IMPROVING ANTIMICROBIAL STEWARDSHIP FOR CONTROL OF MASTITIS IN DAIRY HERDS

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ABSTRACT

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Implementation of better management practices that promote antimicrobial stewardship on farms is needed due to increased concerns with the impact of antimicrobial usage **(AMU)** on development and transmission of antimicrobial resistance from animals to humans. Interventions to reduce AMU can be applied by monitoring antimicrobial treatments at farm-level and identifying diseases that contribute to AMU. Mastitis is the most frequent bacterial disease occurring on dairy farms (USDA–APHIS–VS–CEAH, 2008), and it is well known that the occurrence of mastitis results in major economic losses for dairy farmers (Ruegg, 2005). Intramammary administration is the major route of AMU on dairy farms when using a dose-based metric to calculate AMU. (Pol and Ruegg, 2007; Saini et al., 2012a; Stevens et al., 2016) Dry cow therapy typically accounts for 31 to 70% of intramammary treatments (Pol and Ruegg, 2007; Saini et al., 2012a; Stevens et al., 2016; Schrag et al., 2020b). The overall hypotheses of this dissertation is: 1)the greatest quantity of AMU on large dairy farms is for treatment and control of mastitis, and 2) that AMU and costs on farm can be reduced by reducing the days of treatment for clinical mastitis during lactation or implementing selective treatments at dry-off. The aims of this dissertation are to: 1) quantify AMU on large dairy farms and contrast total AMU by route and active ingredients using both dose-based and mass-based metrics. With these results, we will demonstrate how antimicrobial treatments related to udder health impact on total AMU on large dairy farms. 2) Estimate direct costs of treated and non-treated clinical mastitis using data obtained from commercial dairy farms and contrast variation in treatment costs among

herds. 3) Estimate direct costs at dry-off and potential saving if selective dry cow therapy was used. And 4), evaluate a method to potentially decrease AMU at dry-off by performing a clinical trial to determine if use of an alternative dosing schedule for an immune stimulant (pegbovigrastim) reduces the need for administration of antimicrobials at dry-off.

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INTRODUCTION

Antimicrobials have been used on dairy farms for treatment of bacterial diseases, to promote animal health, increase longevity of cows, and control spread of contagious pathogens. However, antimicrobials need to be used responsibly. Unjustified use of antimicrobials could lead to increased costs of production, decreased profitability, and reduced consumer confidence in dairy products. Implementing treatment protocols that aim to reduce use of antimicrobials on farms without affecting animal health will address consumer concerns and potentially reduce costs of production.

Clinical mastitis is the most common reason for administration of antimicrobials on farms and treatment with intramammary antimicrobials is more frequent in large dairy herds. Large dairy farms are responsible for most of milk produced in the U.S., yet few studies have quantified antimicrobial usage in these herds. Antimicrobial usage can be measured by using different metrics but when antimicrobials are measured using the number of daily doses, intramammary administration of antimicrobials often accounts for most of the antimicrobial usage. Reducing antimicrobials on farm could be achieved by using selective treatments on lactating and dry cows or by simply reducing duration of treatment. With net returns often negative on farms, reducing antimicrobial usage could be beneficial to dairy farmers and increase profitability.

The aim of this dissertation is to quantify and improve antimicrobial stewardship for mastitis by performing applied studies that demonstrate differences in antimicrobial usage and costs of management practices that reduce antimicrobial usage were used.

CHAPTER 1. LITERATURE REVIEW

IMPORTANCE OF MONITORING ANTIMICROBIAL USAGE

The primary concern related with antimicrobial usage (AMU) in livestock is its effect on development of antimicrobial resistance (AMR). Most antimicrobials available for usage in livestock are also used for treatment in human medicine. This increases the risk of transmission co- or cross- resistant organisms (Jensen et al., 2004). Thus, surveillance of AMU helps find risk factors associated with AMU, facilitates control and interventions, and assists in interpretation of resistance-surveillance data (Jensen et al., 2004).

