 and 5, the record will only list treatments on day 1, 2, and 3. This method more accurately reflects actual treatments, but it requires more labor (5x) associated with recording time for each treatment administered. Depending on how the electronic system is structured, it may or may not help in gathering the list of cows that need to be treated. Farms recording treatments using written formats primarily recorded them using this individual dose method.

Further complicating these differences in entry methods, some farms use both methods simultaneously depending on the drug or type of treatment. For example, intramammary

treatments administered in the parlor are often recorded on a notebook, and then entered into the computer on a periodic basis, typically varying from daily to weekly. These treatments are grouped by cow and entered as single lines in the record denoting the number of administrations, e.g., a cow treated with intramammary ceftiofur for 5 days was indicated by one record entry on a single day indicating that 5 doses were administered. On the same farm, sick cows were evaluated and treated in a different location for diseases other than mastitis. In this location, their electronic identification tag was scanned and each individual treatment was recorded daily; a cow treated with 5 days of ampicillin was recorded as a separate entry on each of 5 days.

There were occasionally instances where differences in recording methods arose within the same farm for the same treatment product, e.g. one cow that received 5 days of ampicillin was recorded once as a 5 day protocol, while other cows receiving the same treatment were recorded on each of 5 days. Generally, this type of variation in recording method only occurred for a few cows on a farm. A major emphasis of data analysis was to account for all of these instances and accurately report them.

It is important to note that these differences in documentation methods are driven by unique management needs on individual farms. For example, if a farm has new employees who are still in training they may wish to document each administration of a drug separately in order to detect needs for further employee training. On other farms, the employee doing the treatment is the owner, or a very experienced person. If labor is in short supply, the best approach might be to use a format that minimizes time spent entering treatment records. Due to these differences, it is unlikely that an attempt to create a single required format for record systems would be beneficial to the management of farms. It could potentially be harmful to those already facing labor shortages.

The solution to standardizing the differences in treatment method was a two-step process. First, the treatment record was standardized within the original data row. Second, a function was written in R to group the treatments that were part of a single regimen (meaning they belonged to a single cow and a single treatment event without a gap in time of more than 5 days from the other treatments). This function totaled the values for additive columns such as total mg in order to create a standardized row of data with all the information for a particular treatment regimen.

Standardization of Denominator - Cow Year

In order to facilitate benchmarking across farms and to provide context for the measurements of use, a denominator of adult cow years was chosen for use. Cow Years were calculated from the average inventory of adult dairy cows present on each dairy during a calendar year. This number includes all adult cows who have had at least one calf regardless of their current lactation status. It essentially represents a space on a farm occupied by an adult cow for one year. This space is not necessarily occupied by the same cow for the entire year, and therefore the percentage of spaces occupied by cows of a certain days in milk (DIM) may not be constant during the entire year.

In the farm dairy record systems this denominator is an average count of the number of cows who have a lactation number greater than zero measured at multiple time points throughout the year. The method of obtaining this count varied slightly based on the type of production records available. For farms that utilized Dairy Comp 305 systems (26/32), this number was calculated as a weighted weekly average of the farm inventory of adult cows (LACT>0) for the given year. For all other farms it was calculated as a weighted average of the farm inventory on DHIA test days which varied from 6 to 12 times per year.

Animal days at risk was also considered for use as a denominator. Due to distinct events that happen during the dairy production cycle, all days a cow is present on the dairy do not carry the same disease risk for each adult cow. For example, cows with days in milk of less than 10 have a higher risk of metritis while cows 60-300 days in milk have little to no risk of metritis. Risk for mastitis and lameness occurs throughout the production cycle. Since we are reporting use over a calendar year for all cows present on farm, the average inventory method allowed standardization by number of animals and time at risk for any disease treatment. It should be noted that the denominator used here is not necessarily identical to one which might be used to calculate disease incidence for a specific disease. When considering risk for all diseases lumped together, one yearly inventoried cow can be thought of as nearly equivalent to one animal year at risk. If this assumption is made it should be recognized that the method of calculation is simply a weekly inventory and contains no information about which cows were present between counts. This creates an assumption that the variability with which cows leave and enter the herd is relatively constant and normally distributed. It should also be re-emphasized that the 365 cow days at risk

represented by one animal year might be made up of days contributed by multiple individual animals, not necessarily one individual cow.

The use of milk as a denominator was considered. In some ways the use of milk as a denominator might be more relevant than the number of cows. For example, use per cow might be the same across 2 dairies, while use per 100lbs of milk produced might be lower in one of these dairies. When benchmarking at the dairy level, it may be important to distinguish between dairies based on antimicrobial use in relation to milk production. In a dairy system meat is also a product. A method of appropriately allocating use to one or both products is complex and the details of this have not yet been determined. For this reason, this document only reports use on a cow year basis.

Quality Assurance Procedures

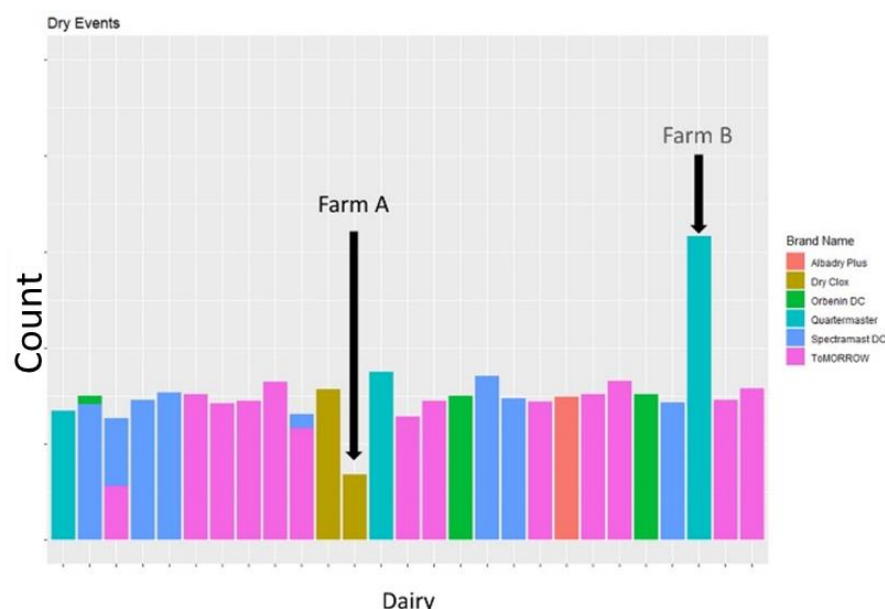
The large amount of data handling necessary for standardization required a great amount of care to preserve data accuracy. Due to the volume of data (165,000 treatment events that originated as greater than 1 million rows of data), the assessment of quality assurance was predominantly performed graphically and by utilization of the skimr package and skim function in R to look for missing values in the data set. 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.

Although farms were entered into the database individually, as soon as the data were standardized it was compared to other farms in the system. Initially the main categories of Dry Cow treatment, No Treat, Non-Antimicrobial, Intramammary, Non-Intramammary, and Unknown were evaluated at the farm level to identify outliers.

All extreme values were investigated to ascertain if the value was consistent with original data or if it was an error in the data standardization process. If outliers required further investigation, data were graphed in different manners to identify the source of the problem or confirm data accuracy. For example, **Figure 2.2** shows dry cow treatments by farm colored by drug. This allowed the determination that selective dry cow therapy was being practiced on Farm A, while there was an error in the code for standardization of the number of administrations on Farm B. Once the main categories were validated, outliers in the regimen parameter description plots (Chapter 3 – Regimen parameter descriptions) were colored by farm and investigated.

Because these plots are at the cow level, this allowed for the quality of data to be examined at a very granular level.

Figure 2.2 Identification and Exploration of Extreme Values



Output Measures Available from the Standardized Record

The reason for record standardization is to derive final data that can be consistently summarized in ways that provide an accurate picture of antimicrobial use, while making as few assumptions as possible. The final standardized record contains 7 output columns: Disease Syndrome, Drug Product, Route of Administration, Number of Administrations, Interval Between Administrations, Administration Dose, and Detected Disease Incidence. The Detected Disease Incidence column is used strictly for benchmarking and outcome evaluations. The output generated from this column will be used as a tool in future efforts to associate antimicrobial use with disease incidence and treatment outcome. It will not be included in this report other than to discuss the relationship between detected disease and outcomes in the beginning of chapter 3. The other 6 columns all interact to provide a complete description of antimicrobial use. This interaction is best described by clearly defining the meaning of “Regimen” as it is used in this report.

A regimen describes treatments to one animal for one disease event, and it is product specific. For example, cow number 33 is diagnosed with metritis and treated with 1 gram of ceftiofur HCL, once a day for 3 days (**Figure 2.3**).

If all methods of documentation used a method which captured each individual dose, then a count could be generated from the number of calendar days product was administered. This would be consistent with the proposal by the Infectious Disease Society of America (IDSA) to calculate the number of Days of Therapy (nDOT). The reality of the records available is that many of them list treatments as “protocols” documenting a number of doses. This number of doses can be reliably associated with a known interval between doses. These 2 numbers can be used together to calculate the total “Time Frame” between administration of the first dose and administration of the final dose in one regimen (**Figure 2.4** blue and purple). This method of Time Frame calculation also allows the regimen descriptions to remain objective, avoiding assumptions about the amount of time single injection injectable products might exert an effect.

The column “Administration Dose” provides the number of grams the animal received at each administration generating the output “Grams per Administration” (**Figure 2.4** black). The “Grams per Regimen” is then calculated by multiplying the “Administration Dose” by the Number of Administrations” (**Figure 2.4** red). Route of Administration is also included in the database and is therefore available as a variable by which descriptions of use can be stratified (**Figure 2.4** green). The large arrows in **Figure 2.4** indicate information that is generated as outputs from the standardized data. Although not pictured, each regimen is also linked to a disease and specific drug to allow grouping by these variables in the report.

Figure 2.3 Example Regimen: Treatment for Metritis using 3 Administrations of Ceftiofur HCL, Administered at 24 Hour Intervals

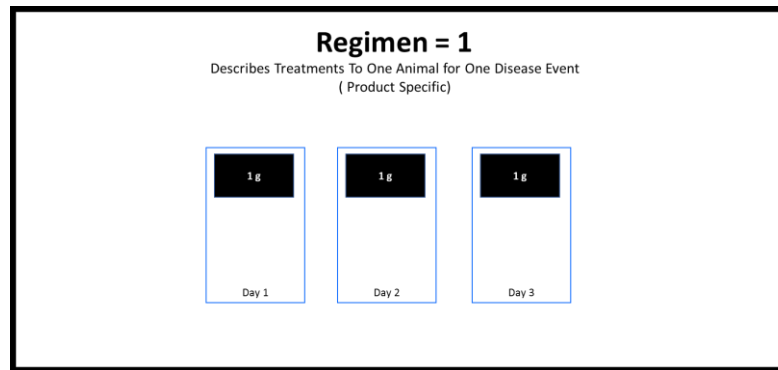
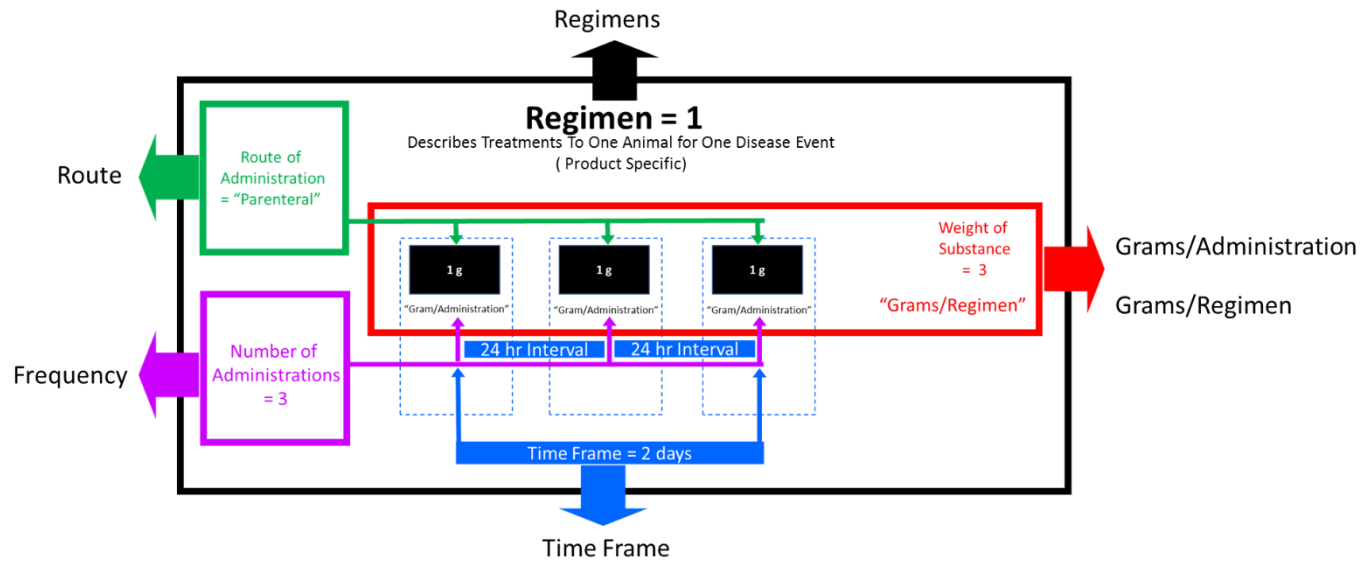


Figure 2.4 Outputs Available From a Completely Described Regimen



Regimens as a Numerator

One advantage of using defined regimens as a measure of antimicrobial use is that they provide a great deal of granularity associated with the use measure. The primary advantage of this is that, if interpreted carefully, they can be used as a proxy measure for detected disease incidence. This means that benchmarks provided to farms based on regimens allow farm management to identify the detected disease associated with use and question the root cause of the level of detected disease. However, if they are to be interpreted in this manner it is important to keep several nuances in mind.

- Relapse or Recurrent Disease - Recall that multiple administrations of the same drug product for the same disease will be counted as “one” regimen provided there is not a gap in therapy of more than 5 days between administrations. This is particularly relevant for mastitis when considering the infection of another quarter during or shortly after the first quarter was treated. If there is a 5 day gap between the first treatment of the newly infected quarter it will be counted as a new regimen, but if the gap is less than 5 days and treatment is with the same drug it will simply increase the Time Frame and number of Administrations, but it will not count as a new regimen.
- Combination Products - It should be noted that some drug products are sold in a manner where 2 drugs are marketed in a formulation where these drugs could not be administered separately. Since they are never administered separately their effect is always due to their simultaneous administration. For this reason they are counted as a single regimen. However, the milligrams are described separately for each active substance in the formulation.
- Polytherapy - There are also times when an animal might be treated simultaneously with multiple products aimed at treating a single disease episode. For example, a cow with severe mastitis might be treated with both parenteral oxytetracycline and intramammary cephalosporin. These instances of “polytherapy” (drugs not packaged together but administered to the same cow) are counted as separate regimens.

In addition to the above nuances the Reason for treatment must also be considered in order to interpret the relationship between Regimens and Detected Disease. For this reason a database column was created assigning each regimen to one of the following “Reason” for use categories.

1. “Other Treatments” – Detected disease results in treatment with one antimicrobial product.
 - a. Example: an animal is diagnosed with metritis and treated with parenteral ceftiofur HCL for 3 days.
2. “Clinical Mastitis” – Mastitis is a disease that occurs at the quarter level rather than the cow level. Therefore, the disease incidence of mastitis at the quarter level is underestimated by regimens as they are calculated at the cow level.
 - a. Example: A cow with disease in a single quarter is counted as one regimen, and a cow with disease detected in 3 quarters is also counted as a single regimen. This difference is apparent only in the regimen description details where the cow treated in 3 quarters received a mg per administration dose that was 3 times that of the cow treated in a single quarter.
3. “Dry Cow” – In dairy production systems, a long acting intramammary product may be used at the beginning of the dry period to control mastitis pathogens rather than waiting for post-calving disease to occur when treatment failure is more likely. Dry cow therapy is administered for 2 reasons; to cure subclinical infections and to prevent new infections during the dry period. Some farms treat all animals (commonly referred to as “blanket” dry cow therapy) and other farms select animals for treatment based on their milk quality and treatment history (“selective” dry cow therapy). While this use is related to disease pressure, the relationship is more complex than for clinically detected disease and differs from farm to farm based on which specific pathogens predominate and the incidence of chronically infected cows.

As summarized in **Table 2.4**, treatments in the category Other Treatments have a 1:1 relationship between the number of regimens and the incidence of detected disease. However, treatments in Dry Cow or Clinical Mastitis categories are not necessarily 1:1.

Table 2.4 Relationships Between Detected Disease Incidence and Regimens based on Reason for Use

Description	Reason for Use	Ratio of Detected Disease to Reported Regimens
All other treatments that were not administered by intramammary route	Other Treatments	1:1
Intramammary treatment for clinical mastitis. Identified by the product used in conjunction with information from the Survey and Protocols.	Clinical Mastitis	$\geq 1:1$ (1-4 quarters)
Intramammary treatment at the end of lactation. Usually identified in the record as “Dry” but on a few farms the only available method for detection was by looking at “string changes” to the String identified as Dry. Distinction between “Blanket” and “Selective” is not documented in the database but known to farm management.	Dry Cow (“Blanket”)	$< 1:1$ (exact number dependent on multiple factors affecting incidence of subclinical mastitis and risk of new infection)
	Dry Cow (“Selective”)	1:1

When blanket dry cow treatment is used, it is assumed that some of the treated animals have no detectable disease and therefore the relationship of Regimens to Detected Disease is greater than 1. This can vary significantly by farm based on the amount of subclinical mastitis present. If 50% of the cows are subclinically infected with mastitis then the ratio of Regimens to Detected Disease is 2:1. However, if only 20% of cows are subclinically infected the ratio is 5:1. Since selective dry cow therapy employs diagnostics to identify subclinically infected individuals the relationship is then 1:1.

Mastitis is a disease that happens at the quarter level, but regimens are reported at the cow level. Therefore, the relationship between detected disease and reported regimens for Clinical Mastitis depends on the number of quarters treated. If clinical mastitis is detected in a single quarter then the relationship is 1:1. However, if multiple quarters are simultaneously identified as infected the number of regimens is still one since regimens are defined at the cow level.

The use of polytherapy occurs occasionally, but makes up a very small percentage of use on most farms. Therefore, instances of polytherapy use are not identified or reported separately in the database format. It should simply be noted that if the number of regimens is interpreted as a proxy for disease incidence these instances of polytherapy would slightly inflate the estimation of disease incidence in any category.

Due to the relationships described above, Regimen measures are reported in a stratified manner that allows more straightforward interpretation of each regimen as it relates to detected disease pressure. The stratifications are labeled in graphs and tables as: “Dry Cow”, “Clinical Mastitis”, and “Other Treatments”.

Grams as a Numerator

The amount of active substance by weight is a common measure of antimicrobial use and can be calculated from the regimen descriptions. It is presented in the following report due to its commonality of use, rather than for its utility in driving stewardship. The exact relationship between the grams of drug used and the magnitude of resistance selection pressure is unknown. Measurements that sum grams of multiple substances are not directly useful for stewardship decisions at the farm level as this measure is heavily influenced by drug potency. However, it is perhaps the most straightforward measure to define, and is reported here to allow comparisons to other measures.

Weight in this report is defined as the weight of active drug product listed on the product label. Treatment records frequently document use as a volume of product or count of dose sized packages. The brand specific product label was used to convert this volume or count to mg of active drug substance. For procaine penicillin G the label indicates drug amount as International Units (IU) rather than mg. These products were converted to mg using a conversion factor of 1666.67 IU per mg of procaine penicillin G.

Final Measures Reported

The process of data collection, standardization, and quality assurance required significant time and resources. While this process was outlined as essentially linear, it should be noted that, in reality, the process was extremely iterative with many loops back to the original data, re-standardization, graphing, and another loop back around. The standardization process was initially

performed manually using sort functions in Excel. This was necessary to learn and understand the nuances of the data. For subsequent years, the process of data cleanup shifted gradually to the use of regular expressions and “stringr” codes in R to make the process more efficient and accurate. Without initial efforts at intimate understanding of the data, standardization could not have been adequately achieved. The standardization process is what allowed the data to be useful for describing antimicrobial use practices and methods of measurement within these systems. It also allowed for measures to be stratified by numerous variables, allowing patterns of use to emerge. Original farm record data included 163,693 documented treatment regimens. Of these, 97,336 were antimicrobial treatments, and the others were non-antimicrobial therapy or detected disease documented as no treatment. These treatments are summarized as follows. Chapter 3 describes antimicrobial use on participating farms by measures of Regimens per Cow Year along with the complete regimen descriptions for all antimicrobial products administered. Due to the commonality of reporting antimicrobial use by weight of active substance Total Grams per Animal Year is also reported in chapter 3. This allows Chapter 4 to explore multiple common measures of use including Regimens, Grams, Days of Therapy, Defined Daily Doses, and Defined Course Doses. The nuances of each of these measures within dairy systems are discussed in Chapter 4. Unanswered questions are discussed in Chapter 5 to provide a basis for future research priorities.

Chapter 3 - Measurements of Antimicrobial Use on Participating Dairy Farms

As discussed in chapter 1, all measures of antimicrobial use currently available have distinct benefits, drawbacks, and confounders. The primary objective of this chapter is to describe antimicrobial use as completely and accurately as possible by reporting measures that inform research priorities for driving antimicrobial stewardship at the farm level. All measures are reported using a denominator of cow years. Fully described regimens are reported as the main numerator. Due to common use as a measure of antimicrobials, total grams of active substance are also reported. This measure will be used in chapter 6 to demonstrate characteristics of different measures of use.

All values are reported as composite values for total use by year, as well as stratified by disease and drug class. These stratifications are further subdivided by the three categories of reason for use: “Dry Cow” therapy, “Clinical Mastitis” therapy, and “Other Treatments”. Dry Cow therapy is defined as intramammary treatment at the end of lactation aimed at curing chronic mastitis infections and preventing new mastitis infections. Clinical Mastitis therapy is defined as intramammary treatment of cows identified as having clinical mastitis by farm definition. Other treatment is defined as all other non-intramammary treatments for all diseases (including systemic treatments for mastitis).

The main overarching methods of reporting are “Study Level” where use is summed across all dairies for each category, and “Dairy Level” where use was summed for each category within each dairy and results are reported as means and medians with descriptions of variation.

“Study Level” - Reporting of Metrics Related to All Dairies Combined

This reporting method does not attribute antimicrobial use to a particular dairy, but rather sums total use in each category across all dairies. When calculating values for Study Level measures the following steps occurred. All steps were performed independently for 2016 and 2017.

- **All regimens of each antimicrobial product** recorded in the standardized treatment record were summed across all dairies. Although occasionally reported as composite totals, each regimen maintained its association with disease and drug class within the

database. Due to the differing relationship to detected disease incidence, the following use categories were also maintained as each value was calculated.

- Dry Cow
- Clinical Mastitis
- Other Treatments
- **All grams of each antimicrobial product** recorded were summed across all dairies within the same use categories as above.
- **All cow years** were summed across all dairies to derive the total cow years represented in the data.
- **The total regimens** for each antimicrobial product recorded within each category were divided by the total cow years to derive the final metric of regimens / cow year for each drug within each category.
- **The total grams** for each antimicrobial product recorded within each category were divided by the total cow years to derive the final metric of grams / cow year for each drug within each category.

“Dairy Level” - Reporting of Metrics as Means, Medians, Standard Deviations, and Ranges

This reporting method first calculates the designated reporting metrics within each dairy. Next, the mean, median, standard deviation, and range was calculated for each reporting metric in each use category for the dairies reporting this use. Drug class is not reported at this level due to extreme variation in the number of dairies using each class. The number of dairies reporting for the indicated subcategory is also reported.

- **Regimens of each antimicrobial product** recorded in the standardized treatment record were summed for each dairy individually. Each regimen maintained its association with disease and drug class within the database. Due to the differing relationship to detected disease incidence, the following use categories were also maintained as each value was calculated.
 - Dry Cow

- Clinical Mastitis
- Other Treatments
- The total grams of all antimicrobial products recorded were summed **within each dairy** for each of the use categories described above.
- Cow years were summed **within each dairy** to derive the total cow years for that dairy.
- **Within each dairy**, the regimen total within each category was divided by the total cow years to derive the final metric of regimens/cow year within each use category for that dairy.
- **Within each dairy**, the gram total within each category was divided by the total cow years to derive the final metric of grams/cow year within each use category for that dairy.
- The counts of dairies reporting use in each use category were determined for each category reported.
- Mean, median, standard deviation, and range were calculated for each reporting metric in each use category for the dairies in which the data indicated use in that use category. Calculations only included the dairies reporting use of any antimicrobial in that use category. The reported mean, median, standard deviation, and range values do not include any zero (nonuse) values in the calculations.

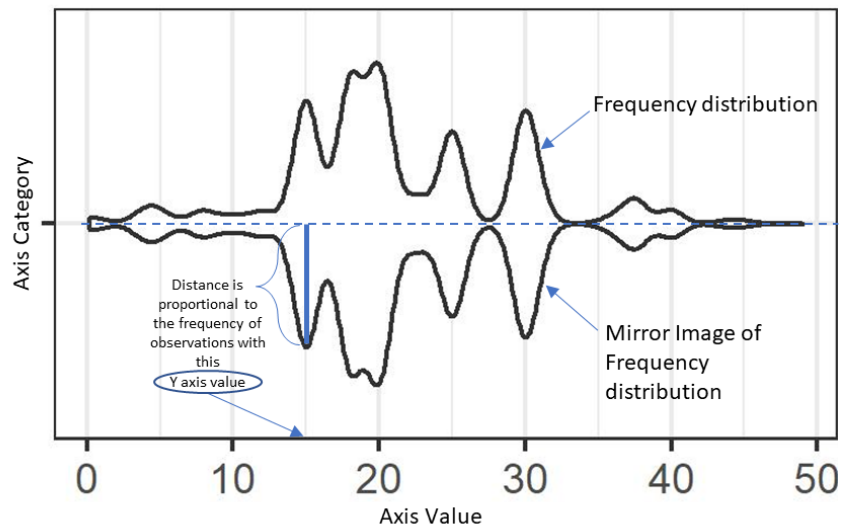
Graphical Reporting

The data presented in the following chapter is reported both in table and graphical format. Graphical reporting methods include bar charts, boxplots, violin plots and combination plots. **Figure 3.1** shows annotated examples of the more complex plot types. Violin plots are basically density plots with the mirror image plotted on a center line. They are a way to more accurately visualize distributions of data. Box plots are a way to visualize the summary parameters of a distribution as well as the outliers. Combination plots are a way to view violin and box plots simultaneously.

Figure 3.1 Descriptions of Graphical Figure Types

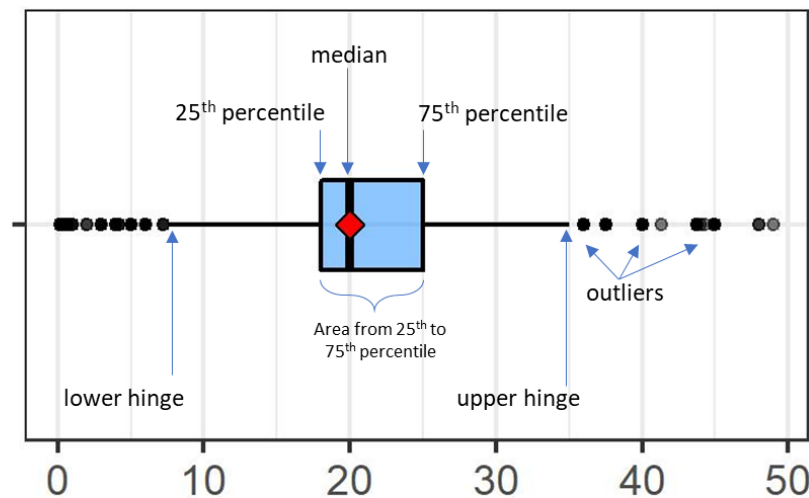
Violin Plots

Smoothed frequency distribution plots. The parameters of the plot are set such that total width of each category is the same. Therefore the magnitude of the distribution is interpretable only within categories and not across categories.