In recent years, the emergence of AMR has become an even more serious issue, especially due to the lack of new antimicrobials. The Center for Disease Control and Prevention (CDC) estimates that AMR is responsible for approximately 2 million illnesses per year, with up to $20 billion spent for direct healthcare costs (CDC, 2013). Exposure to antimicrobials is known to be related to the development of resistance, but the relationship between AMR and use of antimicrobials in production agriculture is not well defined. To have a better understanding on how spread of AMR occurs, quantification of AMU at herd-level is necessary. Antimicrobial quantification started in the mid-1960s by Arthur Engel in Sweden and Pieter Siderius in Holland due to the importance of comparing AMU among countries and regions (WHO, 2003). Their study demonstrated significant difference in sales of antimicrobials in six European countries, motivating the World Health Organization (WHO) to organize the first meeting on drug consumption in 1969. This first study did not allowed comparisons of drug utilization data among different countries due to variation of sources and forms of data. Yet, researchers in the United Kingdom, Norway, and Sweden developed a measurement unit called daily dose, which was further renamed as defined daily dose.

Studying AMU is not only necessary to understand risk factors that drive AMU but also benefits farm profitability. Increased information about the quantity of AMU on individual farms would help veterinarians and government agencies better understand relationships between AMU and development and transmission of AMR.

ANTIMICROBIAL RESISTANCE

Antimicrobial therapy become available for use approximately 100 years ago and usage has drastically increased since 1950's. Antimicrobial therapy has been used to improve animal welfare and increase longevity (Johnston, 1998; Hao et al., 2014). However, the use of antimicrobials in livestock has become a threat due to emergence of AMR and possible transmission from AMR genes from animals to humans (FDA, 2021).

Antimicrobials act by killing (i.e., bactericidal) or inhibiting bacteria growth (i.e., bacteriostatic). Resistance arises when a previously susceptible bacteria develops mechanisms which prevent bacteriostatic or bactericidal effects of a given antimicrobial. Antimicrobials act by inhibiting formation of cell wall, protein, DNA, or other targets. Beta-lactams are the primary class of antimicrobials used for treatment of adult cows (USDA–APHIS–VS–CEAH–NAHMS, 2018), and their activity damages the development of bacterial cell walls (Boothe, 2022). Resistance of an organism to antimicrobial occurs through two mechanisms: 1) intrinsic resistance or 2) acquired resistance. The first type of resistance is the innate ability of a bacteria to resist against activity of antimicrobials (e.g., Gram-negative). This could be caused by the lack of affinity of a drug to certain bacteria, inaccessibility of a drug into the bacteria cell, or innate production of enzymes that inactivate the drug. Acquired resistance is when a bacterium that was previously susceptible to an antimicrobial develops the ability to resist against an antimicrobial activity by a mutation or acquisition of a resistant gene. Emergence of acquired

resistance is driven by selection pressure based on exposure of bacteria to antimicrobials (CDC, 2021).

Relationships between intramammary (IMM) antimicrobials (measured in ADD) administered for treatment or prevention of mastitis and AMR have been studied for different mastitis causing-pathogens and different results were found for Gram-positive and Gram-negative bacteria (Saini et al., 2012c, 2013). The study evaluating AMR with Gram-negative bacteria demonstrated that IMM treatments using βeta-lactams increased the odds of intermediate or resistant *E.coli* to ampicillin, aminoglycoside, and trimethoprim-sulfa, but no association was found based on usage of other IMM antimicrobials and resistance in other genera (Saini et al., 2012b, 2013). In contrast, when AMR was evaluated for non-aureus staphylococci, AMR was associated with systemically but not IMM usage in dairy cows (Nobrega et al., 2018). Use of ceftiofur (a broad-spectrum 3rd generation cephalosporin) is concerning because the WHO has classified third generation cephalosporins as a critically important class for human health (WHO, 2018a). Responsible usage guidelines encourage use of narrow spectrum antimicrobials when appropriate.

INTRAMAMMARY INFECTION ACROSS LACTATION

Bulk tank somatic cell counts in the U.S. have reduced approximately 41% in the last two decades and averaged 178,000 cells/mL in 2020 (Norman et al., 2021). Reduction in bulk tank somatic cell counts is a consequence of changes in management practices and control of contagious pathogens that were known to remain subclinical and cause persistently high SCC in affected cows.