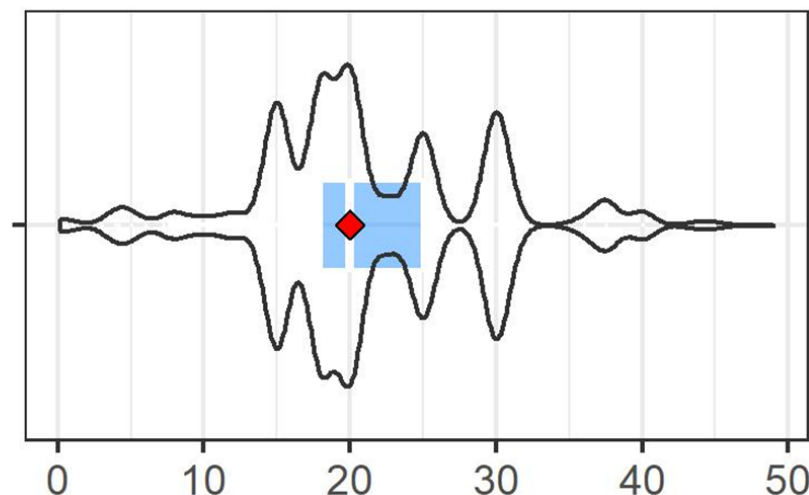
Box Plots

Represent distribution as percentiles and outliers. The red diamond is plotted to additionally emphasize the median. An outlier is defined as a value beyond the hinge. The lower and upper hinges are calculated from the interquartile range. Hinge values = $1.5 \times \text{IQR}$.



Combination Plots

Represent a boxplot and a violin plot of the same data plotted simultaneously. The color of the lines and outliers of the boxplot are changed to a white color to make them disappear and improve readability.



Regimens by Year (2016 and 2017)

The process of data standardization resulted in 97,336 total antimicrobial regimens documented in the treatment records collected from 2016 and 2017 on participating farms. The values of regimens per cow are presented at both the study level and dairy level in **Table 3.2** (by disease). The values of regimens per cow with respect to antimicrobial drug class are presented in **Table 3.1** at the study level. The top row in both tables represents the overall use by year. The final column in both **Table 3.1 and 3.2** is labeled “count of dairies” and represents the number of dairies (in years 2016 and 2017) reporting use in the specified category. As very few dairies were represented by certain drug classes, use by antimicrobial drug class is not reported at the dairy level.

The number of total regimens per cow year are shown in **Figures 3.2** (study level) and **Figure 3.3** (dairy level). The top row in **Table 3.2** displays the calculated values represented in the **Figures 3.2 and 3.3**. The rest of the table rows display all the values represented in **Figures 3.4-3.9** for regimens per cow year at both the study level and farm level. These include all regimens in the 3 main categories described previously (Dry Cow, Clinical Mastitis, Other Treatments) for all medically important antimicrobial drug classes. Non-medically important and non-antimicrobial regimens are not reported here.

There is no mean or median for study level values because this is one single value summed for all cows in the study and divided by all cow years represented. The dairy level values display the variation present at the farm level. It is important to note that when measuring by total regimens, dry cow therapy drives the measure so any change in the percent of cows receiving dry cow therapy may greatly affect this measure when reasons for use are combined.

Figure 3.2 Total Regimens per Cow Year – Study Level

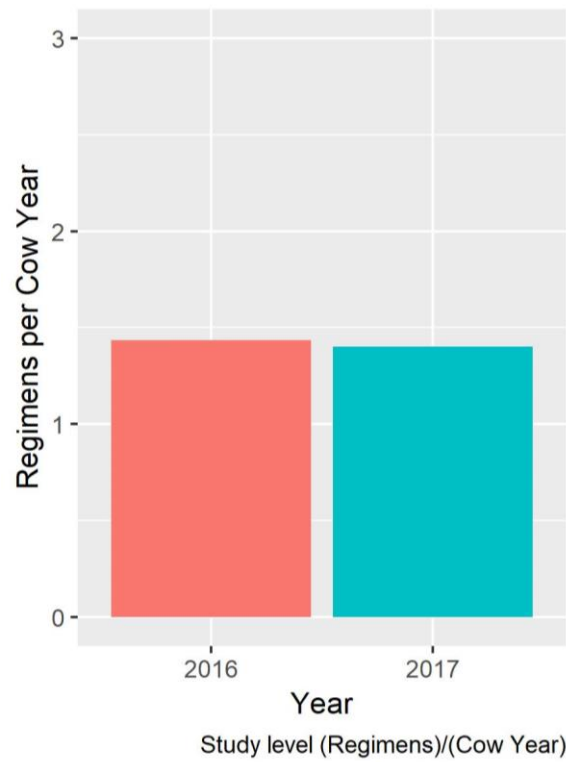


Figure 3.3 Total Regimens per Cow Year – Dairy Level

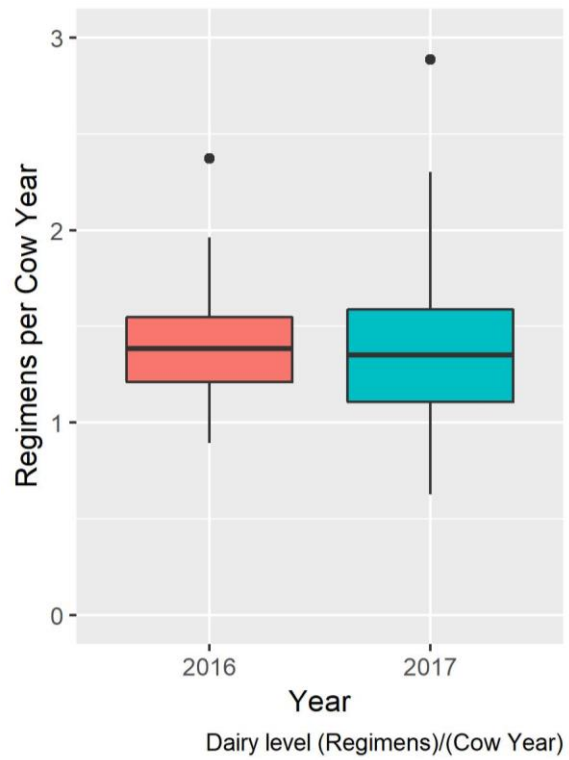


Table 3.1 Regimens per Cow Year by Antimicrobial Class and Reason for Use at Study Level

Regimens per Cow Year				
Antibiotic Class	Reason for Use	Year 2016	Year 2017	Count of Dairies
		Study Level	Study Level	
All Classes	All Reasons	1.434	1.403	29
Cephalosporin	Clinical Mastitis	0.260	0.233	29
	Dry Cow	0.542	0.486	20
	Other Treatment	0.250	0.256	28
Penicillin	Clinical Mastitis	0.017	0.034	13
	Dry Cow	0.142	0.178	13
	Other Treatment	0.125	0.127	28
Aminocoumarin	Dry Cow	0.014	0.010	2
Aminoglycoside	Dry Cow	0.030	0.017	3
Amphenicol	Other Treatment	0.0003	0.0003	4
Lincosamide	Clinical Mastitis	0.025	0.025	17
Macrolide	Other Treatment	0.00006	NA	2
Sulfonamide	Other Treatment	0.014	0.013	5
Tetracycline	Other Treatment	0.016	0.023	19

Table 3.2 Regimens per Cow Year by Disease and Reason for Use at both Study Level and Dairy Level

Regimens per Cow Year														
Reason for Use	Disease Syndrome	Year 2016						Year 2017						Count of Dairies
		Study Level	Dairy Level					Study Level	Dairy Level					
			mean	median	sd	minimum	maximum		mean	median	sd	minimum	maximum	
All Reasons	All Diseases	1.434	1.400	1.384	0.354	0.894	2.371	1.403	1.397	1.350	0.461	0.626	2.887	29
Clinical Mastitis	Mastitis	0.302	0.316	0.301	0.206	0.009	0.890	0.292	0.301	0.292	0.203	0.014	0.733	29
Dry Cow	Mastitis	0.727	0.730	0.735	0.077	0.438	0.851	0.691	0.701	0.719	0.111	0.301	0.826	29
Other Treatment	Unknown	0.092	0.109	0.039	0.180	0	0.779	0.111	0.132	0.044	0.225	0	0.872	24
	Respiratory	0.039	0.053	0.032	0.081	0.001	0.379	0.050	0.076	0.038	0.170	0.003	0.855	25
	Other	0.058	0.031	0.012	0.063	0.001	0.267	0.027	0.019	0.014	0.027	0	0.115	19
	Metritis	0.117	0.132	0.097	0.107	0.001	0.372	0.120	0.122	0.104	0.081	0.003	0.317	27
	Metabolic	0.002	0.011	0.005	0.015	0.001	0.037	0.001	0.005	0.004	0.004	0.001	0.011	7
	Mastitis	0.041	0.065	0.020	0.103	0.001	0.403	0.047	0.086	0.018	0.142	0.001	0.599	24
	Lameness	0.030	0.032	0.028	0.024	0.002	0.112	0.039	0.043	0.031	0.040	0.001	0.167	24
	Displaced Abomasum	0.014	0.023	0.012	0.034	0.001	0.134	0.016	0.023	0.018	0.030	0	0.137	19
	Digestive	0.013	0.020	0.015	0.018	0	0.050	0.008	0.012	0.005	0.015	0.001	0.038	13

Regimens by Disease Syndrome

Use is presented in association with the detected disease syndrome treated to facilitate thought about strategies that may be helpful in reducing use by reducing detected disease or optimizing regimens. **Figures 3.4** (study level) **and 3.6** (dairy level) display regimens per cow year for each disease syndrome. Antimicrobial use stratified by Reason for use are illustrated in **Figure 3.5** (study level) and **3.7** (dairy level). All values represented in these figures are presented in **Table 3.2**.

There are some farms who are culturing all mastitis cases prior to treatment. On these farms, cases of mastitis are identified, cultured, and treated (or not treated) according to culture results. This diagnostic method identifies animals not likely to benefit from treatment, and so they are not treated. These instances of “No Treatment” are not reported here, but are reported in benchmark reports for participating farms. Reporting decisions for “no treatment” or “non-antimicrobial” treatment are helpful in differentiating instances when use is low due to good management practices such as culture-based treatment allocation, from instances when low use is the result of poor disease detection potentially leading to a decrease in animal welfare or system sustainability, i.e. recording disease detection with the decision to not use an antimicrobial for treatment demonstrates that low use is not due to a lack of disease detection.

Consistent with other studies, this study found that dry cow therapy for mastitis is the primary driver of use when measured by regimens (Treatment Incidence) documented in the treatment record.⁹⁶ When use is measured by Regimens the highest use is for Dry Cow treatments, followed closely by use for Clinical Mastitis. The highest value for a single disease syndrome within the Other Treatment category is for Metritis, but this use is about 1/3 of the use for Clinical Mastitis, and less than 1/5 of the use for Dry Cow.

The current data set for 2016 includes only one farm using selective rather than blanket dry cow therapy. In 2017 there were 3 farms using selective dry cow protocols. When viewing the benchmarks some producers were confused by their calculated value for Dry Cow use. They were assuming that since they are using "blanket" dry cow therapy the value would be closer to 1 indicating that all lactating cows get treated. However, the calculated value is logically not near 1, even if all cows achieving dry cow status are treated. The average cull rate on U.S. Dairy farms is about 30%, the animals that get culled do not receive dry cow treatment as they are sold prior to

completion of their current lactation. Therefore, the value of 0.730 Regimens per Cow Year in 2016 is a very logical use measurement in this category.

Overall use for Dry Cow therapy decreased from 2016 to 2017 within participating farms. While it is tempting to attribute this reduction to farms switching from blanket to selective dry cow programs it is unlikely that this is the main driver of use. Most farms that switched to selective dry cow were small, and therefore contributed less to the study level use. This change in the amount of dry cow use at the study level from 0.73 to 0.69 Regimens per Cow Year (**Table 3.2** row 3, study level) is more likely related to changes in culling practices on some of the larger farms. However, at the dairy level the farms representing the outliers at the low end of Dry Cow therapy are indeed those using "selective" Dry Cow protocols: one farm in 2016 and 3 farms in 2017 (**Figure 3.7**).

There is a considerable amount of variation at the dairy level for all disease syndromes (**Figures 3.6 and 3.7**). This variation is due to a combination of variation in disease pressure, case definitions, and the application of case definitions. When use is measured at the dairy level there is a large amount of variability between dairies. The extreme values seen for a few dairies in the "Unknown" disease category are due to a record keeping style that was aimed more directly at residue avoidance and documented treatments and disease using two separate data entries. For treatments with intramammary products these could easily be tied to the Mastitis disease syndrome. For other disease syndromes, recorded disease and treatments had to be matched by date and cow number. For some farms this worked well, but for 3 dairies the disease syndrome was not always documented resulting in extreme values in the "Unknown" disease category.

Regimens reported under the disease syndrome of Mastitis within the Other Treatment reason for use are systemic antimicrobial treatments of cows with mastitis (**Figures 3.5 and 3.7**). This is an infrequent occurrence compared to intramammary treatments for this disease. There is a great amount of variability between farms for Clinical Mastitis regimens per animal year. Farms at the low end of these values were questioned to confirm that use was indeed this low. These farms are likely over represented in this convenience sample due to the number of herds who chose to participate that have extremely good udder health and milk quality. Several farms had average bulk tank somatic cell counts of less than 80,000. The other factor contributing to this value is that there are some farms who choose not to treat the majority of cows with mastitis due to the predominant pathogens present not responding to treatment. Since non-antimicrobial regimens

are not reported here, this also lowers this value compared to the actual detected disease incidence because if they are treated with non-antimicrobial drug (i.e. flunixin) it is not reported.

To those familiar with diseases in dairy cattle at first the value for lameness seems very low. However, it should be remembered that the values reported here only include antimicrobial regimens, and do not report non-antimicrobial regimens as the benchmarks do. Many lameness treatments consist of only corrective trimming or mechanical drainage of an abscess. When antimicrobials are used for lameness frequently it is topical application of a small amount under a bandage. As described in chapter 2 the variability in record keeping for lameness made this topical use difficult to capture on some farms. When the non-antimicrobial regimens are not reported then this value is only a measure of the incidence of lameness where antimicrobials were used for treatment, and does not provide a proxy measurement for the overall incidence of lameness.

Figure 3.4 Regimens per Cow Year at Study Level by Disease Syndrome

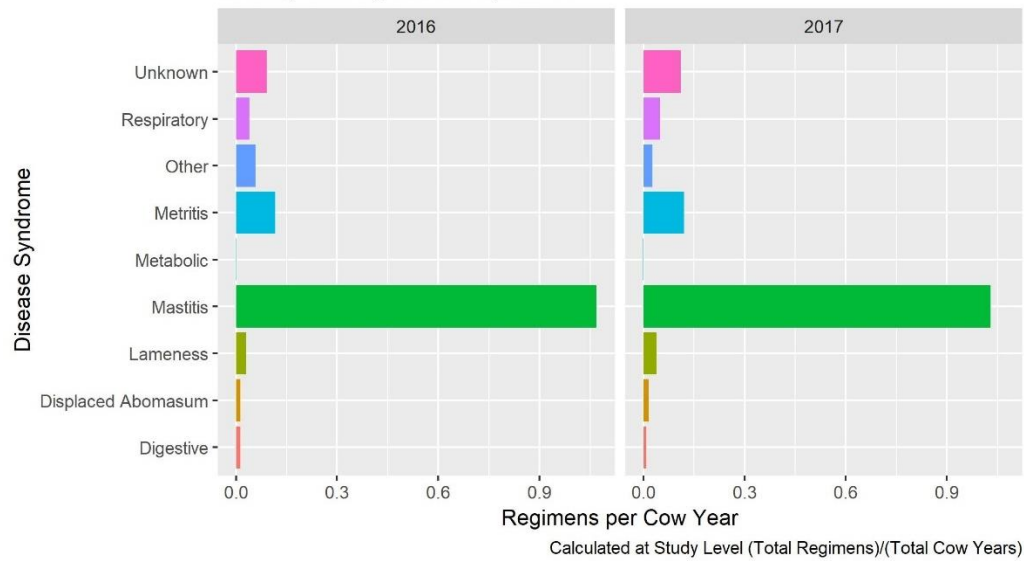


Figure 3.5 Regimens per Cow Year at Study Level by Disease Syndrome and Reason for Use

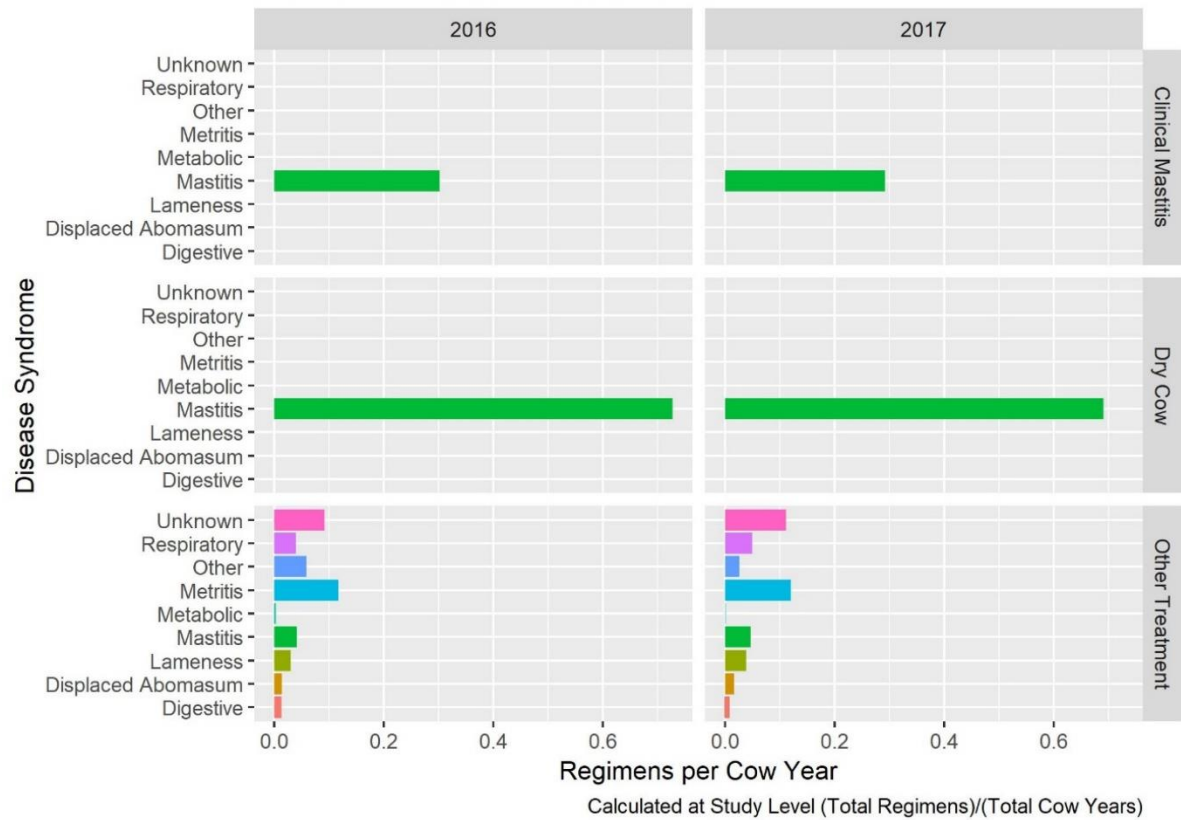


Figure 3.6 Regimens per Cow Year -Dairy Level by Disease

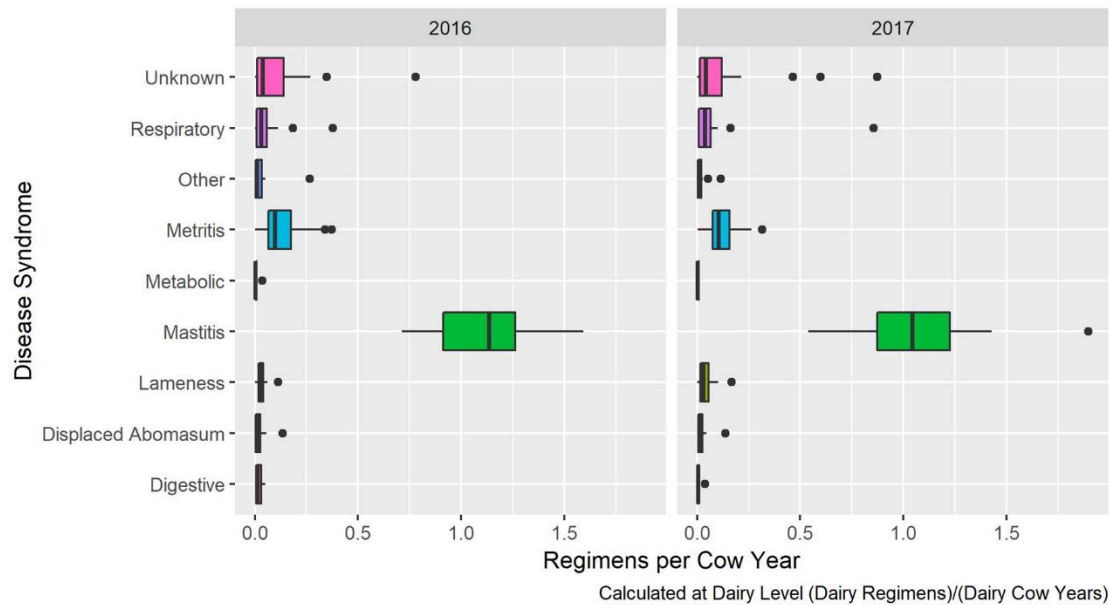
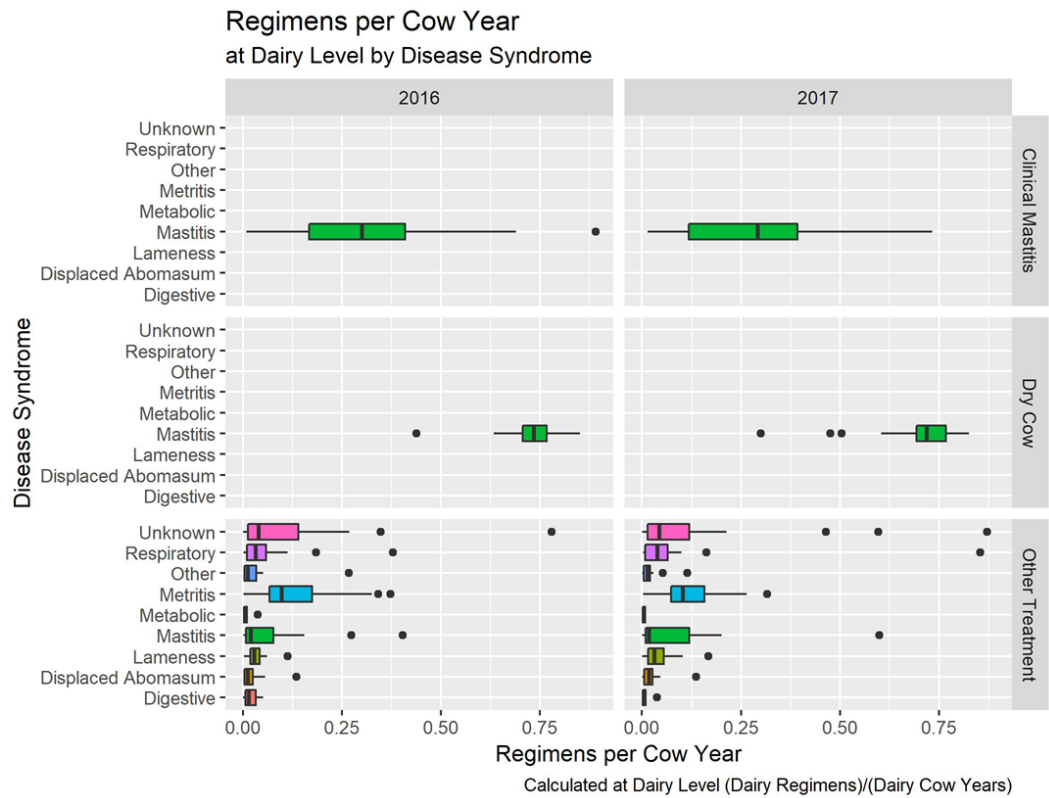


Figure 3.7 Regimens per Cow Year -Dairy Level by Disease and Reason for Use



Regimens by Drug Class

Dairies vary in their specific drug choices used for therapy. Due to this variation, most farms use only a few specific drug products. For this reason, every dairy does not contribute to use in every antimicrobial drug class (**Table 3.1**, column “Count of Dairies”). Therefore, use by class is described only at the study level, as use at the dairy level is not readily interpreted. It is important to remember that some drug classes include multiple products, while other drug classes may include only a single product. This makes it challenging to interpret use divided by class, as use across classes may represent a very different number of dairies or drug products. **Table 3.1** shows the values for Regimens per Cow Year by antimicrobial class stratified by relationship to detected disease. **Figure 3.8** (study level by Class) and **Figure 3.9** (study level by Class and Reason) show Regimens per Cow Year stratified by Class and Reason for use.

Cephalosporins and penicillins are represented in all categories of Reason for use. All dairies use cephalosporins, and all but one record the use of penicillins.

Aminocoumarins and aminoglycosides are each represented by a single combination product that also contains a penicillin. Both products are used only for Intramammary Dry Cow therapy.

Lincosamides are also represented by a single product labelled for Clinical Mastitis. According to survey data another lincosamide product is occasionally used topically in a footbath or as a foot spray for control of digital dermatitis. While this product appeared in the purchase data, its use was not recorded during the 2016-2017 time frame and surveys indicated that dairies routinely used either copper sulfate or formalin in their footbaths rather than a lincosamide.

Macrolide use represented an extremely small amount of use. It is likely that these individual animals were actually treatment record errors at the dairy level where a calf treatment was erroneously assigned to an adult cow number. Since we have no way to confirm that this was indeed a recording error, we report it here in the interest of transparency. Macrolides are not illegal for use in adult dairy cows but their use is extralabel and rarely indicated as the milk and meat withdrawal times are impractically long.

It is informative to compare **Figures 3.8 and 3.9** that show Regimens per Cow Year with **Figures 3.20 and 3.21** that show Grams per Cow Year. These comparisons illustrate that as priorities are identified for antimicrobial stewardship programs the type of measurement used to quantify use warrants careful consideration.

Figure 3.8 Regimens per Cow Year - Study Level by Antimicrobial Class

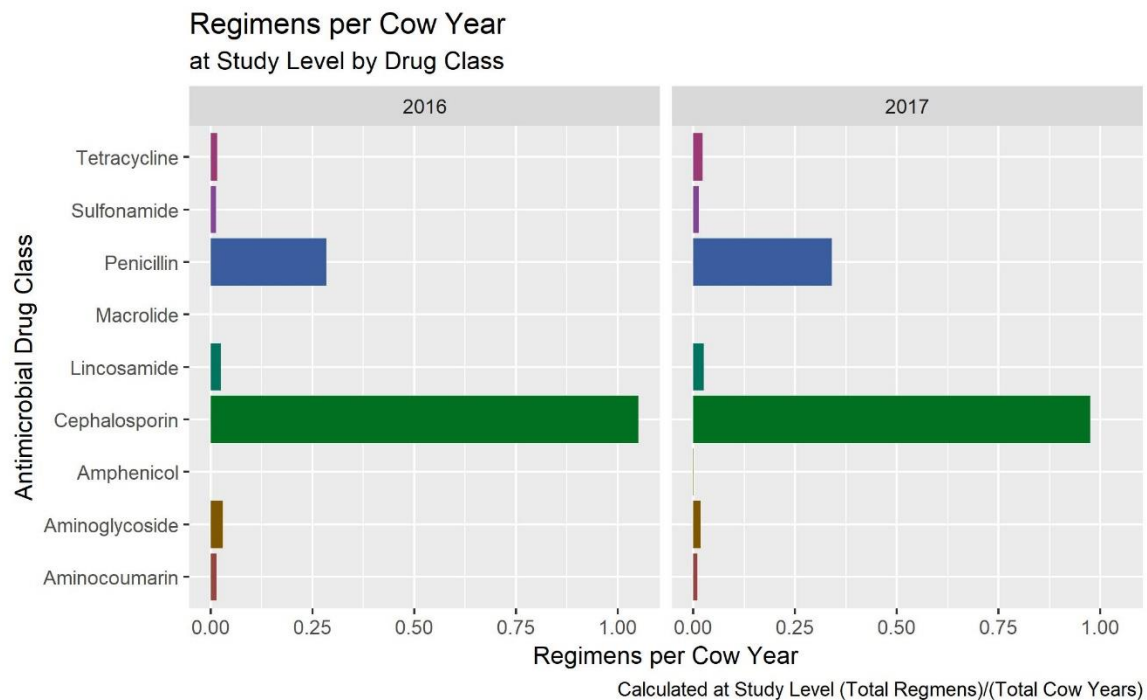
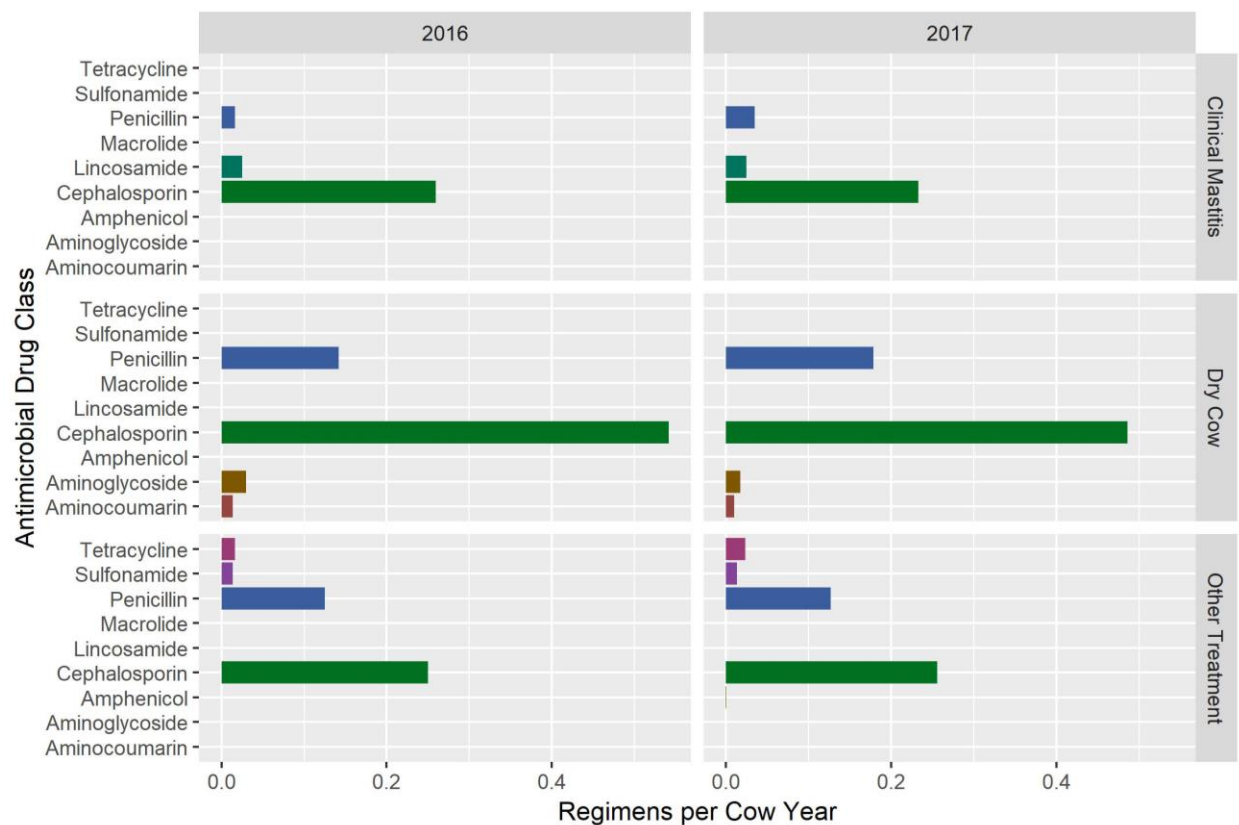


Figure 3.9 Regimens per Cow Year - Study Level by Antimicrobial Class and Reason for Use



Regimen Parameter Descriptions

As shown in **Table 3.3** and **Figure 2.4**, regimens are described by 5 parameters: drug product, route of administration, number of administrations, time frame between first and last administration, and amount of drug product administered.