Dairy cows are susceptible to mastitis across their entire lactation. Intramammary infection (IMI) occurs when the immune system is not successful in combating pathogens that

invade the teat canal (Sordillo, 2018). Mastitis is classified based on the presence (clinical) or absence (subclinical) of clinical signs. The inflammatory response is dependent on duration of infection, host immune status, and pathogen virulence (Sordillo, 2018; Erskine, 2020). Detection of mastitis is commonly performed during milking through fore-stripping or is based on observation of monthly individual cow SCC. Subclinical mastitis if often detected based on SCC monitored by dairy herd improvement organizations or California Mastitis Test. A threshold of ≥200,000 cells/mL for composite SCC test or score ≥ trace for CMT is often used to identify infected cows (McDougall et al., 2022). When using a threshold of ≥ 200,000 cells/mL on composite milk, false negatives may happen as a high SCC from one quarter can be diluted from the low SCC of other quarters. Subclinical mastitis prevalence varies from 5% to 75% in infected cows and from 2% to 40% in infected quarters (Erskine, 2020).

Clinical mastitis is normally detected based on presence of clinical signs during fore-stripping. According to a national survey, the percentage of cows affected annually with mastitis in small, medium, and large herds was 25.6%, 16.4%, and 26.9%, respectively (USDA–APHIS–VS–CEAH–NAHMS, 2014). In the U.S., Gram-positive and Gram-negative opportunistic environmental pathogens cause most clinical mastitis (Ruegg, 2018). On most dairy farms, a large proportion of clinical cases are bacteriologically negative or are caused by Gram-negative pathogens or *Streptococci*. In herds where contagious pathogens has been effectively controlled, a goal for the incidence of clinical mastitis should be 1 to 2 cases/100 milking cows per month and severe cases should not exceed 1–2 cases/100 milking cows /year (Erskine, 2020).

During dry period, mammary gland goes from periods of extreme susceptibility to extreme resistance against mastitis causing pathogens (Oliver and Sordillo, 1989). Active involution occurs in the first 2 days after dry-off and continues up to 21 days (Zhao et al., 2019).

However, in high producing cows, milk synthesis never completely stops, and teat sphincters could take up to 30 d after cessation of milk to completely close, increasing vulnerability to mastitis (Oliver and Sordillo, 1989). During periods of intense physiological changes (e.g., early dry-off and colostrogenesis), mammary glands are particularly susceptible to new IMI, and infections acquired during dry period are known to persist through next lactation. Consequently, monitoring the incidence of mastitis during this period is essential to maintain productivity in the subsequent lactation. In studies evaluating IMI dynamics across dry period have reported a prevalence of 10 to 50% of mammary gland quarters have developed IMI by the end of a lactation and are mostly caused by non-aureus staphylococci (Green et al., 2005; Pantoja et al., 2009; Scherpenzeel et al., 2014).

CONSIDERATIONS WHEN MEASURING ANTIMICROBIAL USAGE

Among various sources used to monitor AMU, on-farm records (paper or electronic records), medical waste, and sales data are the most used resources. For many years, sales data along with AMU pattern has been of interest of health regulation authorities and pharmaceutical industry (Chauvin et al., 2001). Sales data has been the primary source used for commercial purposes, but this source is also used to monitor AMU. Sales data represents the volume of a product introduced into the market or purchased. In the U.S., antimicrobial administration in livestock is monitored by the FDA Center for Veterinary Medicine that publishes an annual report including the total amount of antimicrobials sold for use in food-producing animals (FDA, 2020a). This report separates animal species by cattle, swine, chicken, turkey, and others but lacks a denominator for all species. Dairy and beef cattle are characterized as one group, making interpretation of the data on a commodity basis difficult. While sales data are important to monitor the volume of antimicrobials in industry, sales data cannot be used as a reliable indicator

of AMU and AMR development (Bright-Ponte, 2020). Sales data does not represent administration of antimicrobials to animals (Sanders et al., 2020) and comparison of total weight of antimicrobials sold among different species is not encouraged due to difference in number of animals among species and body weight for example (FDA, 2020b).