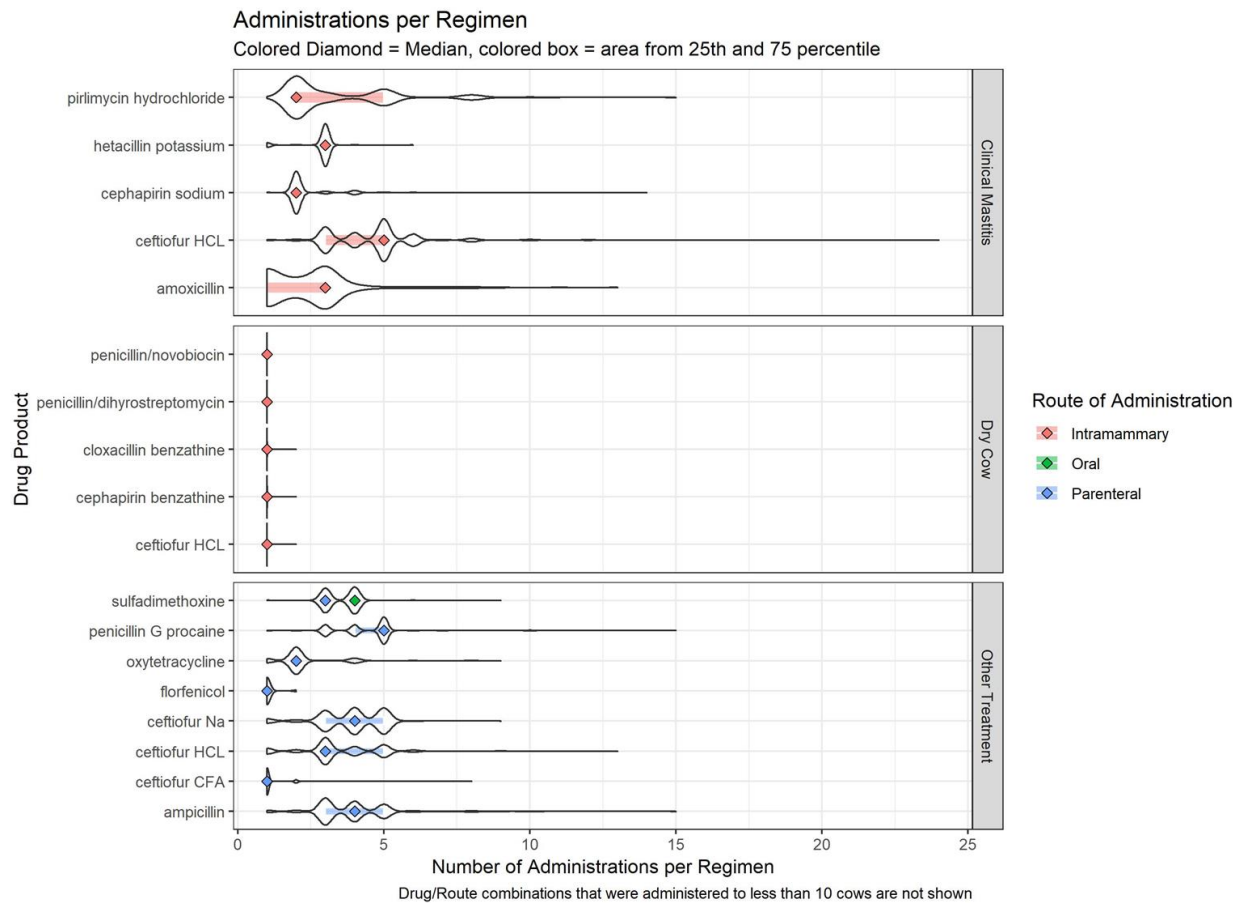
Table 3.3 Regimen Parameter Definitions

Parameter	Description
Drug Product	Active antimicrobial substance. Usually a single drug, unless the product is sold in a formulation that combines multiple antimicrobials. In this case the combination product is counted as a single regimen, but the regimen parameters are defined separately for each drug in the mixture.
Route	Route by which the treatment was delivered. Common routes include: intramammary, parenteral (IM, SC, IV), oral, or topical.
Number of Administrations	The number of occasions that drug is administered to the cow.
Time Frame (Days)	Calculated using the interval and number of administrations. It represents the time in days between the first administration and the last administration. $(\text{Interval}) \times (\text{Number of administrations} - 1)$ Single administrations have an interval of zero hrs, and therefore a time frame of zero days.
Amount (mg)	Grams per Regimen: Total number of mg administered for the entire regimen. $(\text{Number of Administrations}) \times (\text{mg/Administration})$
	Grams per Administration: Dose of antimicrobial administered at a single time point.

The specific regimen parameters for all regimens encountered in participating dairies are shown in **Figures 3.10-3.13**, and **Tables 3.4-3.7**. The figures are displayed first as a group with their descriptions, followed by the corresponding tables.

These parameters are defined at the individual cow level for each antimicrobial product. All regimens encountered in the study are described for both years 2016 and 2017, regardless of their frequency of use. In order to account for this, the far right column in the tables displays the percent of total regimens in the study that are represented by that regimen description. For graphical clarity regimens involving less than 10 animals are not depicted graphically to improve visual clarity, but they are represented in the tables. Individual regimens of greater than 15 administrations involving less than 3 individual cows were identified as outliers. These 12 treatment regimens are not represented in the parameter descriptions below as they distort the description of the majority of treatments.

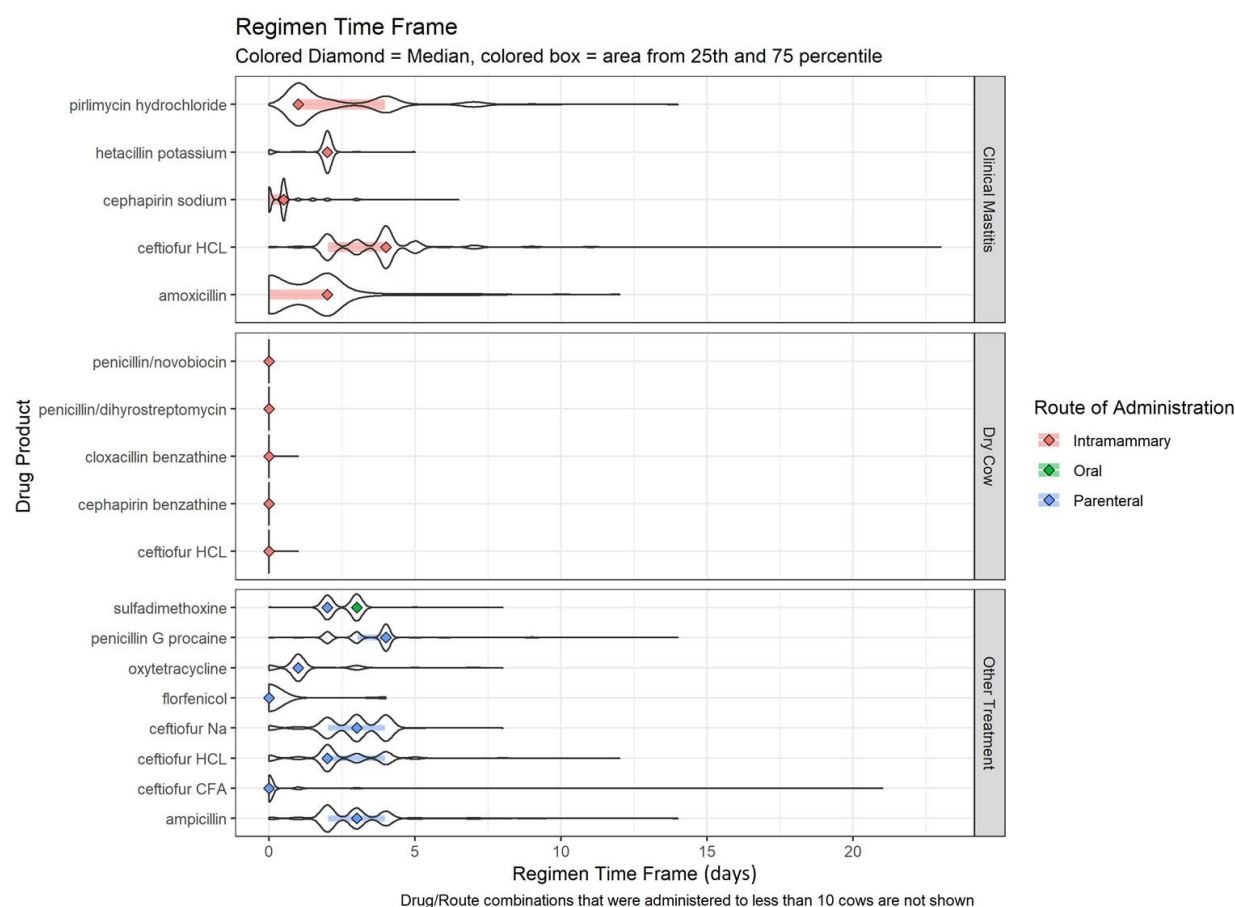
Figure 3.10 Administrations per Regimen



There is variation in the number of administrations across reasons for use displayed in **Figure 3.10**. Use in the dry cow category consistently involves only a single administration, while use in treatment of clinical mastitis or other treatments involves multiple administrations demonstrating considerable variability between regimens within and across products. The very small tail on use for cloxacillin benzathine, cephalapirin benzathine, and ceftiofur HCL is due to what appear to be duplicate documentation of dry treatment on the same cows. These are likely documentation errors rather than actual repeated dry treatments. However, in the interest of transparency, and a desire to characterize treatment records as a source of data, they are reported here.

Recall that treatments were defined as part of the same regimen if there was not more than a 5 day gap. This means that while most administrations happen on consecutive calendar days, not all do. Since most drugs used for treatments on dairies are administered daily, the number of administrations closely reflect the regimen time frames (**Figure 3.11**).

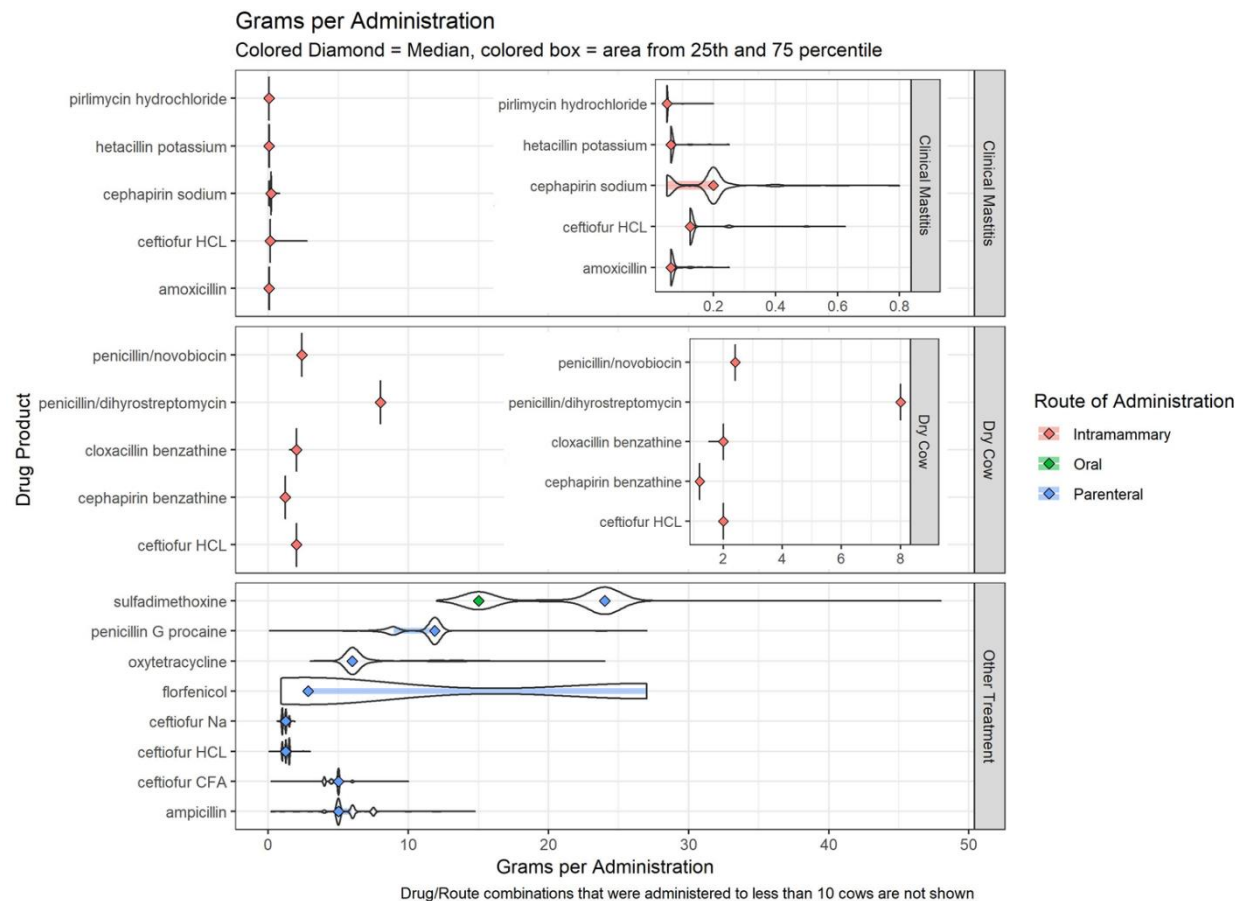
Figure 3.11 Regimen Time Frame



A regimen time frame is the number of days between the first administration and the last administration of a single regimen. For an example of this calculation, see **Table 2.1**. The time frame is calculated as $(\text{number of administrations} - 1) \times (\text{Interval}/24)$. When calculating regimens, a treatment was counted as a new regimen if a gap of more than 5 days existed between administrations of the same drug product for the same disease. It should be noted that there may be some error present in the time frame calculation if a drug product was administered in a manner that had an inconsistent interval. For example, 5 doses of amoxicillin were administered, but 3 doses were given at 24 hr intervals, then one day was skipped and then 2 more doses were given. This would result in a regimen time frame calculation of 5 days when the true time frame was four days. This does happen occasionally, but it is rare.

As demonstrated in **Figure 3.11**, there is variation in the time frame pattern between reasons for use. Variation exists within product for both Clinical Mastitis and Other Treatments, but Time Frame is consistently zero for Dry Cow.

Figure 3.12 Grams per Administration

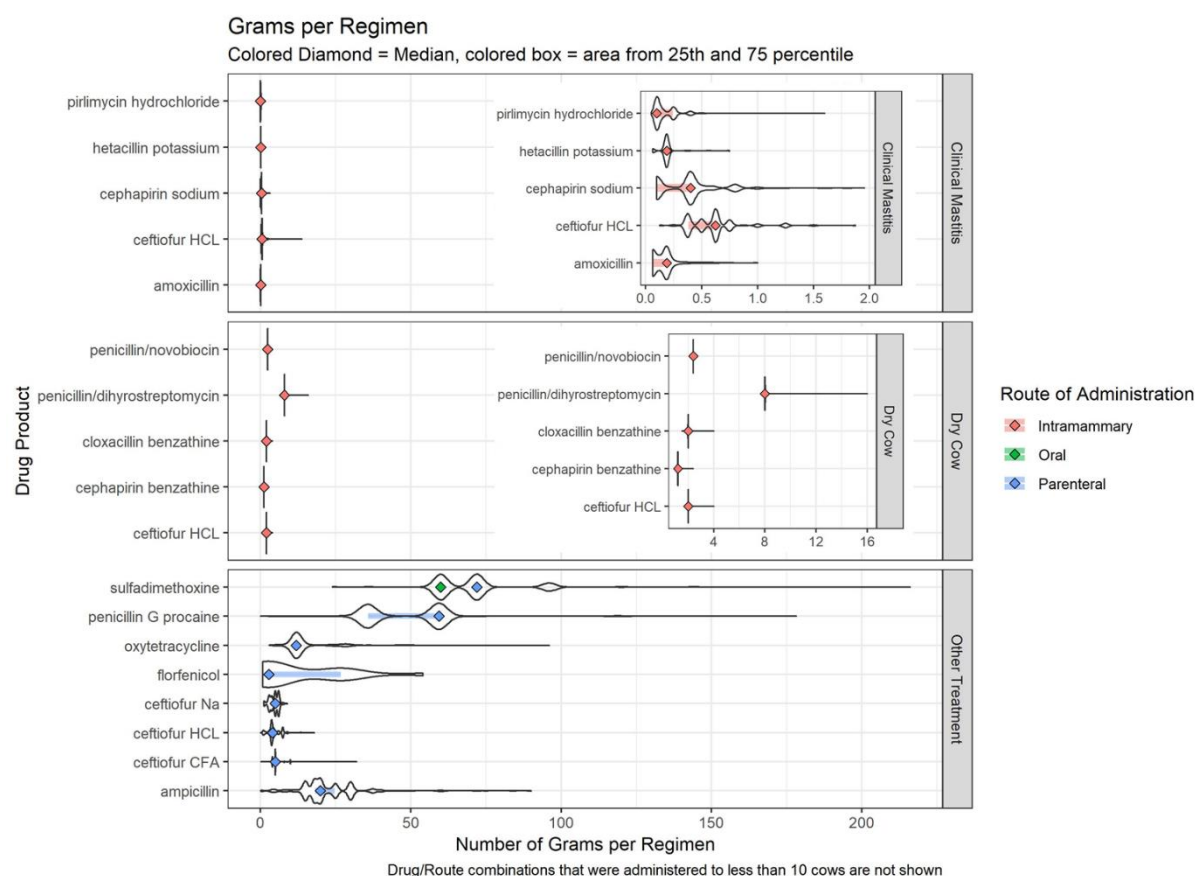


The smaller plots within the main plot are zoomed in views of the same data.

As demonstrated in **Figure 3.12** for intramammary products there is almost no variability when use is for Dry Cow. The small tail seen for cloxacillin is due to a few animals on one farm where they documented drying off 3 quarters rather than 4. This likely occurred occasionally with other products, but it was not found in other farm treatment records. Grams per administration of intramammary use for clinical mastitis varies in predictable amounts based on the number of quarters treated. The amount per tube (per treated quarter) is always the same for a specific product.

The variation in grams per administration is high for many of the products used in the use category of Other Treatment. Most of these products are injectable, and labeled for multiple diseases, which contributes to the variation in dose used.

Figure 3.13 Grams per Regimen



For non-intramammary products both the amount per administration as well as the number of administrations contributes to variability within product (**Figure 3.13**). This is demonstrated by the multiple peaks and long tails observed in the Clinical Mastitis and Other Treatment categories. The scale on the inset graphs should be noted, especially for Clinical Mastitis where the total grams per regimen does not exceed 2 grams.

However, variation in Dry Cow therapy remains low as doses and number of administrations is standard. The small tail seen on several of the dry cow products is due to a very small number of cows who were documented as receiving Dry Cow treatment twice. This is an unlikely and perhaps unintentional event but documented as such.

Table 3.4 Regimen Time Frame

Regimen Time Frame (days)							
Route	Drug	<i>mean</i>	<i>median</i>	<i>standard deviation</i>	<i>minimum</i>	<i>maximum</i>	Percent of Total Regimens
Intramammary	amoxicillin	1.49	2	1.72	0	12	0.240%
	ceftiofur HCL	1.43	0	2.04	0	23	35.321%
	cephapirin benzathine	0	0	0	0	0	14.422%
	cephapirin sodium	0.73	1	0.90	0	7	3.501%
	cloxacillin benzathine	0	0	0.01	0	1	8.931%
	hetacillin potassium	1.81	2	0.62	0	5	1.594%
	penicillin/dihyrostreptomycin	0	0	0	0	0	6.631%
	penicillin/novobiocin	0	0	0	0	0	3.351%
	pirlimycin hydrochloride	2.33	1	1.93	0	14	1.771%
Parenteral	ampicillin	2.85	3	1.34	0	14	5.474%
	ceftiofur CFA	0.20	0	0.75	0	21	6.675%
	ceftiofur HCL	2.53	2	1.39	0	12	9.969%
	ceftiofur Na	2.86	3	1.11	0	8	1.384%
	florfenicol	0.20	0	0.89	0	4	0.021%
	oxytetracycline	1.16	1	0.96	0	8	1.405%
	penicillin G procaine	3.40	4	1.21	0	14	3.519%
	sulfadimethoxine	2.33	2	0.74	0	8	0.728%
	tilmicosin	0	0	NA	0	0	0.001%
	tulathromycin	0	0	NA	0	0	0.001%
Ophthalmic	ceftiofur HCL	0	0	0	0	0	0.003%
	oxytetracycline	2.00	2	0	2	2	0.004%
Oral	sulfadimethoxine	3.05	3	0.46	3	7	0.231%
Uterine Infusion	oxytetracycline	0	0	0	0	0	0.003%

Table 3.5 Administrations per Regimen

Administrations per Regimen							
Route	Drug	<i>mean</i>	<i>median</i>	<i>standard deviation</i>	<i>minimum</i>	<i>maximum</i>	Percent of Total Regimens
Intramammary	amoxicillin	2.50	3.00	1.71	1	13	0.240%
	ceftiofur HCL	2.43	1.00	2.04	1	24	35.321%
	cephapirin benzathine	1.00	1.00	0.04	1	2	14.422%
	cephapirin sodium	2.41	2.00	0.87	1	14	3.501%
	cloxacillin benzathine	1.00	1.00	0.03	1	2	8.931%
	hetacillin potassium	2.81	3.00	0.62	1	6	1.594%
	penicillin/dihyrostreptomycin	1.00	1.00	0	1	1	6.631%
	penicillin/novobiocin	1.00	1.00	0	1	1	3.351%
	pirlimycin hydrochloride	3.33	2.00	1.94	1	15	1.771%
Parenteral	ampicillin	3.84	4.00	1.33	1	15	5.474%
	ceftiofur CFA	1.12	1.00	0.36	1	8	6.675%
	ceftiofur HCL	3.53	3.00	1.38	1	13	9.969%
	ceftiofur Na	3.86	4.00	1.11	1	9	1.384%
	florfenicol	1.05	1.00	0.22	1	2	0.021%
	oxytetracycline	2.23	2.00	0.97	1	9	1.405%
	penicillin G procaine	4.40	5.00	1.21	1	15	3.519%
	sulfadimethoxine	3.33	3.00	0.74	1	9	0.728%
	tilmicosin	1.00	1.00	NA	1	1	0.001%
	tulathromycin	1.00	1.00	NA	1	1	0.001%
Ophthalmic	ceftiofur HCL	1.00	1.00	0	1	1	0.003%
	oxytetracycline	3.00	3.00	0	3	3	0.004%
Oral	sulfadimethoxine	4.05	4.00	0.46	4	8	0.231%
Uterine Infusion	oxytetracycline	1.00	1.00	0	1	1	0.003%

Table 3.6 Grams per Administration

Grams per Administration (g/Administration)							
Route	Drug	<i>mean</i>	<i>median</i>	<i>standard deviation</i>	<i>minimum</i>	<i>maximum</i>	Percent of Total Regimens
Intramammary	amoxicillin	0.07	0.06	0.02	0.06	0.25	0.240%
	ceftiofur HCL	1.27	2.00	0.91	0.13	2.75	35.321%
	cephapirin benzathine	1.20	1.20	0.00	1.20	1.20	14.422%
	cephapirin sodium	0.17	0.20	0.10	0.05	0.80	3.501%
	cloxacillin benzathine	2.00	2.00	0.02	1.50	2.00	8.931%
	hetacillin potassium	0.07	0.06	0.03	0.06	0.25	1.594%
	penicillin/dihyrostreptomycin	8.00	8.00	0.00	8.00	8.00	6.631%
	penicillin/novobiocin	2.40	2.40	0.00	2.40	2.40	3.351%
	pirlimycin hydrochloride	0.05	0.05	0.01	0.05	0.20	1.771%
Parenteral	ampicillin	5.62	5.00	1.33	0.20	14.75	5.474%
	ceftiofur CFA	4.72	5.00	0.55	0.20	10.00	6.675%
	ceftiofur HCL	1.28	1.25	0.24	0.05	3.00	9.969%
	ceftiofur Na	1.19	1.25	0.19	0.60	1.90	1.384%
	florfenicol	11.21	2.85	11.99	0.90	27.00	0.021%
	oxytetracycline	6.86	6.00	2.24	3.00	24.00	1.405%
	penicillin G procaine	11.00	11.88	2.16	0.09	27.00	3.519%
	sulfadimethoxine	23.86	24.00	1.43	12.00	48.00	0.728%
	tilmicosin	13.50	13.50	NA	13.50	13.50	0.001%
	tulathromycin	0.60	0.60	NA	0.60	0.60	0.001%
Ophthalmic	ceftiofur HCL	0.38	0.50	0.22	0.13	0.50	0.003%
	oxytetracycline	0.20	0.20	0.00	0.20	0.20	0.004%
Oral	sulfadimethoxine	15.20	15.00	1.74	15.00	30.00	0.231%
Uterine Infusion	oxytetracycline	3.33	2.00	2.31	2.00	6.00	0.003%

Table 3.7 Grams per Regimen

Grams per Regimen (g/Regimen)							
Route	Drug	<i>mean</i>	<i>median</i>	<i>standard deviation</i>	<i>minimum</i>	<i>maximum</i>	Percent of Total Regimens
Intramammary	amoxicillin	0.17	0.19	0.13	0.06	1.00	0.240%
	ceftiofur HCL	1.47	2.00	0.70	0.13	13.75	35.321%
	cephapirin benzathine	1.20	1.20	0.04	1.20	2.40	14.422%
	cephapirin sodium	0.42	0.40	0.30	0.10	3.20	3.501%
	cloxacillin benzathine	2.00	2.00	0.05	1.50	4.00	8.931%
	hetacillin potassium	0.19	0.19	0.09	0.06	0.75	1.594%
	penicillin/dihydrostreptomycin	8.00	8.00	0.14	8.00	16.00	6.631%
	penicillin/novobiocin	2.40	2.40	0	2.40	2.40	3.351%
	pirlimycin hydrochloride	0.17	0.10	0.11	0.05	1.60	1.771%
Parenteral	ampicillin	21.77	20.00	9.36	0.20	90.00	5.474%
	ceftiofur CFA	5.28	5.00	1.76	0.20	32.00	6.675%
	ceftiofur HCL	4.54	4.00	2.10	0.05	18.00	9.969%
	ceftiofur Na	4.59	5.00	1.48	1.25	9.00	1.384%
	florfenicol	12.56	2.85	15.00	0.90	54.00	0.021%
	oxytetracycline	14.79	12.00	7.87	3.00	96.00	1.405%
	penicillin G procaine	48.34	59.40	15.71	0.09	178.20	3.519%
	sulfadimethoxine	79.42	72.00	18.34	24.00	216.00	0.728%
	tilmicosin	13.50	13.50	NA	13.50	13.50	0.001%
	tulathromycin	0.60	0.60	NA	0.60	0.60	0.001%
Ophthalmic	ceftiofur HCL	0.38	0.50	0.22	0.13	0.50	0.003%
	oxytetracycline	0.60	0.60	0	0.60	0.60	0.004%
Oral	sulfadimethoxine	60.81	60.00	6.94	60.00	120.00	0.231%
Uterine Infusion	oxytetracycline	3.33	2.00	2.31	2.00	6.00	0.003%

Grams by Year (2016 and 2017)

Weight of active substance is commonly used as a measure of antimicrobial use. Due to this commonality, the total weight of active substance is reported here for comparative purposes. It is a simple summation of the total grams per regimen across years, disease categories, and antimicrobial drug classes. The number of total grams per cow year are shown in **Figures 3.14** (study level) and **Figure 3.15** (dairy level). Row 1 in **Table 3.8** displays the values for grams per cow year at both the study level and farm level. These summarized totals include all treatments in the 3 main reasons for use described previously (Dry Cow, Clinical Mastitis, Other Treatments) for all medically important antimicrobial drug classes. Non-medically important antimicrobials and non-antimicrobial regimens are not reported here.

Figure 3.14 Grams per Cow Year - Study Level

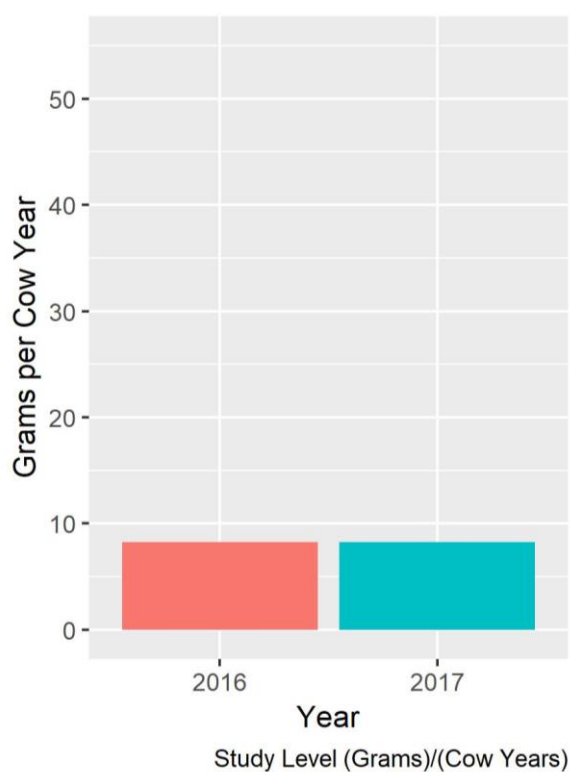


Figure 3.15 Grams per Cow Year - Dairy Level

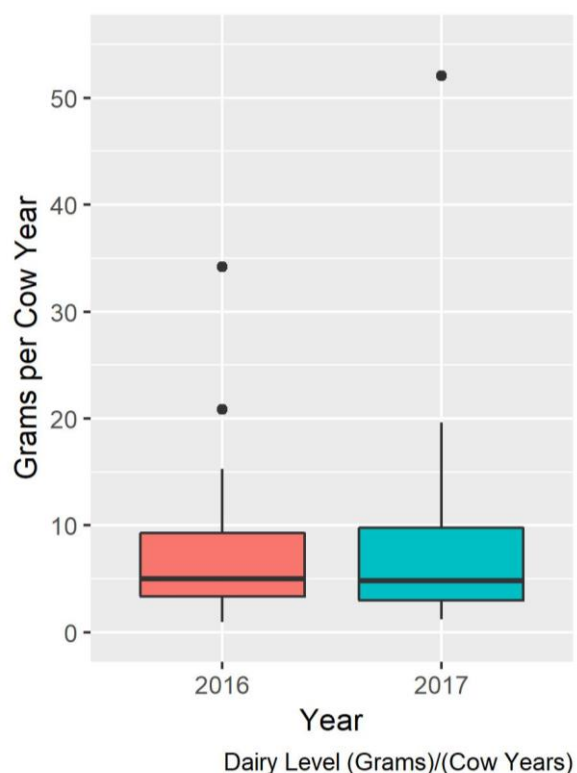


Table 3.8 Grams per Cow Year - by Antimicrobial Class and Reason for Use

Grams per Cow Year				
Antibiotic Class	Reason for Use	Year 2016	Year 2017	Count of Dairies
		Study Level	Study Level	
All Classes	All Reasons	8.25	8.25	29
Cephalosporin	Clinical Mastitis	0.168	0.136	29
	Dry Cow	0.897	0.818	20
	Other Treatment	1.191	1.234	28
Penicillin	Clinical Mastitis	0.003	0.006	13
	Dry Cow	0.455	0.456	13
	Other Treatment	4.033	4.055	28
Aminocoumarin	Dry Cow	0.044	0.031	2
Aminoglycoside	Dry Cow	0.236	0.138	3
Amphenicol	Other Treatment	0.005	0.002	4
Lincosamide	Clinical Mastitis	0.004	0.005	17
Macrolide	Other Treatment	0.0004	0	2
Sulfonamide	Other Treatment	0.957	1.050	5
Tetracycline	Other Treatment	0.255	0.320	19

Table 3.9 Grams per Cow Year - by Disease and Reason for Use

Grams per Cow Year														
Reason for Use	Disease Syndrome	Year 2016						Year 2017						Count of Dairies
		Study Level	Dairy Level					Study Level	Dairy Level					
			mean	median	sd	minimum	maximum		mean	median	sd	minimum	maximum	
All Reasons	All Diseases	8.25	7.488	5.028	7.047	0.952	34.171	8.25	7.910	4.816	9.670	1.232	52.039	29
Clinical Mastitis	Mastitis	0.17	0.189	0.156	0.152	0.003	0.554	0.15	0.167	0.151	0.145	0.004	0.484	29
Dry Cow	Mastitis	1.63	1.625	1.432	1.295	0.770	6.323	1.44	1.570	1.391	1.290	0.361	5.991	29
Other Treatment	Unknown	0.95	1.048	0.308	1.799	0.002	7.129	1.20	1.424	0.304	2.503	0.004	8.281	24
	Respiratory	0.34	0.337	0.197	0.418	0.005	1.625	0.42	0.475	0.270	0.765	0.015	3.660	25
	Other	0.75	0.504	0.134	0.818	0.0004	3.151	0.51	0.351	0.115	0.509	0.012	2.099	19
	Metritis	1.85	2.081	0.989	2.583	0.012	8.231	1.71	1.761	1.303	1.767	0.050	5.911	27
	Metabolic	0.01	0.052	0.034	0.050	0.014	0.140	0.01	0.063	0.023	0.104	0.014	0.275	7
	Mastitis	1.22	2.179	0.296	6.732	0.001	31.088	1.50	3.361	0.417	10.489	0.024	46.177	24
	Lameness	0.77	0.501	0.217	0.902	0.0003	3.571	0.71	0.415	0.180	0.554	0.005	2.056	24
	Displaced Abomasum	0.43	0.633	0.137	1.238	0.004	4.787	0.50	0.549	0.147	1.132	0.002	4.940	19
	Digestive	0.11	0.230	0.151	0.230	0.008	0.703	0.10	0.135	0.032	0.201	0.002	0.495	13

Grams per Cow Year by Disease Syndrome

Figures 3.16 and 3.17 (study level) and **3.18 and 3.19** (dairy level) and **Table 3.9** show total grams per cow year by disease syndrome at both the study level and the dairy level. When measured at the study level (**Figure 3.16**), mastitis is the disease requiring the greatest number of grams, with metritis as the second highest for both 2016 and 2017. It is informative to compare the use measured by grams in **Figures 3.16 and 3.17** with the use measured by regimens in **Figures 3.4 and 3.5**.

Interpretation of grams is challenging when stratified by disease due to the difference in drug potency. This is demonstrated in **Figures 3.18 and 3.19** where there are numerous outliers, with one very extreme outlier. The magnitude of these outliers are influenced by differences in drug potency in addition to differences in disease pressure and choices about duration of therapeutic regimen.

Figure 3.16 Grams per Cow Year - Study Level by Disease

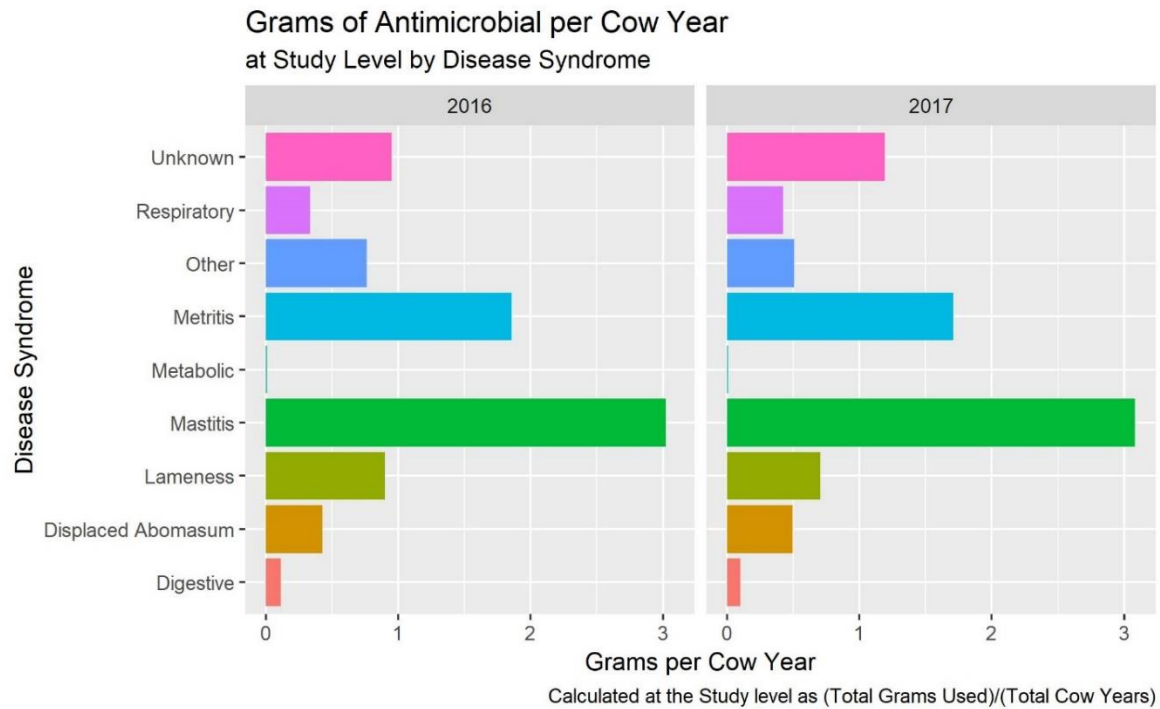


Figure 3.17 Grams per Cow Year - Study Level by Disease and Reason for Use

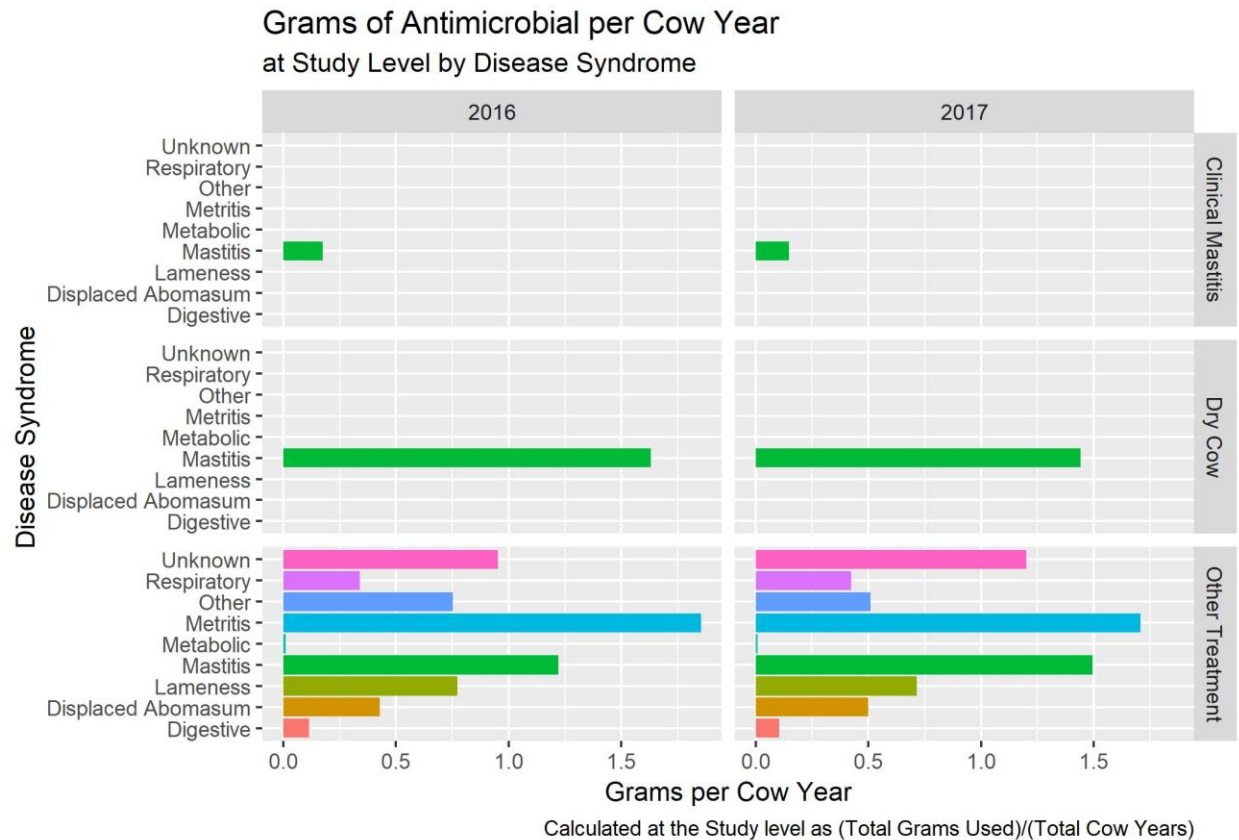


Figure 3.18 Grams per Cow Year - Dairy Level by Disease Syndrome

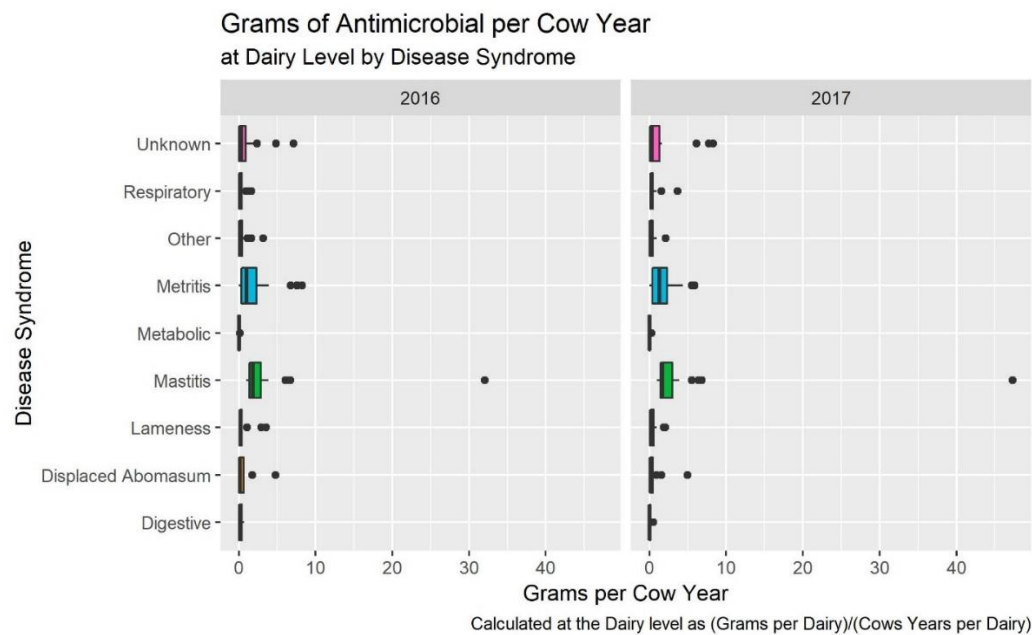
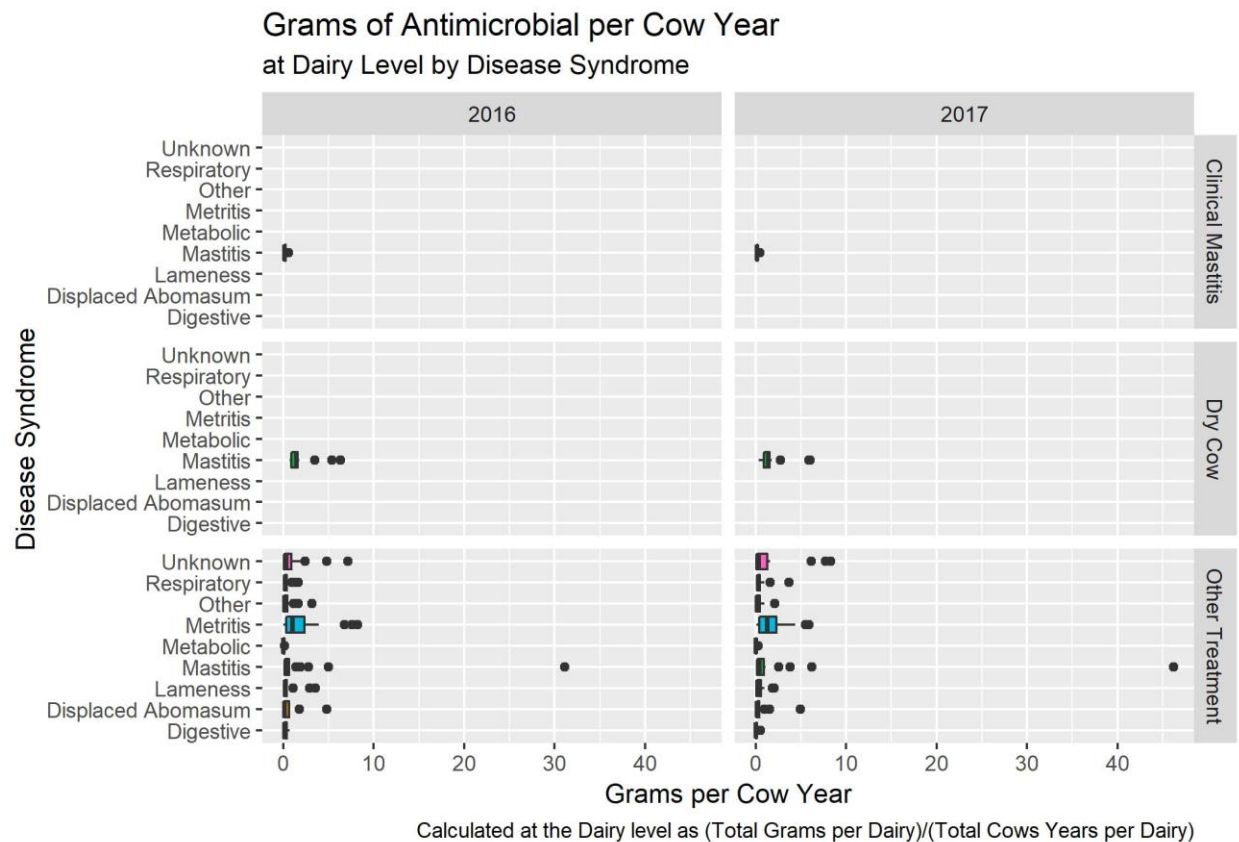


Figure 3.19 Grams per Cow Year - Dairy Level by Disease and Reason for Use



Grams by Drug Class

Describing use at the level of drug class is commonly used as a way to stratify use. The direct utility of this stratification is difficult to interpret at the farm level due to the therapy choices on a single farm falling into only a few classes. Unlike the disease syndromes where most farms are represented in most disease syndrome categories, only small numbers of farms are represented in most drug class categories. For this reason, grams of antimicrobial use by drug class is only reported at the study level in **Figures 3.20 and 3.21** and **Table 3.8**.

Figure 3.20 Grams per Cow Year - Study Level by Antimicrobial Class

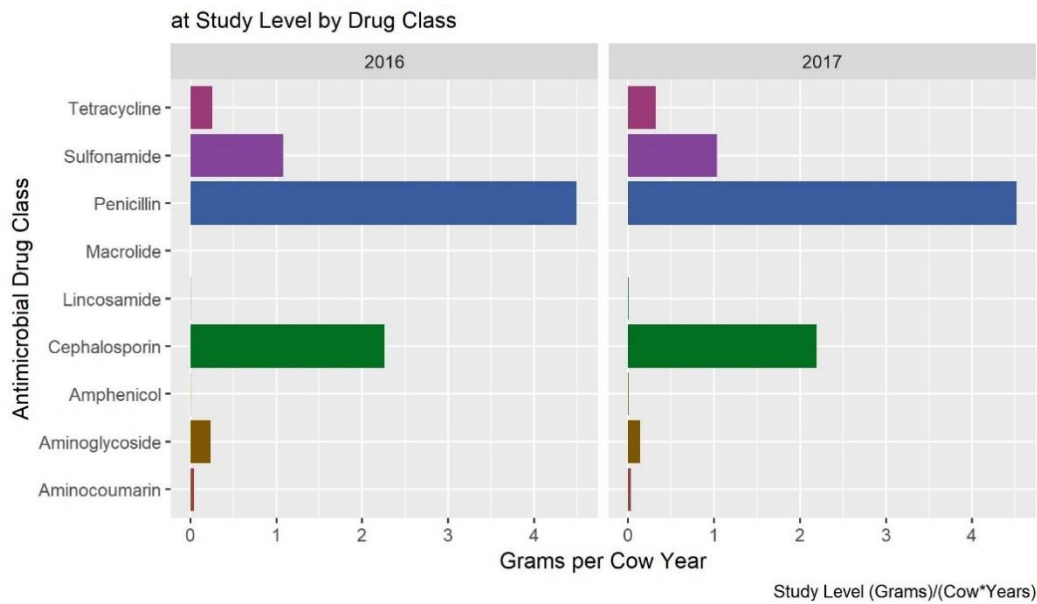
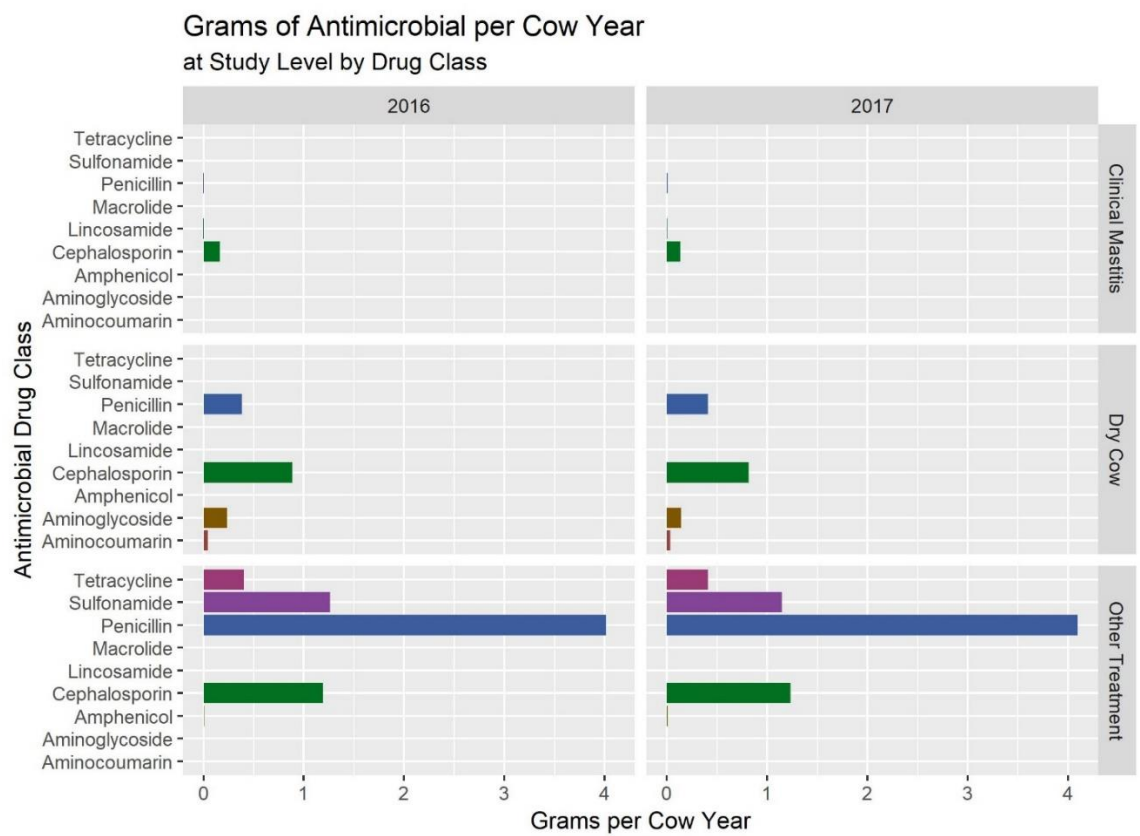


Figure 3.21 Grams per Cow Year - Study Level by Class and Reason for Use



Confounders of Use Measures and Challenges for Interpretation

For all methods of use measurement there are confounding factors that complicate interpretation. Some general mechanisms introducing error and complicating interpretation at the study level are discussed below, including incomplete records, protocol deviations, cow movement, milk production differences, specific pathogens, and farm resource availability.

Incomplete Records

Variability in the completeness of the treatment record causes variability in the accuracy of use estimates derived from these data. Due to the milk withdrawal times associated with antimicrobials, the risk of a residue is relatively high if treatments are left unrecorded. For this reason, the vast majority of antimicrobial treatments administered get recorded on most farms. However, farms on the low end of estimated use were questioned about specific uses during the second farm visit. Usually, discussion confirmed that treatments had been recorded. However, on one farm it was discovered that the entire record system for parlor mastitis treatments had not been collected. These records were not kept in a permanent manner that allowed retrospective capture. Cows were marked with chalk and put in the “hot” pen when treated. The marking system allows for accurate adherence to withdrawal time, but the information is not available retrospectively for farm level use monitoring. Due to this known omission of a significant portion of treatment data, this farm was removed from the overall analysis. However, they were still presented with yearly benchmark results. It should be noted that in spite of this omission, the farm was not the lowest total user of antimicrobials. There undoubtedly are instances on other farms where occasional treatments are not recorded but there is simply no way to identify these with currently available data.

Protocol Deviations

Many farms have protocols that indicate the number of days an animal should be treated with a particular drug for a particular disease. Some of these farms choose to document use by a method that records all treatments in that protocol as a single entry per protocol, rather than an entry for each treatment administration. This method has potential to over-estimate use when deviation from the treatment protocol occurs. These types of deviations were confirmed in survey

data indicating that often the treatments are stopped on day 2 or 3 if the cow is clinically improved, in spite of the protocol indicating 5 days of treatment.

Cow Movement

Dairies that are expanding, downsizing, or who apply culling practices with differing priorities may notice that these practices serve as confounders of relative antimicrobial use measures. When expansion is occurring, the criteria for culling may be established to make it less likely a cow is culled as there is a system advantage for cows to stay in the herd in order to increase total cow numbers. Even if disease incidence is equal, these expanding systems may choose to treat rather than cull, when a downsizing system would choose to cull rather than treat. The availability of replacement heifers may affect herds in a similar manner even when size remains constant. Herds with many heifers available are more likely to cull an older cow to make space for a heifer. If herds do not have a ready supply of heifers, the older cow is more likely to be treated and remain in the herd. These factors are not related to disease pressure or treatment success, but to system logistical challenges. These factors make comparative interpretation of individual farm benchmarks problematic to interpret without intimate knowledge of the specific farm system.

Differences in Average Days in Milk

Differences in lactation persistency over time can alter the percentage of total animals that are “dry treated” within a calendar year. Farms with long lactation persistency are likely to dry off a lower percentage of total cows in a 1 year time frame than farms that have short lactation persistence. Since dry cow therapy is a major contributor to use regimens, knowledge of lactation persistence or other factors affecting average days in milk on a particular farm aids in the interpretation of benchmark results.