To better understand associations between AMU and development of resistance, data should be collected at farm level (Bright-Ponte, 2020), and when possible, contain information such as the administration dose, antimicrobial active ingredient, duration of treatment, and disease. However, obtaining detailed data is often difficult and most of the time incomplete, making impossible to calculate AMU at national level. Health and management practices on U.S. dairy farms have been characterized at national level (USDA, 2016). Across all herds, the primary reason for farmers to keep a record system is to monitor breeding history and genetic improvements (85.7%), followed by culled cow sales (78.2%), animal health (72.5%), milk production (65.6%), antibiotic withdraw times (64.1%), and others (1.9%). The proportion of farms that maintain a record system increased with herd size. For example, a record system was used to monitor animal health by 95% of large herds (≥ 500 cows) in comparison to 81% and 64% of the medium (100 – 499 cows) and small (30 – 99 cows) farms, respectively. Similarly, usage of computerized records also differed by herd size and adoption of computerized dairy management records increased from about 33% of small farms to 68% of the medium farms and 99% of large farms (USDA, 2016).

Collecting drug waste could be also used to measure AMU on dairy farms. This method has been reported as one of the most precise sources to assess AMU (Nobrega et al., 2017). However, increases labor as empty drug packages must be disposed in drug receptables and consistently collected and might include information bias as all packages might not have been

properly discarded (Stevens et al., 2016). This method becomes potentially impractical for very large herd sizes.

METRICS USED TO MEANSURE ANTIMICROBIAL USAGE

Depending on the purpose, AMU can be summarized in dose, mass, or prescription of an antimicrobial for a specific population over a period (Mills et al., 2018). Among metrics used to quantify AMU, animal daily dose (ADD) is characterized as a dose-based metric and it is widely adopted for quantification of AMU in animals (Jensen et al., 2004). ADD is dependent on the label instructions for each antimicrobial and represents the dose that an animal would receive per day if following the label instructions. An important distinction of this metric is that it does not account for difference in potency among antimicrobials. Total AMU within a population is usually expressed as the number of ADD per 1,000 animals per day, but could be converted to ADD per cow per year by dividing by 2.74 (1,000/365), and is calculated as follows:

$$\frac{Amount\ of\ drug\ used\ in\ one\ year\ (mg)}{DDD(mg) \times 365\ days \times animals\ at\ risk} \times 1000\ animals\ at\ risk$$

$$= Number\ of\ DDD\ per\ 1000\ animals\ per\ day$$

Dose based metrics have been used to measure changes in AMU as the result of national legislative efforts in some countries (Taverne et al., 2015). However, one disadvantage of ADD is that AMU can be underestimated for antimicrobials that have a label frequency of two applications per day. For these antimicrobials, 2 applications per day are classified as one dose daily dose (i.e., 1 ADD). Another disadvantage is that since ADD is dependent on label instructions, comparisons among countries is complicated as labels and dosages vary among countries (Taverne et al., 2015). Other factors that can influence ADD are assumptions on body weight, combination products (e.g., novobiocin- procaine penicillin G or procaine penicillin G-dihydrostreptomycin used for IMM DCT), and calculation of dry cow therapy. Blanket dry cow

therapy is the practice of administering antimicrobials to all quarters of all cows at the time of dry-off and can be calculated by using 4 IMM as 1 ADD or 1 IMM as 1 ADD depending on preference for calculation, which directly impacts estimates of AMU. Differences in IMM AMU have been previously observed in studies that used 4 IMM DCT as 4 doses (Stevens et al., 2016) to studies that used 4 IMM as 1 dose (Pol and Ruegg, 2007; Redding et al., 2019). Stevens et al., (2016) reported that DCT accounted for the majority of AMU. In contrast, Pol and Ruegg (2007) reported that treatment of lactating cows accounted for the majority of AMU. For combination products, ADD has been estimated based on the main substance (Grave et al., 1999; Redding et al., 2019) or alternatively, each compound was summarized to determine the total weight of the combination (Saini et al., 2012a; Stevens et al., 2016). Antimicrobials reported using International Units (e.g., Penicillin G), are usually converted to milligrams as 1,000 IU of penicillin G procaine = 0.6 mg and 1,000 IU of polymyxin B = 0.1 mg (Saini et al., 2012; Prescott and Dowling, 2006), or 1,000 IU of benzyl penicillin G procaine = 0.99 mg (Stevens et al., 2016), or 1,666.67 IU per mg of penicillin G (Schrag et al., 2020a). In previously published studies, body weight has varied from 425 kg (Hyde et al., 2017) to 600 kg (Saini et al., 2012a; Stevens et al., 2016; Redding et al., 2019) or 680 kg (Pol and Ruegg, 2007), and it is important to clearly define the body weight used for the calculation to not underestimate or overestimate overall AMU (Mills et al., 2018).