Specific Pathogens Present

The presence or absence of specific pathogens on a farm can significantly alter the most appropriate disease management strategies. The management of diseases such as mastitis can vary greatly based on the specific pathogens present. Without knowledge of farm specific pathogens it is impossible to create useful and appropriate recommendations for treatments. For example, if

two farms both indicate high usage of antimicrobials for mastitis, there is no single treatment recommendation that would be appropriate for all farms. If most cases were Gram negative, treatment would not be effective. However, if most cases were Gram positive then there is good evidence that treatment is reasonable for many cases depending on the specific pathogen. Farm level knowledge is necessary to determine the most effective disease treatment strategy.

Farm Resource Availability

The lack of certain resources challenges the implementation of recommendations. Farms differ drastically in their availability of facilities, labor, feed quality, “cow sense”, veterinary involvement, and diagnostic services. Therefore, recommendations for disease management must be tailored to the specific farm resources available for implementation. Introducing an on-farm culture system to guide mastitis treatment decisions to a farm that already suffers from a severe labor shortage may cause more harm than good by taking employee time away from other important tasks such as feeding or sanitation. An alternative could be in-house rapid culture services offered by the local herd veterinarian. However, this resource is also not universally available for multiple reasons. Disease management strategies to improve antimicrobial use must take these factors into account in order to ensure that new recommendations do not cause more disease than they eliminate.

Summary

Numerous measurements and methods of stratifying measures were presented. Further discussion of comparisons between methods of measurement is addressed in Chapter 4. Due to the aforementioned confounders of use measures, summarized values of measures only minimally support antimicrobial stewardship unless they can drive questions about disease management at the farm level.

Although summary data were presented in numerous graphs within this chapter it should be recognized that summarized data are similar to measures of “apples and oranges” (see preface). Different milligrams of drugs with different potencies are added together, or different regimens for different diseases with different time frames and frequencies of administration are added together. Until these are used in their more granular form to create farm level benchmarks, they

cannot safely drive change without risk to animal welfare and other resources both natural and economic.

Chapter 4 - Comparative Metrics of Antimicrobial Use

Describing use by regimens provides a granular look at antimicrobial use on dairy systems. However, there is significant cost to achieve this granularity. While technology offers some solutions to gathering data efficiently, there are still challenges to managing granular data. As previously described, there are many confounders of antimicrobial use measures and to assume that the numbers can be interpreted without “boots on the ground” would be somewhat naive. However, it is also true that there are many purposes for monitoring use, with various levels of granularity required for each. This chapter explores the various measures that can be calculated to provide some clarity about their relationship to each other.

The different measurements presented here are generated from the same data source, i.e., treatment records supplemented by other information as needed to completely define regimens. If data were acquired from multiple sources, it is recognized that even within a single calculated measure there could be significant variation in the calculated value from one data source vs. another. The following comparisons all use treatment data as the source of all information for comparisons. This eliminates confounding by source and all factors affecting use measures described in chapter 3.

Calculations of Measures for Comparison

Comparison of different use metrics in this study is possible due to the granularity of information provided by regimen descriptions. One of the reasons that regimens was chosen as the basic measure is that it allows calculation of many other common measures of antimicrobial use. The Infectious Disease Society of America (IDSA) recommends calculations of the number of Days of Therapy (nDOT) for evaluation of human antimicrobial use.⁷⁸ While the format of the original data reported for this dairy study does not allow for exact identification of calendar days, the standard format allows a very close approximation to be made by multiplying the number of administrations by the administration interval and dividing by 24 hours to get an output in days.

Although this calculation is based on treatment records, it requires more assumptions than the calculation of time frame. For single injection products, such as ceftiofur crystalline free acid (CCFA) or 200 mg/ml and 300 mg/ml oxytetracycline products, the days of therapy provided do not match the number of calendar days on which the treatment was administered. In order to

remain consistent with the intent of the IDSA recommendations for using DOT rather than DDD, an adjustment was made to account for single administration with a treatment interval of zero days. The complete rules used for this assignment are shown below:

1. If drug states a duration of effective therapy, that duration is used. (e.g. 200 mg/ml oxytetracycline states 72 hrs so DOT = 3)

2. For dry cow treatments:

- a) if there is an equivalent lactating cow formulation, dry cow therapy was assigned a duration of twice the lactating cow recommended dosing interval.

- b) if no equivalent lactating cow formulation exists, duration was determined by pharmacokinetics, pharmacodynamics, efficacy data and expert opinion on the part of the authors.

It is recognized that the assumptions made in assigning durations of antimicrobial effect are arbitrary. The actual duration of drug effect varies by specific pathogen but is reported here as a single value. The desire to transparently describe use while making as few assumptions as possible lead to the exclusion of reporting total nDOT as a measure of use in previous chapters. However, it is included here in the comparative section for several reasons. First, there are potential situations when the calculation of an intended therapeutic exposure would be indicated, especially in research settings when the intention is to describe the association between use and resistance selection. Second, since it has been recommended by the IDSA, it is possible that this method may be adopted by those creating antimicrobial use monitoring programs. It is therefore included here to point out the necessary assumptions and its relationship to other use measures.

The comparisons between measurements made in this chapter are possible due to high granularity present in treatment record data. It is recognized that antimicrobial use is often calculated from less granular data than treatment records. Data sources such as sales data provide product specific substance weight with less collection and analysis resources. However, this weight is then the only information available to calculate use. It has long been recognized that potency can confound use measurements when they are based on substance weight. For this reason, defined doses are often used to convert the weight measurement into a number of course doses, or a number of daily doses, as described in Chapter 1.

This calculation of number of daily or course doses from a weight of drug requires a definition of the assumed animal weight at the time of treatment and definition of the dose administered (**Equation 1, Figure 4.1**). The dose may be defined as the “daily dose” or the

assumed amount administered per day, or as the “course dose” or the assumed amount administered per course (regimen) of therapy. If these assumptions are accurate, the number of course doses calculated will closely approximate Regimens (or Treatment Incidence). Again if all assumptions are correct, the nDDD should closely approximate the calculated nDOT.

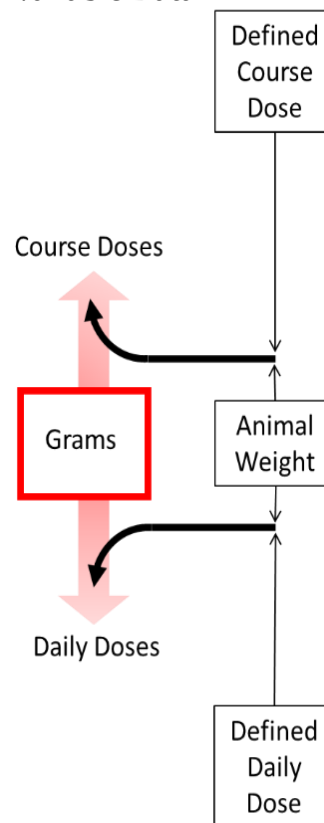
Equation 1 Calculation of defined doses from total milligrams

$$\frac{\text{Total Active Substance Weight (mg)}}{\text{Animal Weight (kg)} \times \text{Defined Dose } \left(\frac{\text{mg}}{\text{kg}}\right)} = \text{Number of Defined Doses}$$

The defined doses used here for comparison calculations are those published by the European Medicines Agency (EMA) as DDDvet and DCDvet.⁷¹ For products not listed by the EMA, the label recommendations in conjunction with common uses within the data were used to assign the defined dose. Due to the structure of the database used for the comparison analysis, the EMA doses for intramammary products were converted to mg/kg doses in order to have consistent units with the oral and parenteral products; e.g. if the standard dose published by EMA was one tube, then the converted dose was the total mg of active substance in one tube divided by the standard weight of a dairy cow, 680 kg. Actual cow weights are rarely measured on a dairy. This estimated standard weight was determined by evaluating the amount of product administered, and back calculating the weight of the animal treated based on the protocol dose (or label dose if protocol didn’t specify). This weight has also been used by other U.S. investigators.⁷⁵

Recalling the variation present in the number of administrations per regimen (**Figure 3.10**), it is apparent that the assumption that all animals received the same amount of drug in association with therapy is not entirely accurate. However, it is the purpose of this chapter to

Figure 4.1 Grams as the Only Available Data



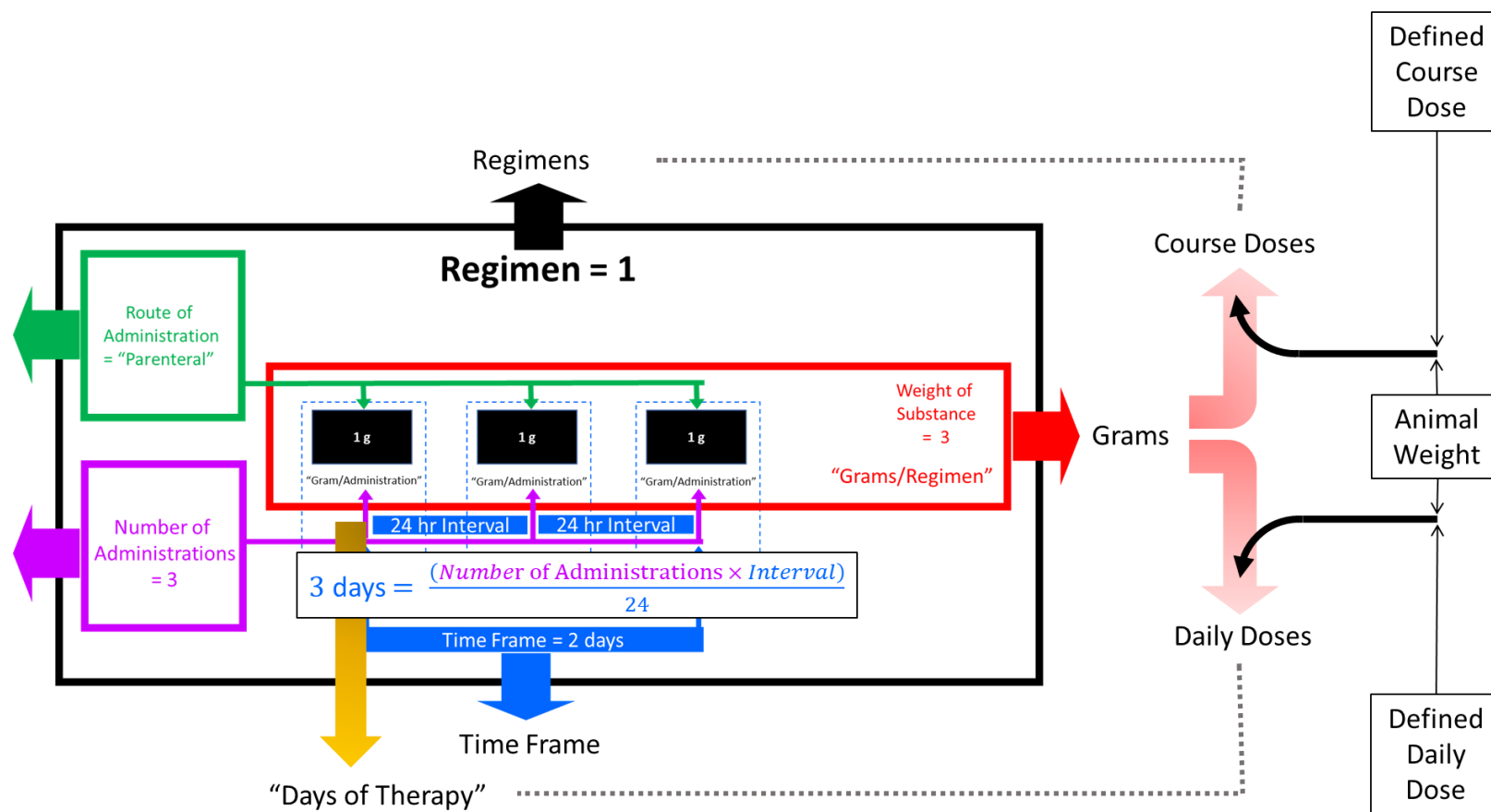
explore the relationship between the standard doses defined by the EMA and the actual doses recorded as administered in U.S. dairy systems. The numerical comparison of EMA vs study-generated mg/regimen and mg per dose are shown in **Table 4.1**.

Table 4.1 Comparison of Defined Doses Generated from Data to Those Published by EMA

Drug Product	Route of Administration	Daily Dose			Course Dose			Reason for Use
		EMA Defined (mg/kg)	Study Median (mg/kg)	Percent Difference from EMA	EMA Defined (mg/kg)	Study Median (mg/kg)	Percent Difference from EMA	
amoxicillin	Intramammary	0.09	0.09	0%	0.28	0.28	0%	Clinical Mastitis
ceftiofur HCL		0.18	0.18	0%	0.55	0.92	67%	
cephapirin sodium		0.29	0.29	0%	0.88	0.59	-33%	
hetacillin potassium		0.09	0.09	0%	0.28	0.28	0%	
pirimycin hydrochloride		0.07	0.07	0%	0.22	0.15	-33%	
ceftiofur HCL		1.47	1.47	0%	2.94	2.94	0%	
cephapirin benzathine		0.88	0.88	0%	1.76	1.76	0%	Dry Cow
cloxacillin benzathine		1.47	1.47	0%	2.94	2.94	0%	
dihydrostreptomycin (penicillin)		5.87	5.87	0%	11.75	11.75	0%	
novobiocin (penicillin)		2.35	2.35	0%	4.70	4.70	0%	
penicillin (dihydrostreptomycin)		5.87	5.87	0%	11.75	11.75	0%	
penicillin (novobiocin)		1.17	1.17	0%	2.35	2.35	0%	
sulfadimethoxine	Oral	40.00	22.03	-45%	185.00	88.11	-52%	Other Treatment
ampicillin	Parenteral	11.00	7.34	-33%	37.00	29.37	-21%	
ceftiofur CFA		1.00	2.45	145%	6.60	7.34	11%	
ceftiofur HCL		1.00	1.84	84%	4.00	5.87	47%	
ceftiofur Na		1.00	1.84	84%	4.00	7.34	84%	
florfenicol		13.00	1.05	-92%	40.00	4.19	-90%	
oxytetracycline		6.50	8.81	36%	23.00	17.62	-23%	
penicillin G procaine		13.00	17.44	34%	46.00	87.22	90%	
sulfadimethoxine		30.00	35.24	17%	90.00	105.73	17%	

Qualitative relationships between measures are shown in **Figure 4.2**. The grams and regimens were calculated as described in Chapter 3. The number of Daily Doses and the number of Course Doses were calculated from the Total Grams measured from the regimens. This allows a comparison of the measures without source confounding the comparison. All measures were calculated at the farm level. For further exploration they were also subdivided by reason for use.

Figure 4.2 Relationships Between Measures



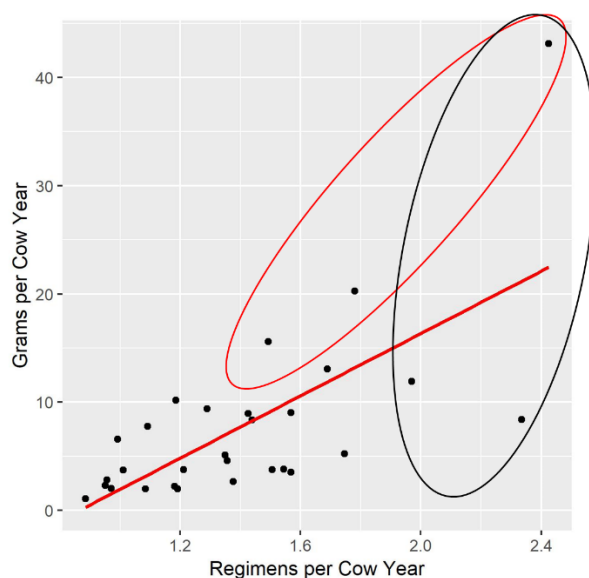
Can One Measure be Used to Estimate Another?

Due to the logistical challenges and cost associated with gathering and analyzing granular data from treatment records, it is reasonable to question whether or not estimates from easier-to-collect data (e.g. sales data) might be useful estimations of use. The granular data in this study provide a basis for answering this question. The measures included in the comparison are Regimens, Grams, nDDD, nDCD, and nDOT. They were chosen because they are representative of the numerous types of measures that have been used to describe antimicrobial use.³⁸

Farm Level Use

Although direct comparisons of active substance weight is confounded by drug potency, this measure is still very commonly used to account for antimicrobial use. The difference in potency is only relevant when multiple drugs are chosen for treatment and those drugs have significantly different potencies. If the differences are small, then this measure is less affected by drug potency and should correlate well with other measures. To explore the effect of potency within dairy systems, antimicrobial use measured as Regimens was compared to Grams at the farm level in **Figure 4.3**.

Figure 4.3 Comparison of Measurement by Regimens vs Grams

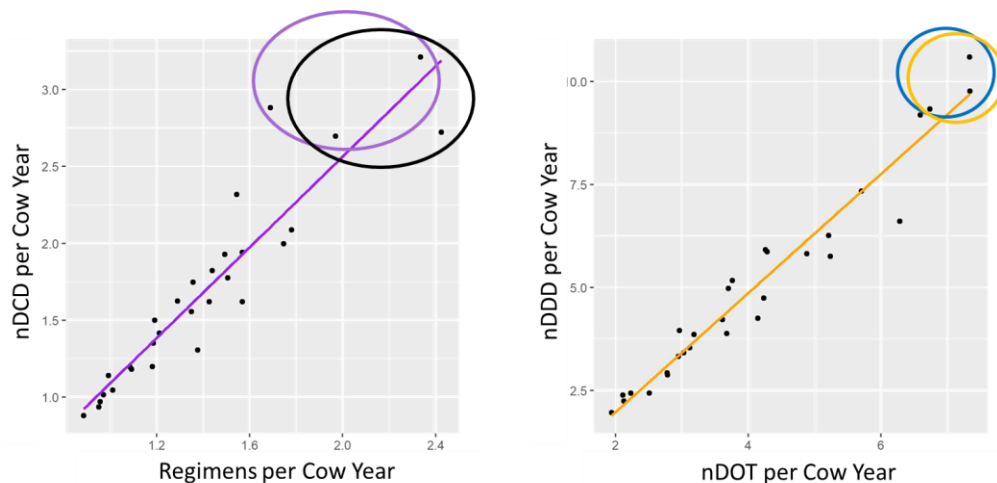


Grams vs Regimens

Each dot in **Figure 4.3** represents an individual dairy. The red circle encompasses the 3 dairies with the highest measured Grams per cow year, and the black circle encompasses the 3 dairies with the highest measured Regimens per cow year. It is clear that there may be differences in which dairies will be identified as high vs low users when measured by Grams vs Regimens. In this figure, it is apparent from the actual data that the two different measures would result in different dairies being classified as “highest users”. Although some of this variation exists due to differences in regimens, much of it can be entirely explained by the known issue of potency. So the real question is whether or not Grams can be converted to a measure that then accounts for this difference in potency.

Based on the relationships between metrics described in **Figure 4.2** it is reasonable to compare nDCD to Regimens as they are both measures of treatment incidence. It would also be reasonable to compare nDDD to nDOT as they are both attempts at quantifying number of days bacteria were exposed to a drug. These comparisons are illustrated in **Figure 4.4**.

Figure 4.4 Comparison of Defined Dose Measures to Regimens and nDOT



Defined Doses vs Regimens

The relationship is now improved. The circles encompassing the highest 3 dairies based on nDCD incorporate 2 of the 3 dairies identified as the highest by Regimens. When use between nDDD and Regimens is compared the same 3 dairies are identified as the highest by both measures. The comparison can be further quantified by performing simple linear regression between the measures. The results of this regression analysis are shown in **Table 4.2**. These differences do

not imply that one measure is superior to another. It simply demonstrates that a measure of active substance weight will not always be well aligned with a measure of treatment incidence.

Table 4.2 Linear Regression for Selected Measures

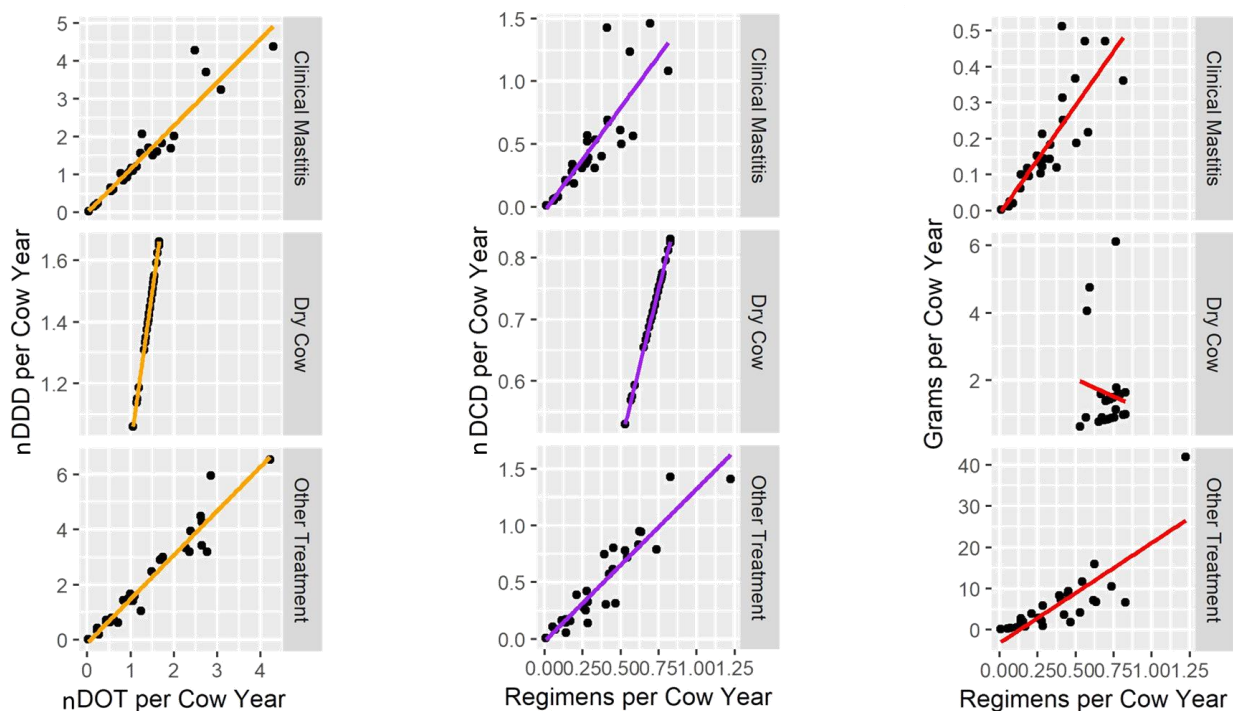
X	Y	Intercept	Std. Error Intercept	Coeff	St. Error Coef	Adj. R-squared	F-stat	p-value*
mg	Regimens	1.29	890	9.20E-06	7.10E-06	0.02	1.69	0.2
nDDD	nDOT	0.64	0.19	6.40E-01	0.03	0.92	339	<0.0001
nDCD	Regimens	0.46	0.07	5.40E-01	0.04	0.85	174	<0.0001

*This is the p-value for the model. Lower p-values indicate a model where X is predictive of Y.

Similar plots were created to determine the relationship between regimens as a baseline and each of the other use measures. Each point in these plots represents an individual dairy. In this instance, the plots were subdivided by reason for use. **Figure 4.5** demonstrates that the relationships differ based on reason for use.

Comparisons between all other combinations of measures can also be made (**Figure 4.6**). To efficiently represent these comparisons a correlation matrix was calculated. This calculation was first performed for all treatments administered. It was then calculated for each treatment reason; Dry Cow, Clinical Mastitis, and Other Treatments. The correlation matrices as shown in **Figure 4.6** display some interesting patterns.

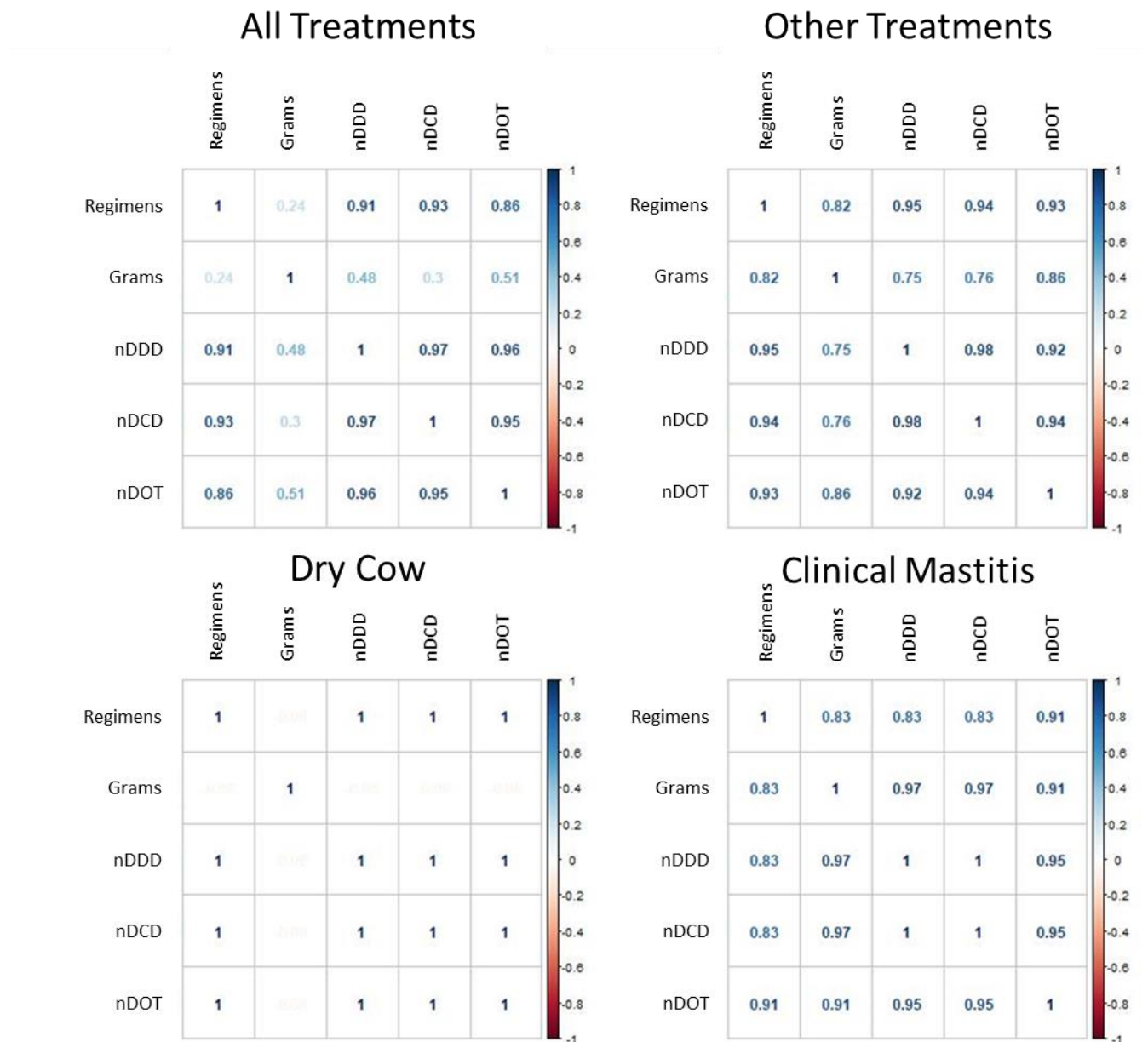
Figure 4.5 Measure Comparisons by Reason for Use



Correlations of Measures

Generally, the measures are well correlated with each other as demonstrated by many correlations of greater than 0.90. Comparisons involving mg are the least correlated, but that is to be expected due to differences in potency. It is notable that for Dry Cow therapy when mg is compared to any other measure the correlation is -0.06 making it too low to be readable in the graphical output. In spite of this all other measures for Dry Cow are perfectly correlated. There are 3 factors contributing to this very consistent relationship. First, the calculation of defined doses is very simple for dry cow treatments. There is little variation in dose per cow; nearly all cows get 4 tubes. There occasionally is a cow that only receives 3 tubes, but this occurs very rarely, and would only contribute to a discrepancy between measures when it was documented in the record. There was only one dairy on the study that documented 3-quartered dry off regimens, and only a small percent of animals on that dairy received this treatment. Second, there is no variation in the duration of this therapy. It happens once, at the time of dry off. Therefore, the definition of a course dose is very simple and accurate for all regimens administered. The third factor is that there is large variation in the drug products chosen for dry cow treatment and these products vary greatly in potency. The most potent product labeled for dry cow therapy results in a dose that totals 800 mg per cow. The least potent results in a dose of 8000 mg. This coupled with the fact that these

Figure 4.6 Correlation of All Measures



treatments occur relatively frequently at the dairy level means that differences of very large magnitude can occur in the number of mg used per cow year. This difference is almost entirely reflective of the differences in drug potency.

For Clinical Mastitis, the accuracy of defined daily doses is similar to that of Dry Cow treatments with near perfect definition of the daily dose being easily accomplished. For clinical mastitis, the grams measure also correlates relatively well with the other measures, 0.97, 0.97, and 0.91 for nDDD, nDCD, and nDOT, respectively. The lowest correlation is seen between regimens and other measures for Clinical Mastitis. This occurs because the daily dose is easy to define; however, the number of days animals are treated varies often from 2-8 days. Therefore, the calculation of the nDCD is not as accurate, leading to less correlation of the defined dose measures with regimens. The mg correlation is much less affected by potency than dry cow therapy due to a smaller difference in potency between drugs commonly used for treatment of clinical mastitis. The most potent dose is 50mg, while the least potent is 200 mg. This results in only a 4x potency difference rather than the 10x for dry cow therapy. In fact, multiple compounds are within a 2x potency range of the least potent drug used for clinical mastitis. This leads to a stronger correlation between regimens and grams since use of any drug chosen for treatment contributes similarly to any other drug chosen.

The relationship of nDOT correlations for clinical mastitis treatment are interesting as well. They are not perfectly correlated with regimens as different regimens have different common durations of therapy. It creates a problem similar to that of potency, where the number of days of treatment is confounded by duration choice.

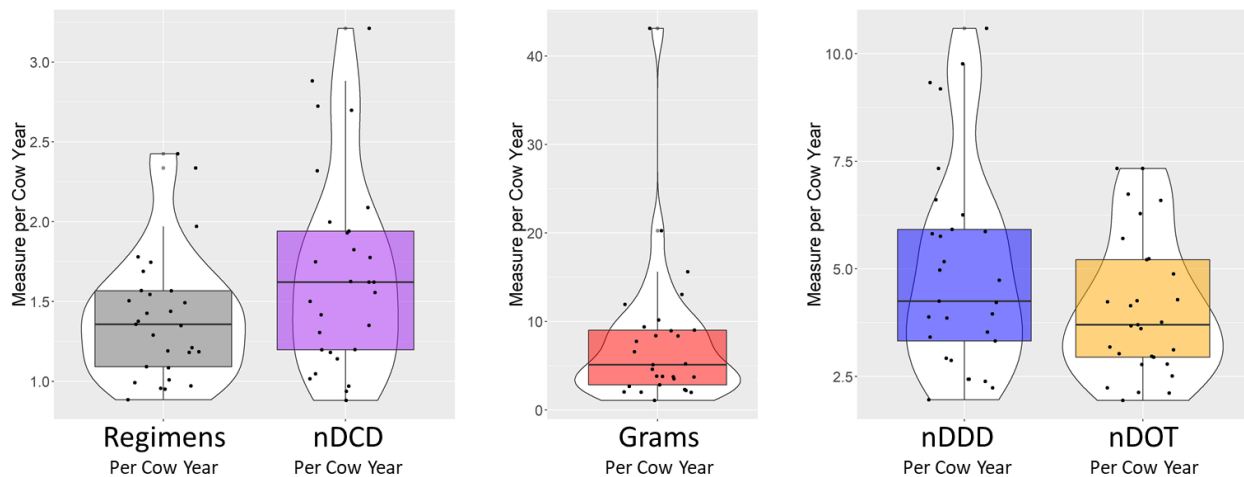
A visual representation of the variation in relationships based on reason for use is presented in **Figure 4.5**. These are the same measurements previously regressed in **Figures 4.3 and 4.4**, but now are subdivided by reason for use. As demonstrated by the individual graphs in **Figure 4.5**, the subsets of correlations for each reason for use have differing effects on the overall use. Due to the high incidence with which dry cow therapy is applied it has a greater effect on the overall correlations between measures than the correlations seen with other measures for use. The effect of “perfect” dose definition is clearly demonstrated by each point falling directly on the line for dry cow therapy, except for the mg comparison.

Overall use

Due to the strong correlation between use measures and the known but definable problem of potency confounding mg measures, the next question explored was that of predicting overall

use, rather than dairy level use. Violin, box, and scatter plots were then created for each measure to graphically show their relationship (**Figure 4.7**).

Figure 4.7 Comparative Distributions of Overall Measures



Recall that both nDDD and nDCD are calculated from grams of antimicrobial used. These graphs demonstrate the reason that defined doses were developed to account for potency. As predicted by the high correlations of these measures, nDCD (purple box) provides a relatively accurate estimate of Regimens (grey box, on the far left), and nDDD (blue box) approximates nDOT (yellow box, on the far right). The numeric descriptions of these comparisons are shown in **Table 4.3**.

It is important to recognize that while grams can be converted to either nDCD or nDDD to estimate the central value for Regimens or nDOT, the raw value of grams has a correlation of less than 0.051 when compared to any other measurement. If modeled as a predictor of regimens the model is not significant. It is unknown which of these measures is most correlated to resistance selection pressure.

Table 4.3 Comparison of Overall Measures

Measure	Mean	Standard Deviation	Median	IQR*	MAD**	Minimum	25 th Percentile	75 th Percentile	Maximum
Regimens	1.37	0.36	1.35	0.45	0.32	0.8	1.11	1.56	2.34
nDCD	1.66	0.61	1.59	0.73	0.55	0.88	1.21	1.94	3.26
Grams	8.63	9.28	5.17	6.29	4.7	1.09	3.01	9.29	42.26
nDDD	4.99	2.34	4.32	2.54	2.18	1.96	3.36	5.9	10.59
nDOT	4.12	1.57	3.73	2.59	1.56	1.94	2.97	5.16	7.49

*IQR = Interquartile Range

**MAD = Mean Absolute Deviation

Effects of Denominators and Assumptions

For purposes of this report, only “cow year(s)” was explored as a denominator of antimicrobial use. It is common for milligram measures to be calculated using a biomass denominator such as PCU. This calculation was not included here because it would have simply changed the numeric value by a constant (1/680 kg), but it would not have changed the relationship of the measures to each other. It is notable that this same relationship is not necessarily true in other livestock systems. In systems with growing animals, the estimates of weight can have more impact on the overall measure. When the number of defined doses can be affected both by an imperfect dose definition as well as an imperfect weight estimation, the correlations between measures in other livestock systems may differ significantly from the ones here, measured in adult dairy cows.

Summary

The degree of correlation that is sufficient for an estimate is entirely dependent on the reason for measuring. If the goal is to estimate overall use described as midpoints and distributions then an amount of error between regimens and nDCDs could be accepted as sufficient. If it is simply for international reporting, a general estimate is probably sufficient. However, if there are going to be cut points set, and consequences for dairies exceeding these cut points then extreme caution should be used to make sure that the measure is indeed identifying dairies that are contributing more heavily to resistance selection rather than simply dairies that are high in a

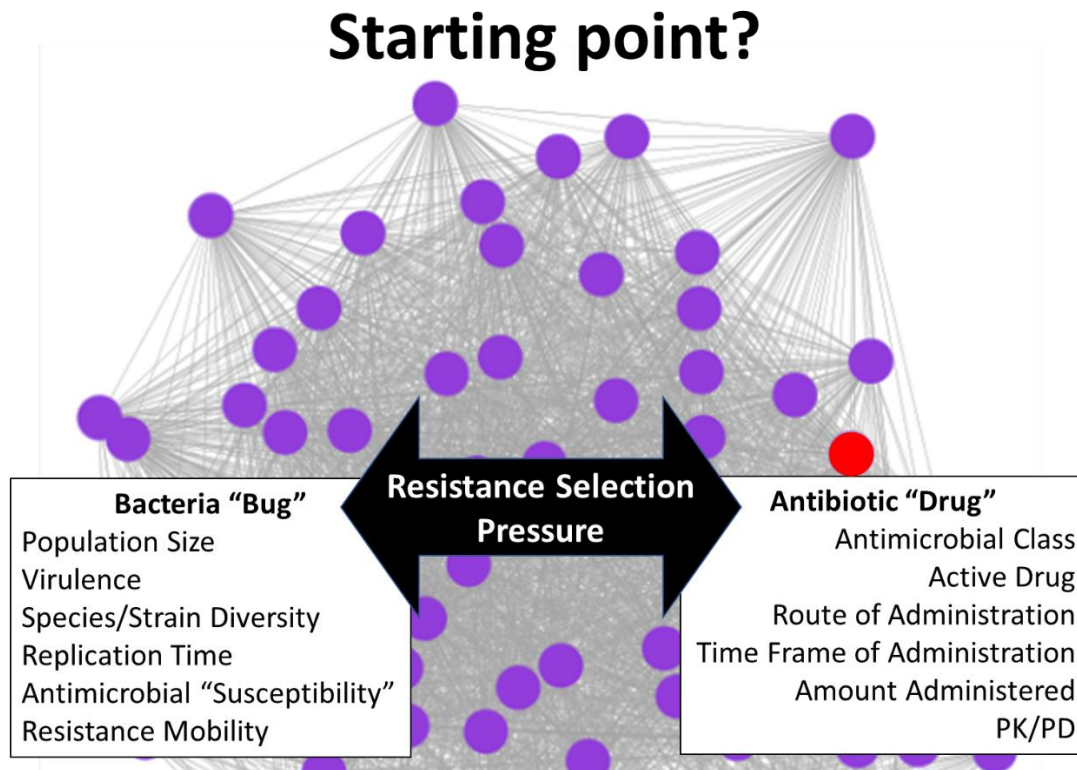
particular measure regardless of that measure's relationship to resistance selection pressure or animal welfare effects. Objective comparisons of differences in selection pressure based on measures and/or categories of use are very limited. At this point, programs with stewardship as their goal should focus on preventing disease, improving diagnostics, and evaluating treatment outcomes. As use measures become more refined, perhaps our ability to identify differences in selection pressure will improve, but for the moment there are more questions than answers.

Chapter 5 - What are the next questions about measuring use?

What is the relationship between use and resistance selection pressure?

Chapter 4 described the relationships between measures in great detail. However, perhaps a more important question is describing how the different measures of use relate to resistance selection pressure. In other words, how can they be used to describe “exposure”? That is the main reason to measure in the first place. Part of the reason that this question was not addressed is that measuring exposure is challenging. The relationship between bacterial populations and antibiotics is not simple and involves many interactions (**Figure 5.1**)

Figure 5.1 Drug measurements are only a small part of a complex interaction between "Bugs" and "Drugs"



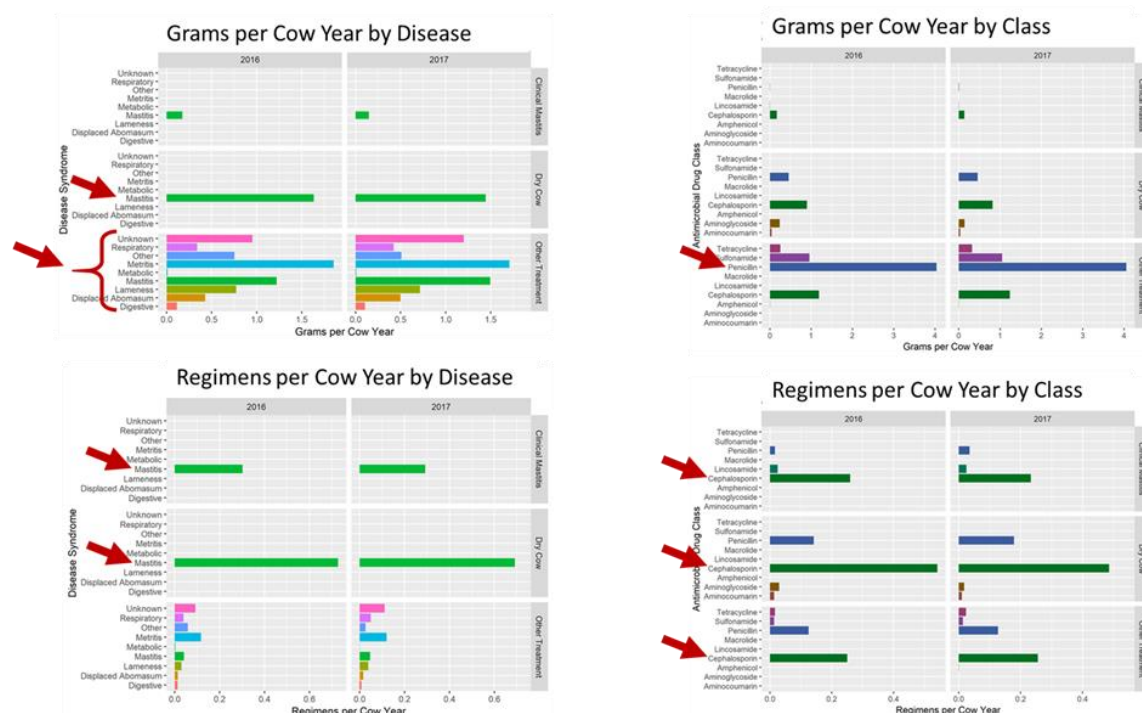
The basic concept of drug use creating selection pressure involves exposure of populations of bacteria to an antibiotic. All the measurements of use thus far have completely ignored the bacterial population. It is really the interaction between “bug” and “drug” that needs further

description. The previous chapters outlined the available data and formats for describing antimicrobial use. The next question is how to fit that with measures of the bacterial population that matter.

- What is the size of the bacterial population exposed?
- What is the diversity of species in the exposed bacterial population?
- What are the genetic resistance characteristics of the exposed bacterial population.
- What is the pathogenicity of exposed bacterial species?
- What is the likelihood that genetics from the exposed population would end up in a pathogenic organism?

A description of parameters aimed at characterizing bacterial populations is essential to objectively evaluating which measures of exposure might matter. Should we focus primarily on the high frequency of Dry Cow regimens that involve a very small number of mg put into a quarter where there is a numerically small non-diverse population of bacteria? (**Figure 5.2**) Or is it important to also focus on the total mg used to treat metritis involving systemic use of drugs like ceftiofur which may affect the diverse bacterial populations of the GI tract? Are grams of penicillin more detrimental cephalosporin regimens.

Figure 5.2 Which matters most? Regimens or Grams? Mastitis or Metritis? Use of Cephalosporins or Penicillin



The combined measurement of both drug use and characterization of a bacterial population would allow exploration of the measures of exposure that are important for resistance selection pressure. Once both sets of measures are available the following questions could be adequately explored.

- Does the magnitude of exposure matter? If so, how?
 - Is it the amount of time exposed?
 - If it is time, which matters more, calendar time or the ratio of calendar time to bacterial doubling time?
 - Does the dose (weight) of drug involved in the exposure matter?
- Does frequency of exposure matter?
 - Single vs. multiple peaks and tails of plasma concentrations
 - Does it matter if one single animal is exposed 10 times vs 10 animals exposed one time?

What is the relationship between use and animal welfare

There is good evidence that antibiotics are effective for treatment of metritis and mastitis caused by certain pathogens. To restrict or remove their use entirely in our current systems would undoubtedly decrease animal welfare. Often, the most heated arguments surrounding antibiotic use do not center around whether a sick animal should be treated, but rather why did the animal get sick in the first place? And how do we know the animal is sick (case definitions)? These animal welfare concerns associated with antibiotic use hinge on the following questions.

- How can we improve prevention of disease?
- Are we using antibiotics out of habit rather than based on a true benefit?
- How can we improve the accuracy of disease diagnosis?
- For which diagnoses do antibiotics positively affect outcomes of disease events?
- Are there times when we could improve the outcome of treatment by changing our therapeutic regimen?
- Are there times when we could change our regimen to reduce resistance selection pressure without sacrificing treatment outcomes?

It would be naive to think that we might reach a point where all infectious disease can be prevented. However, it is not hard to imagine that our livestock systems will continue to find new

ways to decrease disease incidence. Reduced disease incidence offers an opportunity to reduce antibiotic use without sacrificing welfare. Additionally, reduced disease can also benefit overall production and thus system economics provided that the changes needed to reduce disease are not too costly or are offset by a marketing premium.

While there are opportunities for prevention to improve, they do not present themselves to a veterinarian or herdsman nearly as often as the opportunity to treat a diseased animal or to prevent disease within the current system with antimicrobial use. More time is needed for advances in science to clarify the relationship between use and resistance selection, and patience is needed to find opportunities to improve prevention. In the meantime, perhaps there is value in taking advantage of the immediate opportunity to further investigate treatment outcomes. Treatments are documented in data systems that contain information about production parameters such as cull rates, case fatality rates, and milk production. There is opportunity to advance our antimicrobial stewardship recommendations by tweaking our data systems to allow efficient evaluation of treatment outcomes. If treated animals don't remain productive members of the herd, is it worth treating them? Can we identify patterns in treatment success that can point use towards diagnostics that might help distinguish between animals likely to benefit from treatment vs animals whose outcome is not changed by treatment? Rapid culture systems allowing differential treatment of mastitis pathogens are a great start. There are also other disease syndromes for which we can develop tools to help guide treatment decisions.

At the very least, trends in treatment outcomes should be observed. As societal pressure, and therefore market pressure, calls for a reduction in antibiotic use there will be continued pressure to replace known efficacious antibiotic regimens with alternatives. Care should be taken that treatment outcomes are maintained in the face of these alternatives. Simultaneously, treatment outcomes should be evaluated to identify practices where our antibiotic use is not successful or makes no positive difference in outcome, and is therefore unnecessary. It is with outcome evaluation that we can make immediate forward progress in identifying practices of appropriate antimicrobial use. In this manner forward progress can be made without being caught in the ethical dilemma of sacrificing animal welfare for the potential mitigation of a vaguely defined future risk of human pathogen resistance.

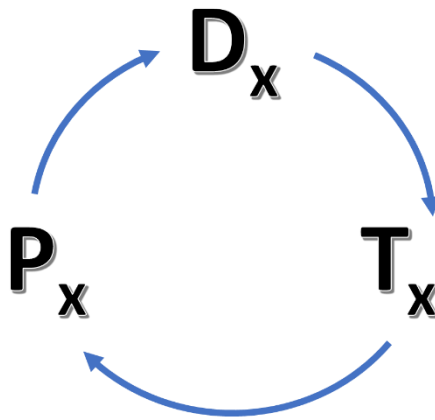
What is the relationship between use and production efficiency?

When does conservation of antibiotics as a resource cost so much in other resources that it becomes nonsensical? Is there a point at which some amount of antibiotic use may be an appropriate tool to allow a system with high output and reasonable production cost to continue? While antibiotics have been the resource of focus in this report there are clearly other resources involved in livestock production. When and where do we draw a line between appropriate use of an antibiotic to improve production practices and inappropriate risk of resistance development? When are antibiotics an appropriate tool for producing more food with fewer animals (which decreases the expenditure of other resources like fuel and water) vs times when antibiotics are abused as a resource to gain market advantage at the cost of exerting more resistance selection pressure than necessary? Again, it appears that a good starting point may be a closer examination of existing data to elucidate treatment outcomes which could guide our use practices. This evaluation happens within the economics of the dairy, allowing conclusions to be economically applicable to the system.

What is “appropriate” use?

As discussed in this dissertation, low “measured” use is not necessarily equal to “appropriate” use. Low use may be due to a lack of disease detection leading to animal welfare issues. High measured use may be due to an appropriate response to increased disease level in a herd. These scenarios cannot be differentiated by simple evaluation of measured use. They can only be determined by boots on the ground and an understanding of the entire dairy system including physical facilities, human resources, financial resources, and intellectual resources available for problem solving. True stewardship is a continued cycle through the process of diagnosis, treatment, and evaluation of disease prevention practices. As these cycles progress, attention must also be paid to other influences on the dairy system such as animal welfare, economics, and the utilization of resources other than antibiotics.

Figure 5.3 - Cycle Necessary to Advance Antimicrobial Stewardship



- **D_x** - Diagnosis of a disease condition likely to benefit from antimicrobial therapy.
- **T_x** - Treatment with a drug regimen that improves disease outcome more than non-antimicrobial treatments
- **P_x** - Revision of prevention practices to further reduce disease incidence.

Crucial to forward progress are repeated revolutions of this circle to provide a path to improved antimicrobial stewardship with continued improvement in the definition of “appropriate” use. It should be noted that rotating this cycle is a continuous daily activity for many veterinary practitioners. Their experience is of exceptional value in improving antimicrobial use practices. Providing practitioners and dairy managers with useful tools for measuring antimicrobial use is one way to advance stewardship within dairy systems.

Chapter 6 - Additional Data Acquired by Survey and Comparison to Data from Records

A survey was administered in year 1 to all participating dairies. The survey was administered to the person or persons willing to participate who had the most direct knowledge of treatment administrations on the dairy. The original intent of the survey was to provide an independent estimate of antimicrobial use, which could be compared to the estimates determined from treatment records. After initial data analysis, it was discovered that the information obtained in the survey contributed so significantly to treatment record standardization of regimen descriptions that a detailed comparison would be confounded by the use of survey information for record standardization. However, at the farm level for regimens per animal year sufficient information was present in each original source to make a comparison. Unless otherwise specified this chapter compares total number of regimens, not just antimicrobial regimens administered. This is due to the added complexity of asking survey questions in a manner that specified antimicrobial vs non-antimicrobial treatments. As demonstrated by the comparisons here, even the simpler comparison of all regimens did not show good agreement between the two sources.

Description of Questions Asked in the Survey

The survey questions were structured in a manner that allowed estimation of regimens by disease from survey results. In order to acquire denominator information, respondents were asked to estimate the average number of lactating cows present in the prior year and the average number of dry cows present in the same year. The number of lactating and dry cows were added together to be used as the denominator of cow years calculated by the survey.

Respondents were also asked to estimate the number of cows treated for each of the disease syndromes. Responses in any format that the respondent felt would be most accurate were allowed. Some dairies would respond with the number of animals treated each week, others with the number per month, and others with a percent treated per year. Respondents were allowed to consult treatment records, if the respondent so elected but the survey did not specifically require it. Five interviewees consulted computerized summaries of treatment records more than 3 times to answer questions.

All but 1 respondent indicated that they used blanket dry cow therapy routinely in 2016 and this dairy used selective dry cow therapy. Two additional dairies began using selective dry cow therapy programs in 2017. These questions helped during the record standardization process to know when further clarification was needed about the method of recording dry cow therapy.

Footbaths are also routinely used to control digital dermatitis and could potentially contain antimicrobials. The survey gathered information about footbath use including product and frequency. No dairies reported using medically important antimicrobial products in these footbaths. All dairies reported that footbaths were used and frequency varied from 1-7 times per week. Products were variable but the most common were copper sulfate, formalin, or a combination of the two.

The survey also included questions about non-medically important antimicrobials, adherence to protocols, product used at the time of dry off, and animal housing.

Comparative Estimates of Use

Comparisons were calculated when both the survey data and the treatment record data were complete, and where calculation of final record metrics did not require input from the surveys to allow comparison of the two measures. Results are presented only for the disease syndromes of mastitis, metritis, lameness, and digestive. The low incidence of the other disease syndromes made it extremely difficult for owners to estimate the number of treatments for these low incidence diseases and the results are not interpretable. The number of dairies included in these comparisons is 28. The dairies excluded are the same as those excluded from the reported use in chapter 3, plus one additional dairy for which survey data was not complete enough for evaluation.

Initially, denominator data gathered from the survey were compared to that gathered from the treatment records. This overall comparison is shown in **Figure 6.1**. The relationship is also evaluated at the farm level in **Figure 6.2**. When the values from each source are put into a linear regression model the adjusted correlation coefficient is 0.9879. There is very little difference between these values both at the dairy level and the study level.

Figure 6.2 Comparison of Denominator Data Gathered by Survey vs Treatment Record

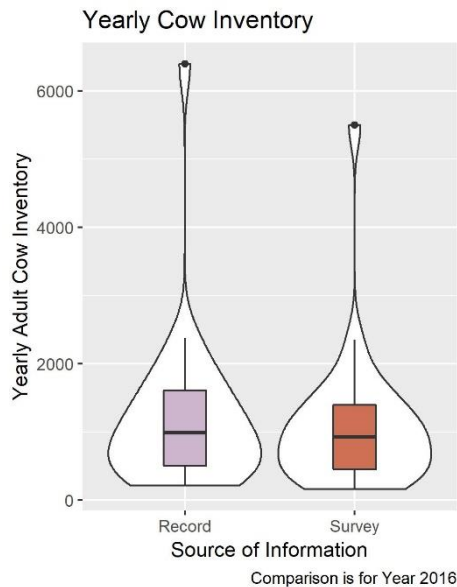


Figure 6.1 Regression of Denominators from Survey vs Record

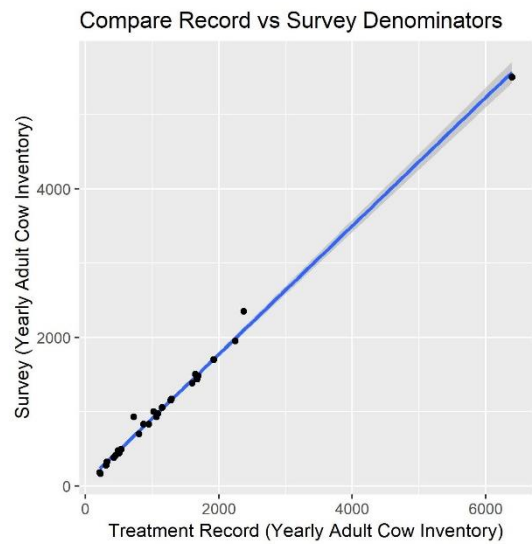


Table 6.1 Correlation of Regimens per Animal Year gathered by Survey vs Record

Disease	Correlation of Survey vs Record (All Treatments)
Metritis	0.51
Mastitis	0.23
Lameness	0.07
Digestive	-0.06

Figure 6.3 Regimens Calculated from Survey and Treatment Record

*Note: Mastitis only includes clinical mastitis, not dry cow.

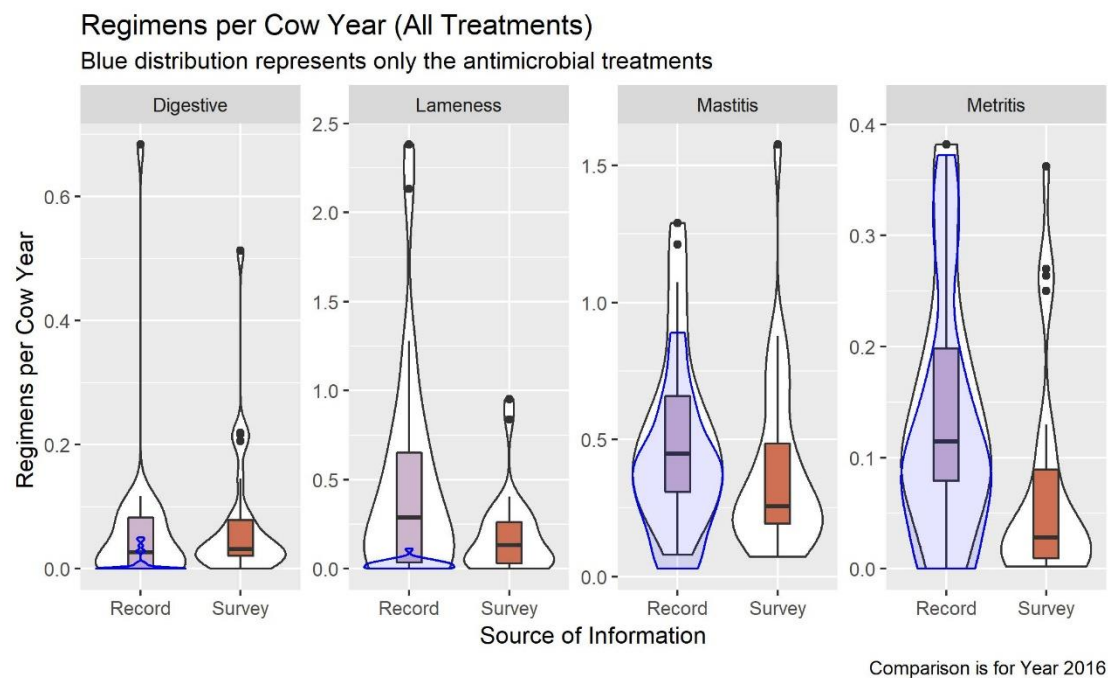


Table 6.2 Regimens Calculated from Survey vs Treatment Record

Disease	Treatment Record (AllTreatments)							paired t-test p.value	Survey						
	Minimum	25th percentile	75th percentile	Maximum	Median	Mean	Standard Deviation		Minimum	25th percentile	75th percentile	Maximum	Median	Mean	Standard Deviation
Digestive	0	0	0.08	0.68	0.03	0.06	0.13	0.703	0	0.02	0.08	0.51	0.03	0.07	0.11
Lameness	0	0.03	0.65	2.38	0.29	0.49	0.61	0.022	0	0.03	0.26	0.95	0.13	0.19	0.23
Mastitis	0.08	0.31	0.66	1.29	0.45	0.52	0.31	0.087	0.07	0.19	0.49	1.58	0.26	0.39	0.33
Metritis	0	0.08	0.20	0.38	0.11	0.15	0.11	0.001	0	0.01	0.09	0.36	0.03	0.07	0.10

The survey also gathered numerator data on the number of regimens administered by disease syndrome. These values were divided by the survey-acquired denominators to provide estimates of regimens per cow year from each source. It is important to note that the survey only asked about clinical mastitis cases, so only the clinical cases from the treatment record were used in the comparison. The overall correlations are shown in **Table 6.1**, indicating that correlation between these measures is not high, with the best for metritis at 0.51.