A mass-based estimate is relatively easier to calculate than ADD and estimates total mass (mg) of a compound per kg of animal body weight on the farm. Differently from ADD, label indications are not used when calculating AMU and it is calculated as follows:

$$\frac{Amount\ of\ drug\ used\ (mg)}{Animal\ body\ weight\ \times animals\ at\ risk} \times 1{,}000\ animals\ at\ risk$$

$$= Total\ mg\ of\ a\ compound\ per\ kg\ of\ body\ weight\ per 1{,}000\ aniamls\ per\ day$$

Differently from ADD, mass metric is highly influenced by the potency of an active ingredient (Jensen et al., 2004), therefore, antimicrobials with lower concentration of active ingredient (e.g., systemic ceftiofur) will have less impact on total AMU than antimicrobials with greater concentration (e.g., oral sulfadimethoxine). The use of a mass-based metric instead of a dose-based metric also is known to alter estimates of AMU based on route of administration (Hyde et al., 2017). This occurs because while IMM is used more frequently than other routes, the concentration of IMM products is lower than the concentration of oral or injectable treatments. In Hyde et al., (2017), AMU was highly influenced by parenteral treatment when using mass metric, but when using ADD, IMM products accounted for most of the antimicrobial doses. The relationship between different metrics and resistance development are unknown and should be explored in future studies. Recently, a new metric called standardized regimens has been described by Schrag et al., 2020a. A standardized regimen is defined at the administration of a single therapeutic treatment per animal per disease. Differently from ADD, this metric does not consider dose recommendations per day and each application per day is measured separately. Another characteristic of a standardized regimens is that treatments performed consecutively for a period of 5 days are classified as a single regimen.

ANTIMICROBIAL USAGE ON DAIRY FARMS

Previously AMU has been quantified in several states of the U.S., including Wisconsin (Pol and Ruegg, 2007), Washington (Afema et al., 2019), Pennsylvania (Redding et al., 2019), and one study that included farms in several states (Schrag et al., 2020b). Total AMU was 14.9 ADD/ 1,000 cow days (5.43 ADD/ cow per year × 2.74) for Pol and Ruegg, (2007), 4.2 ADD/ 1,000 animal days for Redding et al., (2019), and 3.9 REG/1,000 cow days (1.42 REG/ cow per year × 2.74) for (Schrag et al., (2020b). In Pol and Ruegg, (2007) and Redding et al., (2019),

AMU was measured in small to midsized farms and used on-farm audit and online survey respectively. In other studies, AMU was estimated for selected diseases (DCT, clinical mastitis, foot diseases, metritis, and respiratory diseases) and youngstock AMU was included in Redding et al., (2019). In Schrag et al., (2020b) study, AMU data was collected only for adult animals during a farm visit and included midsized to large farms. Several factors make it difficult to compare total AMU among these studies, including differences in metrics, inclusion of youngstock, diseases included, and how AMU for DCT was calculated. However, difference in years among studies, diseases rates, management practices could help explain the difference in AMU observed.

Herd-level comparisons of ADD among studies are difficult because of differences in approved product labels among countries and variation in denominators. However, AMU was quantified in Canada (Saini et al., 2012), Belgium (Stevens et al., 2016), Austria (Firth et al., 2017), the United Kingdom (Hyde et al., 2017), and Argentina (Pereyra et al., 2015). All previous studies have enrolled small or midsized farms. In these studies, except for Pereyra et al., (2015), only adult cows were included and, in some cases, only AMU related to mastitis was measured (Firth et al., 2017). For those studies that considered similar diseases, AMU was 14.4 ADD/1,000 cow-days at Saini et al., (2012), 20.8 ADD/ 1,000 cow-days at Stevens et al., (2016), 3.6 ADD (standardization was not specified) on Hyde et al., (2017), and 14.2 ADD/ 1,000 cow-days at (Pereyra et al., (2015). As previously discussed, difference in AMU could be explained by difference in antimicrobials used, management, and production practices.