Figure 6.3 and **Table 6.2** display the summary estimates for the mean and median values for the two sources. The blue distribution overlaid in **Figure 6.3** indicates only the antimicrobial regimens documented in the treatment record. This highlights some distinct differences between disease syndromes and therapeutic choices. Based on these distributions it can be observed that nearly all cases of metritis and mastitis are treated with an antimicrobial, whereas there is a very small percentage of digestive or lameness cases where an antimicrobial was the therapy chosen.

A paired t-test was performed to quantitatively evaluate the difference between mean regimens per animal year measured by treatment record vs survey (**Table 6.2**, center column). Based on a P value of 0.05, there was a significant difference between means for lameness and metritis, but not for digestive and mastitis.

The relationship was also evaluated at the farm level by plotting the regimens per animal year calculated from both the treatment record and from the survey. Calculated regimens per animal year are shown in scatter plots in **Figure 6.4** to graphically depict their relationship with the regression models shown in **Table 6.3**. The r squared value is low for all models, and the only significant model when all treatments are included is the one for metritis ($P = 0.005$). When only antimicrobial treatments are considered the models for both mastitis and metritis are significant at 0.044 and 0.011 respectively.

It is noteworthy that while the mean estimate of regimens used for metritis was significantly different between survey and treatment record sources, the model predicting survey regimens from treatment record regimens has the highest adjusted r squared value of the diseases compared. This indicates that the accuracy of the mean estimate is low; all survey respondents tended to under-estimate the number of metritis regimens listed in their treatment records. However, respondents on farms with a high number of metritis regimens listed in their treatment records were more likely to indicate a higher number of regimens for metritis in their survey

response. While the r squared value of 0.234 is low it is still numerically greater than the r squared for the other diseases compared.

The relationship between sources was also evaluated by comparing only the antimicrobial treatments from the record with the amount of disease indicated by the survey (**Figure 6.5, Table 6.3**). While more models are significant with this comparison, the r squared value is still very low raising questions about the utility of such a model.

Figure 6.4 Regression of Record vs Survey for all treatments

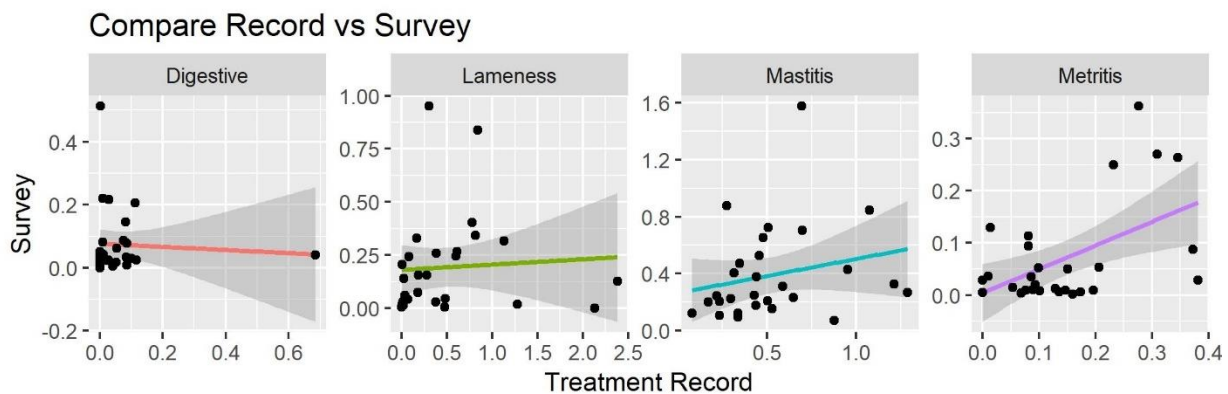


Figure 6.5 Regression of Record vs Survey for only antimicrobial treatments

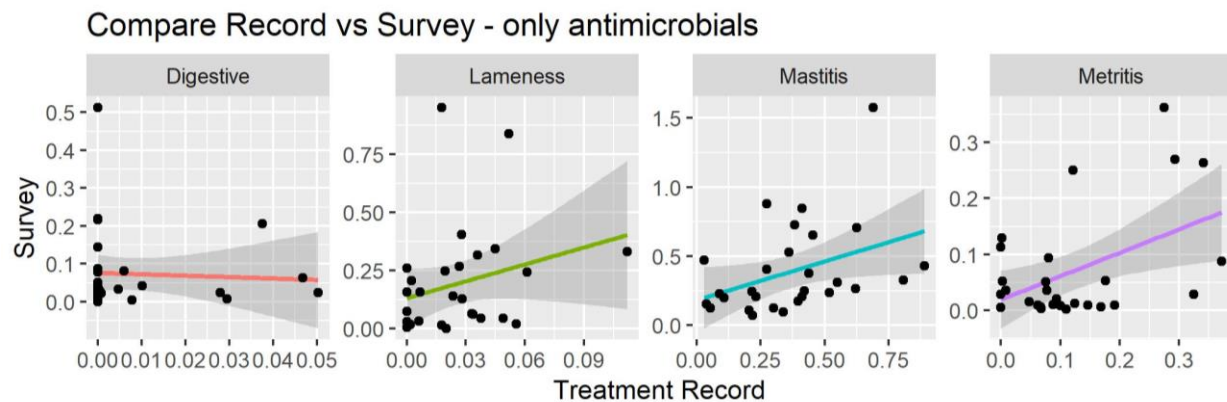


Table 6.3 Linear Regression Models for Treatment Record predicting Survey Regimens per Animal Year

Model	r.squared	adj.r.squared	p.value	AIC	BIC	deviance
Digestive	0.004	-0.035	0.765	-30.58	-26.58	0.444
Lameness	0.004	-0.034	0.739	57.07	61.06	10.157
Mastitis	0.052	0.015	0.244	17.73	21.72	2.492
Metritis	0.263	0.234	0.005	-47.74	-43.75	0.240
Digestive (only antimicrobials)	0.003	-0.036	0.791	-150.01	-146.01	0.006
Lameness (only antimicrobials)	0.071	0.035	0.171	-122.99	-118.99	0.016
Mastitis (only antimicrobials)	0.147	0.114	0.044	-3.77	0.22	1.156
Metritis (only antimicrobials)	0.222	0.192	0.011	-46.31	-42.31	0.253

Limitations and Sources of Error

There are multiple factors contributing to the variation in information derived from each information source:

- There is potential for recall bias in this survey. We administered the survey during summer 2017 but were asking about the calendar year 2016. This time lag may have caused some discrepancies due to changes that had been made but were difficult to recall.
- Treatment events for all diseases other than mastitis are relatively rare. Therefore, especially on small dairies, a difference of a very small number of cows could cause a relatively large proportional difference in estimated regimens per cow year.
- Survey questions asked respondents to estimate treatment frequency for a certain disease syndrome and asked what the protocol treatment was for that disease, but it did not specifically ask for an estimate of how many animals were treated with an antimicrobial. This causes some inherent discrepancy for the models generated based on only antimicrobial regimens. It also makes it difficult to evaluate this particular survey as a tool for estimating antimicrobial use based on regimens.

A pilot survey may have improved results by asking questions in slightly different formats, or by asking for further clarification to ensure the respondent answers were consistent with the question wording. Additionally, more specific questions about case definitions and specific recording habits could have improved the utility of the survey for record standardization, although

these questions likely would not have affected the relationship between the two sources of information.

Respondents struggled to answer the survey questions at times due to the extreme variation in disease pressure that occurs at various points in time throughout the year. For example, some diseases vary seasonally, while others vary with the percentage of cows entering lactation (freshening) at any given time point. The number of cows freshening may be driven by numerous management factors that are unrelated to disease pressure.

Summary

The respondents were able to very accurately report the number of cows present during the year for use as a denominator. Survey estimates of the mean regimens per animal year were not significantly different for mastitis and digestive disease syndromes but were for lameness and metritis. At the farm level, agreement between survey and treatment record were not well correlated indicated by the low r^2 for these models. Multiple factors contribute to these relationships including survey question selection, challenges of estimating infrequent sporadic disease, and recall bias.

Chapter 7 - Resources Required to Achieve Project Goals

Collection of dairy-level antimicrobial use data is resource intensive. This chapter is an estimate of the resources that were used to produce the reported antimicrobial use estimates. It should be noted that there is a degree of uncertainty present in the estimation of time resources necessary for data processing. Most of the work was completed by a graduate student who had little experience with data management software prior to this project. If a scale up process were undertaken by persons skilled in data management, the time necessary to accomplish the task would likely be reduced.

Resources for Dairy Recruitment and Retention

On average, it took 1-2 hours to recruit and maintain a dairy in the study each year. This consisted of phone and email contact time. Additionally, there were approximately 60 minutes of non-visit related contact time per year for each of the veterinarians who identified clients willing to participate.

Resources for Data Collection

Travel to dairies: Dairies in all but 2 regions required travel by air and auto to reach the destinations. In 2 regions travel was by auto only. On average, 4 dairies could be visited each day, but this varied greatly by region.

Time on dairies: Dairy visits ranged from 20 minutes to 2 hours. This variation was predominately driven by the degree to which the respondent wanted to engage in conversation. These conversations were essential to informing evaluation of the data. They were also helpful for maintaining relationships that aided in retention of these dairies for subsequent data collection. Downloading electronic data took less than 5 minutes for all dairies, requiring access to the computer housing the record system.

If written records were present, they were acquired by taking on-farm digital photos. This process proved to be relatively efficient and functioned regardless of the format of the records. Written record formats included spiral notebooks, loose papers, 3 ring binders, and write-in-the-rain record books. As a general estimate, it took approximately 5 minutes to capture treatment data for every 100 cows present on farm.

Resources for Record Upload and Standardization

Initially, record extraction for upload to the database was very time consuming. If a dairy was the first of a record system type, the time was much greater than for subsequent dairies using the same system. A new record system took an average of 18 hours to install the software, explore methods of data extraction, and obtain confirmation from software company representatives that those extraction methods were valid. By the second year of data processing, treatment records could go from the farm computer to the database in an average of 10 minutes if records were in Dairy Comp 305. While travel to the dairies occurred to maintain our relationship, it should be recognized that this is not actually necessary for data transfer. If a consulting version of the dairy record system is in place, records can be transferred electronically.

The greatest amount of time was required for record standardization. Time for standardization ranged from 2 -120 hours per dairy. The challenges of standardization were previously described. To aid in efficiency and reduce potential for errors, this process of standardization was facilitated by the creation of a “key” for each individual dairy. These keys were created by extracting all unique combinations of variables in the original treatment record. Each combination was evaluated and mapped to standardized values. This key was then joined to the original treatment record creating a standardized version of the record for that dairy. The shortest key contained 31 rows, and the longest contained 1,341. On average, key creation took about 1 hour for every 100 rows in the key. While it is possible that parts of the key creation process could be streamlined and automated, some parts will require time spent identifying standardized values from other sources such as protocols, purchase records, or survey data.

The development of the standardization process was iterative (see Chapter 2). There were numerous times when the standardization method was modified. This required repeating some or all of the previous extraction and standardization work for certain dairies. This makes estimations of time necessary per dairy difficult to calculate. There was a significant learning curve, and process modification created greatly improved processes.

Data Clean up and Validation

Once the records had been standardized, it was necessary to validate that the standardized values were correct, and that these values matched with the original record. This was primarily done graphically by plotting subsets of data to look for outliers. The raw data extractions uploaded

to the database totaled 1,332,112 rows; this included all recorded events, some of which were not treatments. Treatment data comprised 637,458 rows. The current method of creating keys is inherently error prone due to the volume of data manually standardized. Therefore, the process of validating these keys are correct were time consuming and currently takes approximately 4 hours per dairy. There is also validation that denominators and products used were properly matched between treatment records and survey data.

Resources for Analysis and Report Creation

Time required for this process is difficult to estimate as the identification of appropriate measures was an iterative process. The specific values reported and the format in which they were reported progressed constantly over the course of the project. Data were stored in an offline SQL database, and outputs were generated using R. Once a final output was identified and the first report was generated, subsequent reports were easily produced if code were written in a manner that facilitated this process.

The most difficult part of this process was validation so that the reported results accurately represent the original data. The most efficient method for validating was to compare benchmark reports and look for outliers. On numerous occasions, an error in a key was identified by this process. This was an iterative process and the necessary time invested is a function of the number of dairies which need to be validated.

As the project progressed, more efficient methods for evaluation were developed. For example, during year 2 a preliminary benchmark report was generated that categorized reported values in a way that facilitated validation graphically in a single output, whereas previous methods required the evaluation of multiple graphical outputs to achieve the same result. In spite of this progress there is still potential for the process to be streamlined and more automated.

Discussion of Scale Up

Subsequent years of data collection and analysis are more efficient than the first, but the collection and analysis of granular use data is resource intensive. An estimate of time to add a new dairy to an existing system can only be attempted if the new dairy has the same record system as an existing dairy, same recording method, and no new formatting challenges. When all of the above are true, it is estimated that a new 500 cow dairy can be added to the system and output

generated in approximately 8-16 hours. However, this assumes no hand entry and an average number of rows in the standardization key. Encountering anything new for which there is not yet a data handling mechanism in place greatly increases the upper estimate of time.

Travel expenses averaged \$215 per dairy per researcher, and included airfare, car rental, lodging, and food. These expenses were calculated from trips which were planned to make very efficient use of time by scheduling dairies conveniently by geography so that travel was minimized. These expenses would significantly increase if compensation to the researcher for time was included. Also, these travel days were often greater than 14 hour days. If the same were attempted with an 8 am to 5 pm schedule there would be less travel efficiency causing the cost to increase.

If it were decided that collection of such granular data was necessary at the national level, more efficient collection could be accomplished by asking the dairies/veterinarians to do the record standardization at the farm level. For example, the columns in the standardized treatment record could be “required data” and the appropriate values for each column could be specified. It would be relatively simple for the dairy database systems to either build in the necessary standard entries or create a report that could be converted. The collection of denominator data would require modification as it should be noted that only approximately 50% of dairy herds subscribe to DHIA testing, which was the source of input data for some dairy record systems in order to calculate denominators for benchmarking.

However, other than the required resources necessary, the largest challenge in scaling this detailed level of reporting up to the national level is that the details of the benchmark data are only useful as a point at which questions can be asked at the dairy level. There are numerous confounders of these data that would result in output that was not interpretable at the national level. The knowledge necessary for appropriate interpretation exists only at the dairy level. Making this level of detail useful to dairies as benchmark reports and also useful to research questions aimed at optimizing use.

Chapter 8 - Plasma concentrations of chlortetracycline achieved by administration of a mineral formulation to adult beef cows

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Abstract

Objective: This study establishes baseline information necessary for future experiments that optimize therapeutic recommendations for Chlortetracycline (CTC) use to control Anaplasmosis.

Materials and Methods: During phase one, 30 cows were allocated to treatment groups and individually received $1.1 \text{ mg} \cdot \text{kg BW}^{-1}$ either in a small amount of feed with observed intake, or by balling gun administration of a gelatin capsule containing the dose. During phase two, 15 cows received an intended dose of $1.1 \text{ mg} \cdot \text{kg BW}^{-1}$ of the same mineral product allocated daily to a pasture mineral feeder. Blood samples were collected at multiple time points and plasma CTC concentration was quantified using HPLC.

Results and Discussion: Individual administration by gelatin bolus resulted in a median plasma concentration of 19 ppb with an interquartile range (IQR) of 11 ppb. Individual administration by observed intake resulted in similar median and range of 20 ppb with an IQR of 8 ppb. These values demonstrated dose linearity with previous literature. When administration was by a single mineral feeder the median CTC concentration was 6 ppb with an interquartile range of 20 ppb. For animals tested in both phases there was a significant difference ($P < 0.01$) in the minimum plasma concentrations achieved when the mineral feeder was used compared to either bolus or observed intake.

Implications and Applications: For future clinical trials administration by bolus offers no advantage over observed intake of CTC dose. The assumption that mean consumption from a mineral feeder will achieve the same therapeutic effect as bunk fed experiments should be further analyzed.

Introduction

Due to concerns about extended administration of medically-important antimicrobials, chlortetracycline (CTC) use in livestock has recently been scrutinized. Since January 2017, its legal use has shifted from an over-the-counter product to one requiring a veterinary feed directive¹⁶. The label for CTC use for anaplasmosis control lacks a defined duration. Revision of label claims to define duration is objective 1.1 in the FDA's report on their 5 year plan for supporting antimicrobial stewardship in veterinary settings.⁹⁷ From 2009-2016, drugs in the tetracycline class consistently constituted 70% by weight of all medically-important antibiotics sold or distributed in the domestic US for use in food-producing animals²⁶. This percentage is much higher than for

any other single class of medically-important antimicrobials. While the potency of tetracyclines is relatively low compared to some other classes of drugs, this overwhelming percentage still indicates common and consistent use. It is important to have substantial evidence documenting the efficacy of recommended CTC regimens. The drug must reach the target pathogen at an appropriate concentration in order for efficacy claims to be legitimate ⁹⁸.

Brock et al. (1957) demonstrated that a bunk fed CTC dose of 1.1 mg·kg BW⁻¹·d⁻¹ prevented clinical disease in animals subsequently challenged with anaplasmosis. This experiment also demonstrated that treated animals did not become carriers of *Anaplasma marginale* as confirmed by blood-transfer inoculation of splenectomized calves. Chlortetracycline was subsequently adopted as a control measure for anaplasmosis in beef production systems ^{99,100}. The use of CTC to eliminate the carrier stage of anaplasmosis was also explored by others. ¹⁰¹⁻¹⁰⁶. Some of these early experiments used tests with inadequate sensitivity for infection detection as compared to modern methods. More recently, Reinbold et al. (2010) used both cELISA and RT-PCR methods to document that a bunk fed CTC dose of 4.4 mg·kg BW⁻¹·d⁻¹ was sufficient to shift infected cattle to a negative test status; carrier elimination was confirmed by blood-transfer inoculation of splenectomized calves.

The label dose for control of anaplasmosis is 1.1 mg·kg BW⁻¹·d⁻¹ for hand-fed products, and the target dose 1.1 to 4.4 mg·kg BW⁻¹·d⁻¹ for free-choice products ⁶⁸. Previous studies of efficacy and related pharmacokinetics of CTC in cattle have examined formulations where CTC was administered by mixing it into a ration or by top-dressing it on feed ¹⁰⁷⁻¹⁰⁹. Pharmacokinetic parameters of CTC in calves at dosages of 4.4, 11 and 22 mg·kg BW⁻¹·d⁻¹ top-dressed on feed twice daily were recently evaluated ¹⁰⁷. In each case, CTC was supplied orally to cattle fed mixed diets in confinement, but not using a mineral formulation. These results were subsequently extrapolated to situations in which CTC was delivered via mineral. To authors' knowledge, no experiments have examined individual plasma concentrations of CTC when the drug was administered as part of a mineral supplement fed to adult cattle.

A common use of CTC is aid in control of active infection of anaplasmosis in adult cattle maintained on pasture. In this setting, CTC is generally administered as a component of a mineral supplement, rather than as a top dress or part of a mixed ration. Multiple consumption studies of free-choice formulations have been performed for this approved use ^{108,110}. Mean CTC intakes from 1.14 to 4.09 mg·kg BW⁻¹·d⁻¹ per cow-calf pair were documented during the experiments that

were used to support the 1996 supplemental approval ¹¹⁰. This is a remarkable dearth of information because animal age, genotype, metabolic state, diet composition, and drug formulation may influence drug absorption and metabolism ¹¹¹; moreover, there is potential for individual variation in consumption when the dose is administered using a mineral feeder in a pasture setting. Therefore, the objective of our experiment was to characterize steady state plasma CTC levels achieved by adult beef cattle treated with a typical dose regimen of a mineral formulation of CTC when intake was known, and to explore concentrations when the same cows were offered this product in a pasture setting typical of production practices for anaplasmosis control.

Materials and Methods

This protocol was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC protocol #3912). The experiment was conducted in 2 phases as depicted in **Table 8.1**. All procedures involving animals were conducted in accordance with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010).

Table 8.1 Treatment descriptions for phase 1 and phase 2.

Treatment	CTC Daily Dose, mg/kg	Number of Animals	Administration Method	Formulation
Phase 1				
DDG	1.1	10 (5 heifers, 5 mature cows)	Individually administered by mixing in 0.34 kg grain supplement.	Medicated mineral mix containing 6.61 g/kg (6000 g/ton) Chlortetracycline
Bolus	1.1	10 (5 heifers, 5 mature cows)	Individually administered by oral bolus.	Medicated mineral mix containing 6.61 g/kg (6000 g/ton) Chlortetracycline
Non-Medicated	0	10 (5 heifers, 5 mature cows)	Individually administered by mixing in 0.34 kg grain supplement	Non- medicated mineral mix.
Phase 2				
Mineral Feeder	1.1 ⁵	15 (mature cows)	Ad libitum access to mineral feeder containing dose sufficient for treatment of group. Mineral was allocated to feeder daily.	Medicated mineral mix containing 6.61 g/kg (6000 g/ton) Chlortetracycline

⁵ Targeted dose per head, intake of mineral not confirmed on an individual animal basis.

Cattle and Husbandry.

The animals chosen for this study were typical of the class of beef animals likely to receive CTC in a production setting. In phase 1 of our experiment, animals were individually administered a daily dose of CTC equivalent to $1.1 \text{ mg} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$. This standardized dose was then used to facilitate interpretation of phase 2 of our experiment, where animal dose was determined by voluntary mineral intake in a pasture setting. The drug formulation used for both phases of the experiment was a commercially-available mineral supplement containing CTC at 6.61 g/kg.

For phase 1 of the experiment Cows ($n = 30$) were housed in two adjacent, earth-floor drylots and fed smooth brome grass hay ad libitum. One drylot contained 15 mature cows (initial BW range = 473 to 715 kg; initial age range = 3 to 8 yr) that were bred via artificial insemination approximately 30 d before beginning phase 1. These mature cows were supplemented daily with 3 kg/hd (as-fed) of a 50:50 (w/w) mixture of dried distillers grain and ground corn. The adjacent drylot contained 15 two-yr-old cows that were nursing their first calves (initial BW range = 364 to 617 kg). These lactating, young cows were supplemented daily with the aforementioned dried distillers grain and ground corn mixture at a rate of 7.5 kg/hd.

For phase 2 of the experiment cattle were housed on native grass pasture with free choice water.

Experimental Design – Phase 1

Animals were blocked by drylot and assigned randomly to 1 of 3 treatments ($n = 10$ cows/treatment): 1) non-medicated mineral mixed in 0.34 kg of the grain mixture (**control**; Purina Wind and Rain™, Land O' Lakes, Inc., Arden Hills, MN) ; 2) balling gun administration of medicated mineral in a gelatin capsule (**bolus**; Purina Wind and Rain™ – CTC Formula, Land O' Lakes, Inc., Arden Hills, MN); or 3) medicated mineral mixed in 0.34 kg of the grain mixture (**DDG**; Purina Wind and Rain™ – CTC Formula, Land O' Lakes, Inc., Arden Hills, MN).

For bolus and DDG treatments, sufficient medicated mineral was dosed to provide CTC at $1.1 \text{ mg} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$. Animals were weighed at 7 d intervals during the experiment to ensure the targeted CTC dose was achieved. All animals were handled once daily for treatment administration. Animals assigned to control and DDG treatments were individually penned at 0700 daily and offered their respective supplements. Animals were observed by investigators until supplement was completely consumed. Animals assigned to the bolus treatment were restrained in

a squeeze chute at 0700 daily. Medicated mineral was delivered orally via hard gelatin capsules⁶ administered using a balling gun⁷.

Animals were acclimated to diets and environmental conditions for 6 wk before blood collection commenced. Initial blood samples were obtained via venipuncture on arrival to confirm no previous exposure to CTC. To ensure that samples were taken when CTC concentrations had reached steady state, treatments were administered for 7 days prior to commencing with sample collections. Thereafter, blood samples were collected via jugular venipuncture on day 7, 10, and 14 (10 mL/sample). Blood was collected at 0700 and 1900 on day 7 and day 14 and at 0700 only on day 0 and 10, for a total of 5 sample collections per animal per treatment group (**Table 8.2**). Throughout the experiment, blood samples (approximately 10 mL) were collected into lithium-heparin tubes (**Table 8.2**). The preferred venipuncture site was the tail, but on days with multiple collections the jugular was used as an alternative.

⁶ Torpac gelatin capsules, Fairfield NJ

⁷ Nebraska style balling gun, Miller Manufacturing, St. Paul MN

Table 8.2 Blood collection schedule for phase 1 and phase 2. Collections A and G were baseline samples to provide evidence that there was no pre-treatment CTC exposure.

Collection	Day	Time	Treatments	Number of Animals Sampled
Phase 1				
A	0	AM	Non Medicated	30
B	7	AM	Bolus ⁸ , DDG ⁹ , Non Medicated ¹⁰	30
C	7	PM	Bolus, DDG, Non Medicated	30
D	10	AM	Bolus, DDG, Non Medicated	30
E	14	AM	Bolus, DDG, Non Medicated	30
F	14	PM	Bolus, DDG, Non Medicated	30
Phase 2				
G	0	AM	Non Medicated	15
H	7	AM	Mineral Feeder ¹¹	15
I	14	AM	Mineral Feeder	15
J	21	AM	Mineral Feeder	15

Experimental Design – Phase 2

After the completion of phase 1 of the experiment and an 80-day washout period, the 15 mature cows¹² from Phase 1 were moved to a single native pasture and offered non-medicated mineral *ad libitum* per product label for 14 d. Following this acclimation period, medicated mineral supplement containing CTC at 6.61 g/kg was fed for 14 d. A single mineral feeder was used to administer only enough mineral supplement for the 15-cow group for 1 day of 1.1 mg·kg BW⁻¹, and product was allocated to the feeder daily consistent with the product label to be *hand fed*.

⁸ Bolus = dosed at 1.1 mg·kg BW⁻¹·d-1-11.1 mg·kg BW⁻¹·d-1, administered in a gelatin capsule by balling gun

⁹ DDG = dosed at 1.1 mg·kg BW⁻¹·d-1-11.1 mg·kg BW⁻¹·d-1, administered via observed intake from individual feed pan

¹⁰ Non Medicated = dosed with non-medicated mineral, administered via observed intake from individual feed pan in phase 1, and voluntary intake in phase 2

¹¹ Mineral Feeder = medicated mineral containing 6.61 g/kg CTC allocated to mineral feeder daily in an amount sufficient to achieve 1.1 mg·kg BW⁻¹·d-1-11.1 mg·kg BW⁻¹·d-1 for each animal in group, voluntary intake.

¹² During the washout period, one cow was removed from the experiment because of a medical condition unrelated to treatment.

Blood samples were collected via tail venipuncture on day 0, following acclimation to non-medicated mineral, as well as on d 7, 14, and 21, following introduction of medicated mineral (**Table 8.2**). After collection, blood samples were immediately placed on ice. They were then transported to the laboratory and centrifuged at $2750 \times g$ at $4\text{ }^{\circ}\text{C}$ for 15 min. Plasma was then removed via pipette and stored at $-62\text{ }^{\circ}\text{C}$.

CTC quantification.

Complete methods for CTC quantification can be found in the supplemental material. In summary, calibration standards, blank, QC and bovine plasma samples were extracted by solid-phase extraction (SPE) using Oasis HLB Prime μ Elution 96-well plates (Waters Co, Milford, MA). Negative control, calibrants, QC plasma or bovine plasma sample were prepared on a 48-well non-tissue culture treated plate (Corning Incorporated, Corning, NY). The 48-well plate was shaken gently at 350 rpm for 10 min at room temperature with the IKA MTS 2/4 Shaker (IKA® Works Inc., Wilmington, NC), and then centrifuged at $1370 \times g$ for 30 min at $20\text{ }^{\circ}\text{C}$ (Thermo Scientific ST16 Benchtop Centrifuge, Waltham, MA).

The analysis was carried out using a UPLC–ESI–MS/MS instrument. The chromatographic separation was performed using an Acquity H Ultra Performance Liquid Chromatography (UPLC) system (Waters, Milford MA, USA) including a heated column compartment, a binary pump, and autosampler. The UPLC column used was an Acquity C18 column Waters HSS T3, $1.8\text{ }\mu$, $2.1 \times 50\text{ mm}$, held at $50\text{ }^{\circ}\text{C}$. The detection and quantification of OTC and CTC was performed using a Xevo TQ-S triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Waters, Milford MA, USA). Data acquisition and data quantification were performed using the Waters MassLynx and TargetLynx software 4.1, respectively. The lower limit of quantitation (LLOQ) of CTC was determined to be 1 ppb according to the FDA analytical validation guidelines.