Among classes of antimicrobials, aminoglycosides, fluoroquinolones, sulfonamides, tetracyclines, and β-lactams have been reported for usage in dairy herds. β-lactams were the primary class of antimicrobial used for treatment of mastitis, reproductive problems, lameness,

respiratory problems, and diarrhea or digestive problems (USDA–APHIS–VS–CEAH–NAHMS, 2018). Of the beta-lactams, ceftiofur is consistently reported as the most frequent antimicrobial used for treatment of bacterial diseases occurring on dairy farms, with 85% of smaller farms using ceftiofur to treat respiratory problems and metritis and 65% of smaller farms using ceftiofur to treat foot infections (Pol and Ruegg, 2007).

Antimicrobial usage in preweaned calves

As compared to adult cows, measuring AMU in PWC can be more complicated as body weight can drastically change among different ages of replacement animals (Pereyra et al., 2015) and due to transfer of replacement animals to remote facilities (rather than remaining on site). In addition, fewer treatment records are maintained for this group of animals (Zwald et al., 2004; Pereyra et al., 2015). According to USDA survey data, digestive and respiratory diseases are the primary diseases in PWC and affect approximately 21% and 12% of the PWC respectively (USDA–APHIS–VS–CEAH–NAHMS, 2018). Of the animals diagnosed with these diseases, 76% of the cases of digestive problems and 95% of respiratory problems are treated with antimicrobials. Among antimicrobials, tetracyclines, cephalosporins, trimethoprim sulfa were the primary antimicrobials used for treatment of digestive problems, and macrolides and florfenicol the primary antimicrobials used for treatment of respiratory problems. At herd-level, AMU has been previously measured for PWC in only a few studies (Pereyra et al., 2015; Redding et al., 2019). Argentinian researchers reported an overall AMU of 0.49 ADD/ PWC per year and included antimicrobials used for treatment of enteritis, respiratory diseases, and navel infections (Pereyra et al., 2015). In Redding et al., (2019), cephalosporins, tetracyclines, phenicols, penicillins, macrolides, sulfonamides, and fluoroquinolones antimicrobials were reported for treatment of diarrhea and respiratory disease in preweaned calves (PWC).

Greater variety of antimicrobials have been used in PWC and understanding how AMU is used for this class of animals is crucial to understand the impact of antimicrobials used in PWC on development of resistance.

Antimicrobial usage for treatment of mastitis

At the national level, clinical mastitis has been reported in all dairy herds (99.7%) and affects nearly 25% of cows (USDA–APHIS–VS–CEAH–NAHMS, 2014). Use of antimicrobials for treatment of mastitis has been reported by 97% of farmers and 86% of affected cows are treated with antimicrobials. Considering that there are approximately 9.4 million milk cows currently in the U.S. (USDA-NASS, 2022), roughly 2.35 million cows are affected by mastitis and about 2 million receive antimicrobial treatment., When using a dose-based metric, IMM administration has been consistently reported as one of the main routes for AMU and accounts for 35 to 71% of total ADD due to year-round presence of mastitis and frequency of treatment (Pol and Ruegg, 2007; Saini et al., 2012a; Pereyra et al., 2015; Stevens et al., 2016; Schrag et al., 2020a). The proportion of IMM AMU for treatment of lactating and dry cows has varied among studies and it is likely caused by difference in how AMU for DCT is calculated (4 IMM tubes as 1 ADD or 1 IMM tube as 1 ADD) and based on the incidence of disease. For example, in Pol and Ruegg, (2007), of all IMM antimicrobials given for treatment or prevention of mastitis, IMM antimicrobials given to lactating cows represented 56% of all IMM antimicrobials while antimicrobials given to DCT represented 44%. However, in Stevens et al., 2016, treatment of lactating cows represented 48% of all IMM antimicrobials, while DCT represented 52%. Among approved antimicrobials for treatment of mastitis, use of IMM ceftiofur is very common and is used to treat about half of all cases of clinical mastitis in the U.S. (USDA–APHIS–VS–CEAH, 2008; USDA–APHIS–VS–CEAH–NAHMS, 2014)

COSTS ASSOCIATED WITH MASTITIS

Mastitis is caused by a variety of bacteria. Treatment recommendations vary based on etiology and magnitude of the inflammatory response. Treatment success is dependent on the etiology, antimicrobial spectrum of activity, and cow related factors such as parity and stage of lactation (Ruegg, 2021). Short and long-term effects of clinical mastitis are often associated with the monetary cost and losses. Therefore, treatment decisions should be made to optimize economic losses (Ruegg, 2020).

Studies evaluating cost of clinical mastitis have used different models and assumptions to calculate costs and these assumptions are usually obtained from literature (Huijps et al., 2008; Pinzón-Sánchez et al., 2011; Rollin et al., 2015). Variables and definitions used to calculate the economic impact of mastitis varies among studies. But milk production loss, drug cost, milk discard, and culling are usually included in the models.

Direct costs of treatment (e.g., milk withhold and cost of antimicrobials) are easy to calculate, however other potential losses (e.g., reproductive losses) are often difficult to estimate and depend on etiology, parity, and days in milk. Milk production costs due to milk withhold and loss in production subsequent to infection are often reported as the primary contributors of economic costs (Seegers et al., 2003; Pinzón-Sánchez et al., 2011). Pinzón-Sánchez et al., (2011), used a decision tree model to estimate expected monetary losses of clinical mastitis based on use of on-farm culture and duration of treatment. Losses were estimated for treatment of first cases of mastitis occurring in a single quarter of a cows within the first 30 DIM. Results from this model demonstrated that treatment costs can widely vary, but overall, costs were linked to extended treatment and milk discard. Milk production costs associated with discard of milk are easy to understand and depends on the total number of days that milk of out of the tank. Loss

of production across lactation is more difficult to estimate and depends on pathogens and inflammatory responses. For cows with an SCC of 500,000 cells/mL, daily milk production has been estimated to reduce approximately 0.7 – 2 kg of milk in primiparous cows and 1.1 to 3.7 kg in multiparous cows (Hagnestam-Nielsen et al., 2009). Milk production loss is the reduction in milk yield and has been reported a reduction range of 5 to 25% (Janzen, 1970).

Long-term effects on productivity are also linked with monetary losses due to mastitis. Presence of subclinical or clinical mastitis during breeding reduced conception rate up to 33% (Fuenzalida et al., 2015). Considering that more than 30% of the first cases of clinical mastitis occurs within the first 60 DIM (Huijps et al., 2008), not only these cases will include costs with treatment but also determine the longevity of this cow on a herd. More recently, Swartz et al., (2021) reported that not only mastitis affects future productivity of a cow but may also negatively affect productivity of future daughters, making this evaluation even more complex.

The perception of mastitis by the farm owners and people that define treatment protocol also influences the costs of mastitis on a farm. About 70% of dairy farmers have been reported to underestimate the cost of mastitis (Huijps et al., 2008). Awareness of the negative monetary losses caused by mastitis are necessary to completely understand the impact of clinical mastitis on profitability and implement strategies to reduce costs with this disease. Ritter et al., (2017) assessed farmers perceptions of management strategies for control of infectious diseases and observed that dairy farmers might overlook existing problems when there is lack of clinical signs. Reports estimating the negative economic impact of disease are available, but it is not certain if farmers are aware of these publications (Ritter et al., 2017). In addition, farmers tended to adopt measures to control mastitis when they had positive perception of effectiveness and these measures were strongly associated with mastitis incidence. Easy access to reports

estimating costs and showing effectiveness of strategies to reduce cost might help in implementation of management practices to reduce incidence of clinical mastitis and strategies to optimize treatment protocols.