Statistical Analysis.

A linear mixed model was created for Phase 1 to explore the effects of potential confounders (animal age and sampling time) and sources of random error (cow and collection) This model was created using the lmer function from the lme4 package in R.

Descriptive statistics for each treatment were summarized showing the mean, sd, median, absolute range, interquartile range (IQR) and mean absolute deviation (MAD) values for each treatment group. Density plots were used to evaluate the distributions of the treatment groups.

Individual animal CTC concentrations are presented as scatter and box plots for both phase 1 and phase 2 to allow visualization of the distribution of plasma concentrations within and among individuals and treatment groups. Additionally, the median, minimum, maximum plasma concentrations were calculated for individual cows. These are presented as a scatter and box plot. Further analysis was performed for the subset of animals that were dosed with 1.1 mg CTC / kg BW daily during phase 1 and were also present during phase 2. For these 9 individuals, the animal level median, minimum, and maximum plasma CTC concentrations were compared between phase 1 and phase 2. Due to the difference in distributions between phases, a Wilcoxon Signed Rank test (`wilcox.test(y1,y2,paired=TRUE)`) was used to compare the known dose administered in Phase 1 with the intended dose administered in Phase 2. Comparisons were made for individual-animal median, minimum, and maximum plasma CTC concentrations.

Due to the lack of literature data indicating which plasma concentration parameters might be most predictive of CTC-dose efficacy, several parameters were selected to demonstrate the potential variation in expected clinical outcomes, as dependent upon plasma CTC concentration. The CTC concentrations selected for evaluation were 5, 10, 15, and 20 ppb. Each animal within each treatment group was evaluated for achievement of each of 3 parameters at the selected concentrations. The 3 parameters evaluated were:

- animal median concentration greater than selected concentration
- animal maximum concentration greater than selected concentration at least once
- animal minimum concentration always above selected concentration

The percentage of animals within treatment group achieving each parameter was then calculated for each selected concentration.

Results and Discussion

Linear mixed model.

For phase 1, a mixed linear model was created to explore the effects of cofactors. Estimates for model parameters are presented in **Table 8.3**. Treatment was the only significant predictor of plasma CTC concentration ($P < 0.01$; **Table 8.3**). Animal age (young vs older cows) and collection

time of day (am vs pm) were not significant at $\alpha = 0.05$ ($P = 0.39$). Blood collection time (am vs pm) tended ($P = 0.07$) to slightly influence plasma CTC concentration (estimate = 2.6 ppb, Table 3).

The small effect of collection time of day might be due to the difference in sample collection time relative to trough concentrations. The am sample was collected at the steady state trough just prior to dose administration. However, the relationship of the pm sample to a steady state peak is not clear. The elimination half-life of CTC is estimated at 16 hrs. using a one compartment open model with first order absorption and elimination, and at 35 hrs. using a non-compartmental analysis (NCA).¹⁰⁷ The same study calculated a time at maximum concentration (T_{max}) of 38 hours when the dose regimen was 2.2 mg/kg administered every 12 hours. Data for T_{max} is not available for a once daily dose of 1.1 mg/kg.

Table 8.3. Mixed linear model parameters

Model Variable - Fixed Effects		Estimate, ppb	Standard Error, ppb	P-value
Treatment Group	Bolus ¹³	19.7	1.5	< 0.01
	DDG ¹⁴	18.8	1.5	
	Non-Medicated Intercept ¹⁵	-1.1	1.5	
Animal Age	Young	1.1	1.2	0.39
AM vs PM	PM	2.6	1.4	0.07
Model Variable - Random Effects		Variance	Standard Deviation	
Cow	Intercept	6.4	2.5	
Collection	Intercept	3.3	1.8	

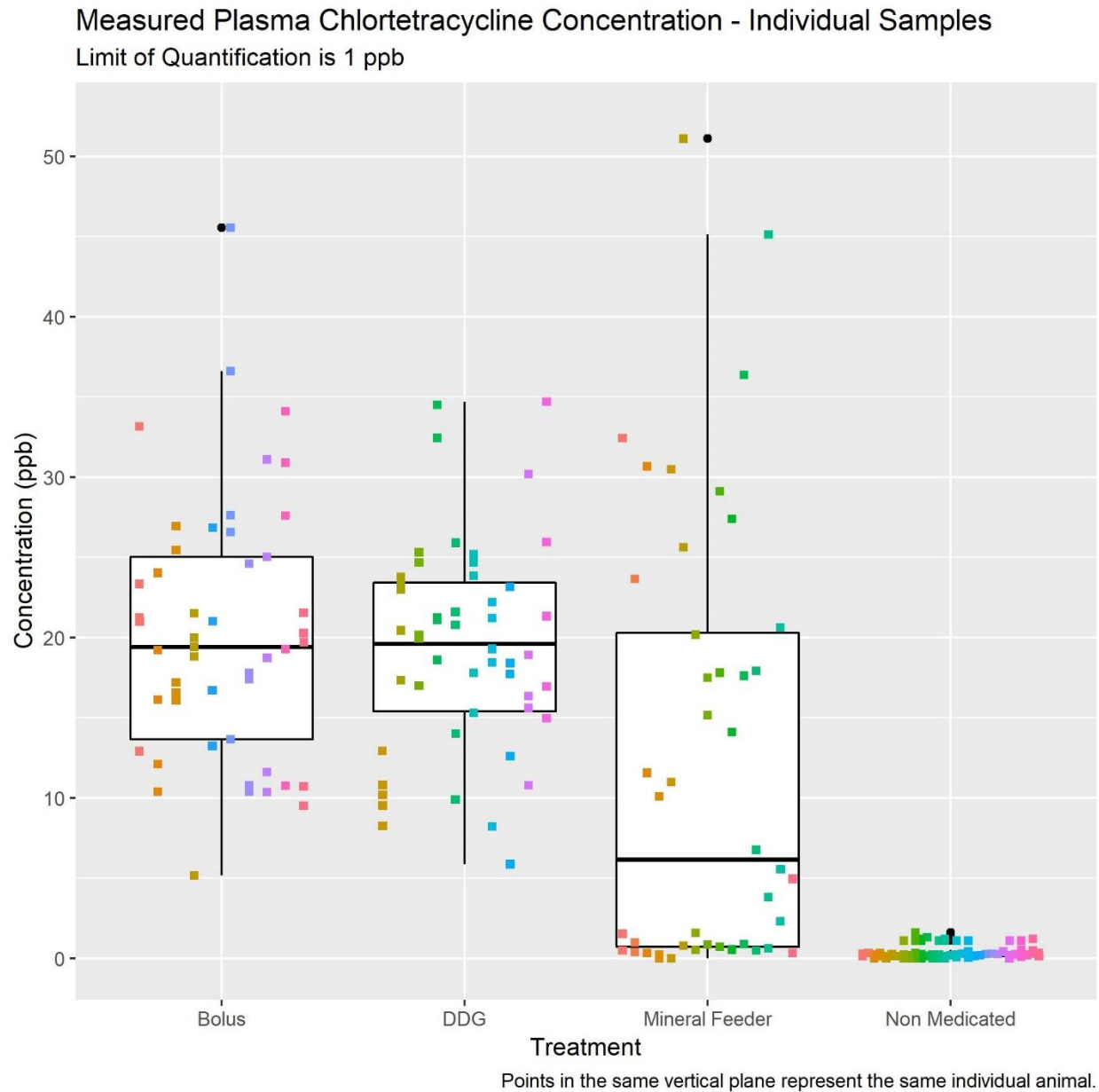
In the model parameters y = phase 1 plasma concentrations. This model was generated to explore the effects of possible cofactors (e.g., time of day and animal age) and to describe the amount of variability associated with known confounders (e.g., cow and collection).

¹³ Bolus = dosed at 1.1 mg·kg BW⁻¹·d⁻¹ CTC, administered in a gelatin capsule by balling gun

¹⁴ DDG = dosed at 1.1 mg·kg BW⁻¹·d⁻¹, administered via observed intake from individual feed pan

¹⁵ Non Medicated = dosed with non-medicated mineral, administered via observed intake from individual feed pan

Figure 8.1 Individual Sample Scatter and Box Plots



Scatter-and-box plots of plasma concentrations measured at steady state. For box plots, the dark center line represents the median, outside edges of the box represents interquartile range, and the whiskers represent the range. Outliers are depicted as black dots. Each vertically associated column (color) of points represents the concentration results for an individual animal.

Both individual animal and blood-collection event were modeled as sources of random error in plasma CTC concentrations (**Table 8.3**). The variation attributed to cow was 6.4 ppb, with a standard deviation of 2.5. Variability between individual animals cannot be avoided or greatly minimized via experimental design. Authors assume that variability in plasma CTC concentration that occurs between animals on the same oral CTC regimen is likely to be greater in field settings due to less accurate BW estimations when scales are not available to calculate dosages. Notably, the variance in plasma CTC concentration attributed to blood-collection event was roughly 50% of the magnitude of that observed to occur between individual animals (3.3 ± 1.8 ppb vs 6.4 ± 2.5 ppb).

Plasma concentrations.

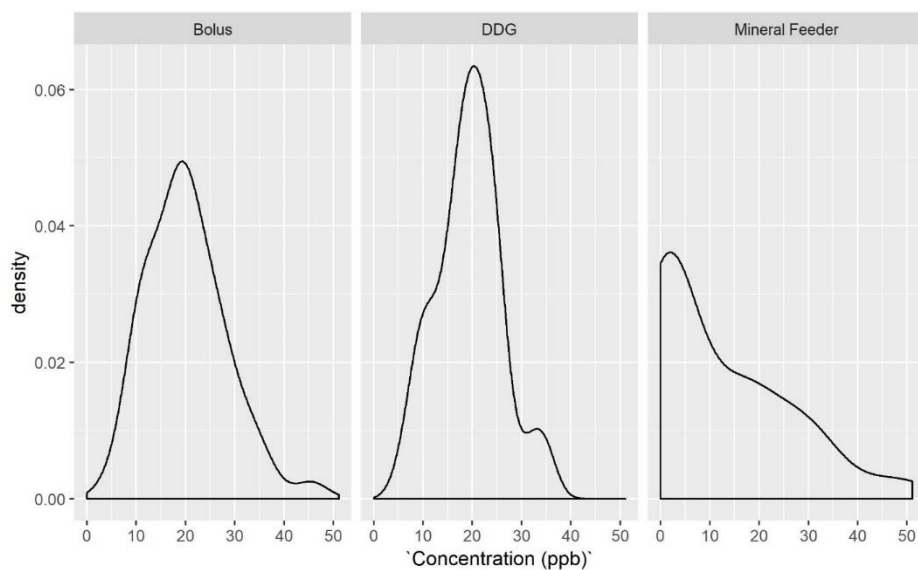
Plasma concentrations for both phase 1 and phase 2 are summarized by treatment in **Table 8.4**, and depicted as a box and scatter plot by treatment in **Figure 8.1**. As seen in Figure 2, the distributions of the plasma CTC concentrations differed between phase 1 and phase 2. Administration by bolus or DDG at 1.1 mg·kg BW-1·d-1 resulted in reasonably-normal distributions of plasma CTC concentrations, whereas administration by mineral feeder resulted in a right-skewed distribution. Further evidence for skewed distribution can be noted in the large number of points at or below the quantification limit in the box plot for the Mineral Feeder treatment in **Figure 8.1**.

Table 8.4. Summary of chlortetracycline plasma concentrations for each treatment group in both phase1 and phase 2.

Plasma concentrations of chlortetracycline, ppb							
Treatment	Mean	SD	Median	Minimum	Maximum	IQR	MAD
Bolus ¹⁶	20	8	19	5	46	11	9
DDG ¹⁷	19	7	20	6	35	8	6
Non Medicated ¹⁸	BLQ	BLQ	BLQ	BLQ	2	NC	NC
Mineral Feeder ¹⁹	NC	NC	6	BLQ	51	20	9
BLQ = Below Limit of Quantification, 1 ppb							
NC = Not calculated due to the skewed distribution of values for this treatment, or BLQ values							

Figure 8.2 Density Plots

Density plot of the distribution of plasma concentrations obtained by treatment. The x-axis is plasma concentration of chlortetracycline in ppb and the y-axis is the density of measured plasma chlortetracycline concentration values



¹⁶ Bolus = dosed at 1.1 mg·kg BW⁻¹·d⁻¹, administered in a gelatin capsule by balling gun

¹⁷ DDG = dosed at 1.1 mg·kg BW⁻¹·d⁻¹, administered via observed intake from individual feed pan

¹⁸ Non Medicated = dosed with non-medicated mineral, administered via observed intake from individual feed pan in phase 1, and voluntary intake in phase 2

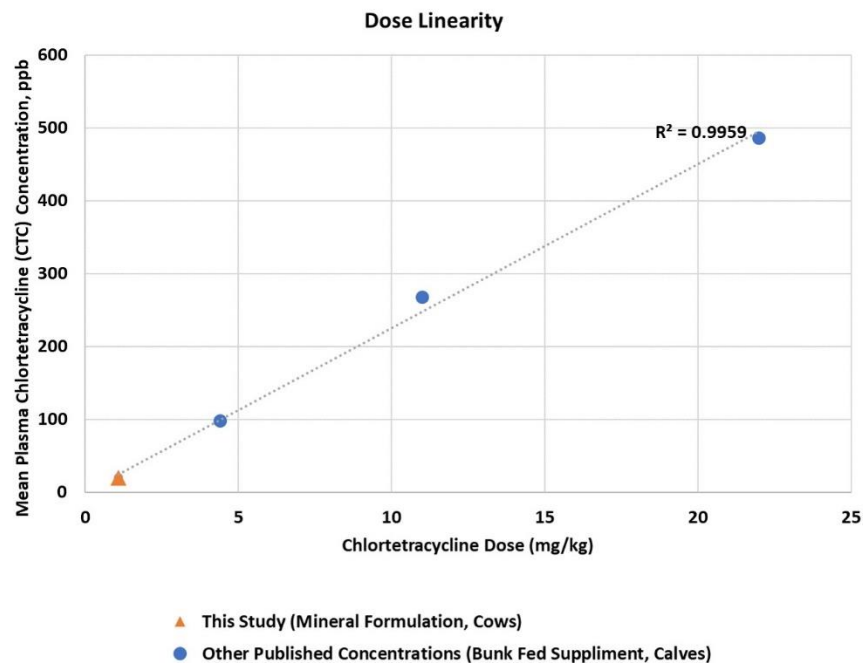
¹⁹ Mineral Feeder mineral containing 6.61 g/kg CTC allocated to mineral feeder daily in an amount sufficient to achieve 1.1 mg·kg BW⁻¹·d⁻¹ CTC for each animal in group, voluntary intake.

Dose linearity.

Despite the differences in animal age, product formulation, and dosing method, plasma CTC concentrations observed in this experiment are consistent with steady state concentrations reported by Reinbold et al. ¹¹². As shown in Figure 3, plasma CTC concentrations detected in phase 1 of our experiment when a mineral formulation of CTC was delivered (dose = 1.1 mg·kg BW-1·d-1) demonstrated dose linearity when compared to CTC administration as part of a bunk fed supplement formulation (dose = 4.4, 11, or 22 mg·kg BW-1·d-1). This suggests that the bioavailability of the mineral formulation is not drastically affected by strong ions in the formulation, and thus likely achieves plasma concentrations consistent with other formulations that were used in previous efficacy experiments.

Figure 8.3 Dose Linearity

Comparison of mean plasma concentrations achieved in this study with other published plasma concentrations achieved with different doses and product formulations. Other published mean concentrations are from Reinbold et al. 2010 at doses of 4.4, 11, and 22 mg/kg.

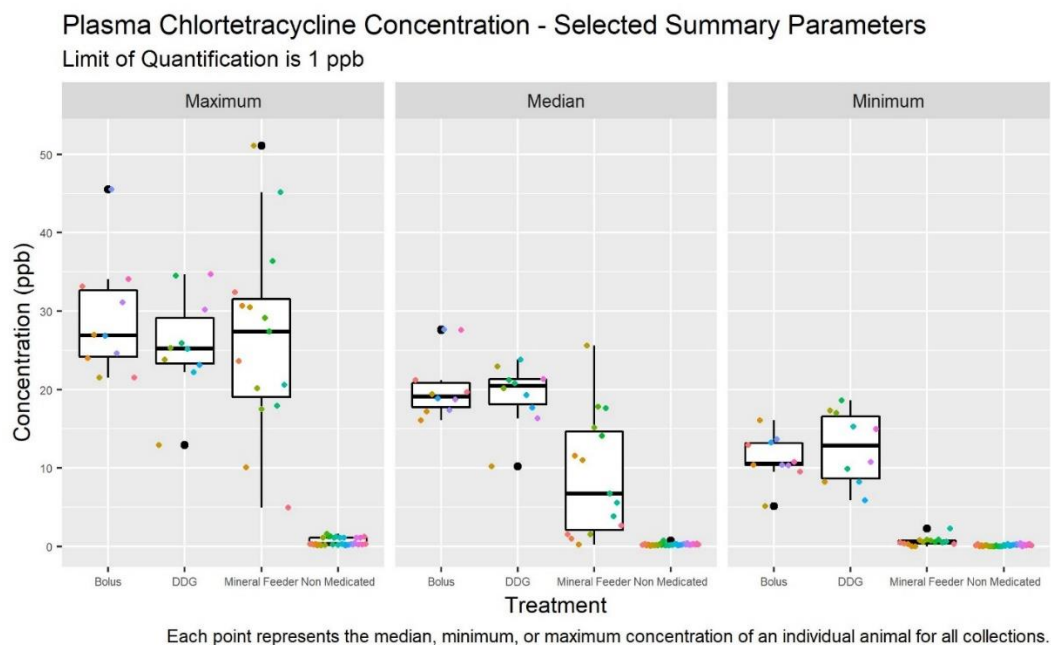


Parameters of interest.

Although consumption studies with the mineral formulation exist, previous studies evaluating the efficacy of CTC for anaplasmosis control did not use mineral formulations. In previous consumption studies for mineral formulations the mean consumption of the group was used to estimate intake by individuals. Figure 4 shows plasma CTC values summarized at the cow level by several different parameters, maximum, median, minimum. The mean was not calculated due to the non-normal distribution of concentrations in the mineral feeder group. Our data indicate that it is possible that the mean value is not the only parameter that should be evaluated if non-individually dosed consumption data is used to estimate likelihood of drug efficacy. Figure 4 allows visualization that while the maximum concentrations differ very little, the minimum concentrations visually appear much lower for the mineral feeder administration compared to Bolus or DDG.

Figure 8.4 – Summary Parameter Scatter and Box Plots

Scatter-and-box plots of selected parameters describing plasma concentrations summarized at the individual cow level



Tables 9.5 and 9.6 show the median, maximum, and minimum plasma CTC concentrations were calculated for each cow receiving chlortetracycline during both Phase 1 and Phase 2 (A). The parameter values were evaluated using a Wilcoxon Signed Rank test to determine if there was evidence of a difference between phase 1 and phase 2 for each tested parameter (B).

Table 8.5 Summary Parameters for Individuals

Selected plasma CTC concentration parameters calculated at the individual animal level, ppb						
	Maximum		Median		Minimum	
	Phase 1 ²⁰	Phase 2 ²¹	Phase 1	Phase 2	Phase 1	Phase 2
Animal A	33.145	32.418	21.228	1.522	12.902	0.483
Animal B	24.028	30.672	16.107	11.553	10.391	0.327
Animal C	26.942	10.093	17.2	0.218	16.091	<0.1
Animal D	12.913	30.495	10.196	10.98	8.243	<0.1
Animal E	21.498	51.1	19.418	25.62	5.15	0.786
Animal F	25.31	17.502	20.147	15.155	17	0.848
Animal G	34.494	36.383	21.247	17.606	18.6	0.885
Animal H	25.903	17.925	20.785	6.755	9.895	0.498
Animal I	21.53	4.946	19.7	2.6375	9.493	0.329

Table 8.6 Wilcoxon Signed Rank Test for Each Summary Parameter Between Phase 1 and Phase 2

Parameter	Wilcoxon Signed Rank - V	P-value	Pseudo median	95% Confidence interval
Median	39	0.05	8.8	-0.6, 16.9
Maximum	23	1	0.6	-14.4, 12.3
Minimum	45	< 0.01	11.3	8.2, 16.1

²⁰ Phase 1 refers to Phase 1 of the experiment where cattle were treated with 1.1 mg·kg BW⁻¹ individually administered by gelatin bolus, or observed intake

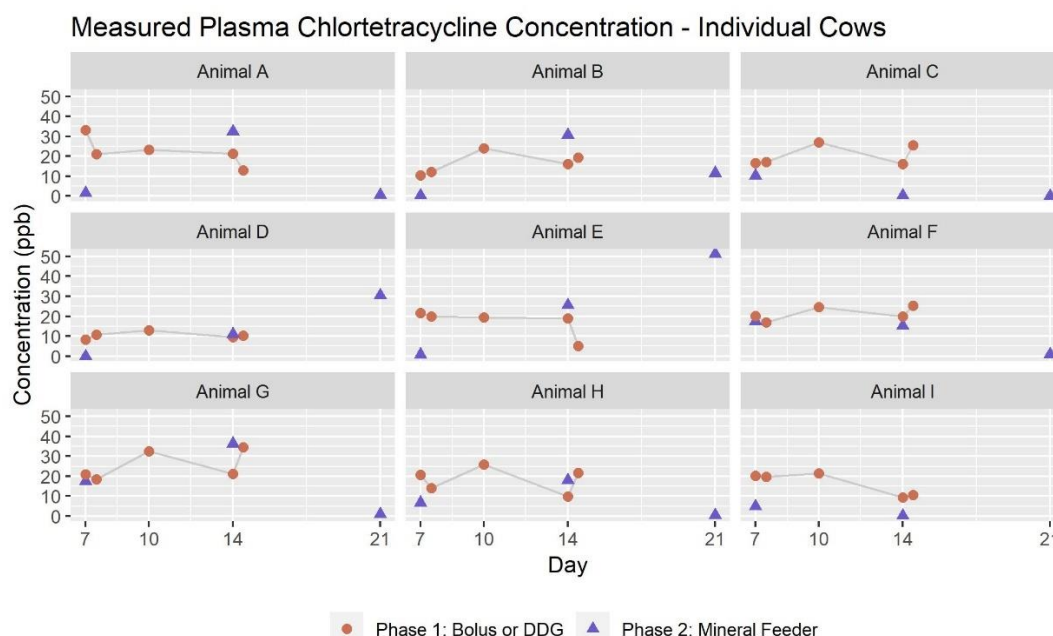
²¹ Phase 2 refers to Phase 2 of the experiment where cattle were treated with 1.1 mg·kg BW⁻¹ administered by allocation of CTC product to a pasture mineral feeder in a sufficient amount to treat the group for one day at the specified dose.

In order to test this difference between treatment groups, analysis was limited to those animals that received a known dose of CTC in phase 1 and were still present in phase 2 (**Table 5.A, Figure 5**). This eliminated the potential of individual cow effect confounding the analysis. Differences between CTC dosing methods in phases 1 and 2 of our experiment were tested using the Wilcoxon Sum Rank test (Table 5.B). The minimum detected CTC plasma concentrations differed ($P < 0.01$) between the two methods of administration. At 90% confidence, the median CTC concentration also differed ($P = 0.05$); however, the detected CTC maximum showed no evidence of a difference ($P = 1.0$

Parameter Importance

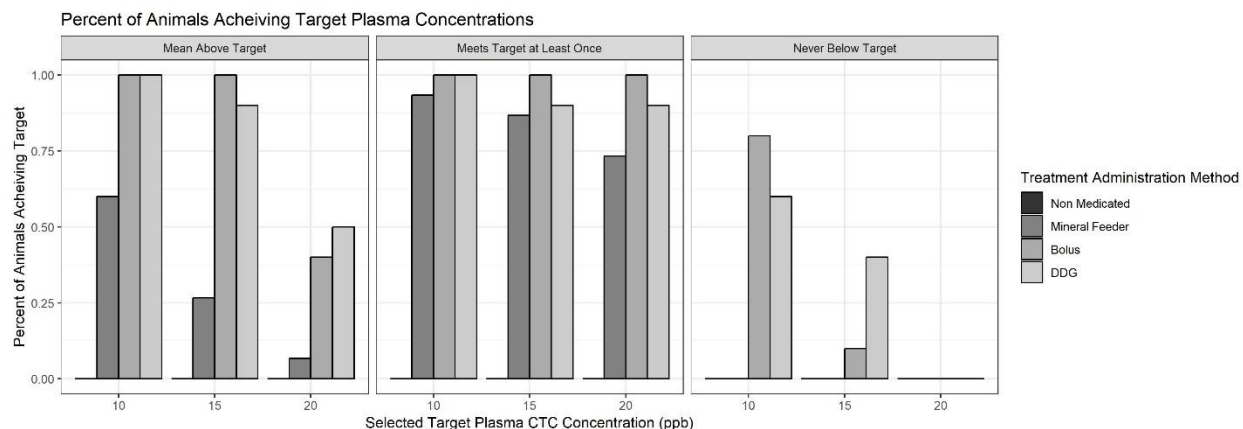
The most important pharmacodynamic parameters for effective treatment of *A. marginale* with CTC are unknown. Parameters such as time above a minimum concentration, maximum concentration, or total area under the exposure curve could be relevant. However, their appropriate application to this scenario remain undocumented. Though chlortetracycline was not tested, the related compound oxytetracycline has. One in-vitro study compared the effect of chlortetracycline to 2 other drugs by observing changes it caused to *A. marginale*. In short term cultures, this study found oxytetracycline the least effective.¹¹³

Figure 8.5 Compare Individual Samples Phase 1 vs Phase 2



Plasma concentrations of chlortetracycline for phase 1 and phase 2. Each sub graph represents an individual cow identified by tag number. Cows who received non-medicated mineral in phase 1 are not shown. The line between points for phase 1 is included for visual clarity to link that group of samples, not due to the expectation that values between points would fall on this line

Figure 8.6 Percentage of Individuals Meeting Target Parameters



Example plasma concentrations were selected to represent potential therapeutic target concentrations that may be identified in future studies. The different sub graphs represent the several possible therapeutic goals associated with the target concentrations

Implications for regimen optimization.

These results may have implications for the establishment of target plasma concentrations of CTC for anaplasmosis control. Due to the complexity of culturing *A. marginale* in vitro, the use of standard methods such as pairing pharmacodynamic targets with mean inhibitory concentrations (MICs) to help establish dose regimens and target concentrations are not currently practical. Therefore, clinical trials will be necessary to establish appropriate target plasma concentrations for control of active anaplasmosis infections, as well as elimination of carrier states. For example, it is possible that for the control of active infection the median plasma concentration might be best correlated to positive clinical outcome but, for elimination of carriers, the minimum concentration might be the parameter that is most important. Due to the lack of data about specific parameters, several parameters were explored to model the effects different therapeutic targets might have on

the percent of a population that is protected from disease. As demonstrated in Figure 6 the mineral treatment group appears to offer less protection for all selected parameters, but especially if maintenance of a minimum concentration is necessary for efficacy.

Discussion.

The variation observed in the mineral feeder treatment group warrants further study. Consumption of mineral from a mineral feeder in a pasture setting is known to be variable and may be influenced by factors such as mineral-feeder location, season of the year, and pasture size.¹¹⁴; however, every attempt was made to minimize such factors in our experimental approach. Subsequent experiments should examine difference in distribution of plasma CTC concentrations to ascertain if it is an artifact in this particular trial with only one pasture, one group of cattle, one type of mineral, and one type of mineral feeder. The label of the mineral product used in our experiment required that the amount of mineral offered to cattle be allocated to the feeder each day, as performed for this trial. In a typical production setting, it is a common practice to fill the feeder with an amount of an appropriate mineral product sufficient for the group for numerous days. Therefore, consumption in a production setting where a free-choice mineral formulation is used may vary somewhat from this trial.

Applications

Administration of a mineral formulation CTC product resulted in very similar plasma concentrations across subjects when intake was encouraged by adding grain to the product and observing intake, compared to using a gelatin capsule to ensure ingestion. Previous experiments documenting efficacy of CTC for anaplasmosis at 1.1 mg·kg BW⁻¹·d⁻¹ were performed using bunk fed (non-mineral) supplement, and some studies individually fed animals. This study suggests that dose linearity is maintained with the mineral formulation when compared to bunk administered supplements, implying similar bioavailability in spite of formulation differences. Although the intended dose was the same, there is indication that voluntary intake in a pasture setting should be further studied. The variation in minimum plasma concentrations achieved in this single pasture setting differed from those achieved when the same animals were administered a known dose. The significance of these differences for maintaining adequate disease control are unknown but should be further explored as they potentially leave animals inadequately treated.

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