SELECTIVE DRY COW THERAPY

For decades, *Streptococcus agalactiae* was the most important pathogen causing mastitis in dairy cows and combined with adoption of management practices that limited transmission, use of antimicrobials was fundamental to successfully control this pathogen (Ruegg, 2017). In addition, the use of IMM antimicrobials at all quarters during dry-off have helped reduce incidence of mastitis during dry period and subsequent lactation by treating subclinical mastitis at dry-off. As selective dry cow and lactating cow therapy programs for clinical mastitis are increasingly adopted (Ruegg, 2018), it is likely that AMU attribute to IMM therapy on dairy farms will decline. Judicious use of antimicrobial is made by not treating animals that are not likely to benefit from antimicrobial treatment. Several studies have evaluated decisions-based treatment using antimicrobial at dry-off with the overall objective of reducing AMU. In general, selective DCT is based on using tests to identify quarters that are likely infected and using antimicrobials only to treat those quarters.

Blanket DCT is extensively adopted by U.S. dairy farmers (USDA–APHIS–VS–CEAH–NAHMS, 2014) and was originally implemented as part of a mastitis control program due to the lack of an economically screening test to identify IMI at dry-off and help control the high prevalence of subclinical IMI in cows (Eberhart and Buckalew, 1972). With better udder health practices, control of important contagious Gram-positive bacteria, and access to screening tests to identify IMI at dry-off, the need for blanket DCT has decreased. Selective DCT is another option available for treatment of subclinical mastitis at the end of lactation and in these programs

antimicrobial treatment is based on assessment of the IMI status of the cow or quarter (Godden et al., 2016).

Studies have evaluated the effect of different antimicrobials used at dry-off (Arruda et al., 2013) or different screenings test (e.g., SCC at dry-off, microbiological results, or CMT test) to implement selective DCT (Scherpenzeel et al., 2014; Rowe et al., 2020; McDougall et al., 2022). When selective DCT was implemented without knowledge of IMI at mammary gland, an increase of IMI was found in the subsequent lactation. For researchers who used the history of subclinical or clinical mastitis as well as the presence of mastitis at dry-off to make decision on selective DCT treatment, had successful outcomes no increase in IMI in the subsequent lactation (Rowe et al., 2020; McDougall et al., 2022). In addition, use of selective DCT has resulted in a 55% reduction in AMU for herds that qualify for and adopt this strategy (Rowe et al., 2020).

STRATEGIES TO IMPROVE MAMMARY GLAND IMMUNITY

Several decades of research indicate that cows become immunocompromised during the peripartum period (Drackley, 1999; Sordillo, 2018). Approximately 80% of the cows lose body condition during the first 30 d in milk (Middleton et al., 2019) and decreased dry matter intake is associated with lipid mobilization release in the form of NEFA from the adipose tissue (Grummer et al., 2004). Increase in NEFA levels are associated with impartment of immune system and susceptibility to postpartum diseases (Hammon et al., 2006). Inclusion of strategies that enhance immunity of dairy cows during period of increased disease susceptibility could help reduce AMU on farms.

Immunomodulation is used to alter host immunity with the purpose of boosting immune defenses against pathogens without risking toxicity or tissue damage (Sordillo and Streicher,

2002). Effectiveness of immunomodulators for maintaining mammary gland health is difficult due to variety of bacteria and different modes of pathogenesis (Sordillo and Streicher, 2002).

Cytokines are important in the modulation of leukocyte and endothelial cell population and are responsible for growth and differentiation of effector and memory T lymphocytes as well as the activation of B lymphocytes (Sordillo, 2005). Treatment of the mammary gland with recombinant granulocyte colony stimulating factor or granulocyte-macrophage colony stimulating factor resulted in an increase in neutrophil phagocytosis and bactericidal activity which could increase the resistance of the mammary gland to invading pathogens.

The use of cytokines that stimulate the innate immune system of periparturient cows have been previously investigated (Canning et al., 2017; Zinicola et al., 2018). The use of a pegylated recombinant bovine granulocyte colony-stimulating factor (pegbovigrastim) has been labeled for usage in peripartum dairy cows around calving time. The primary objective of this product was to stimulate the production and differentiation of neutrophils by progenitor cells in bone marrow (Canning et al., 2017) to reduce the incidence of clinical mastitis post-calving. Increases in neutrophils count have been consistently reported among studies evaluating the effect of pegbovigrastim during peripartum period (Canning et al., 2017; Zinicola et al., 2018; van Schyndel et al., 2021). However, results obtained from these studies indicate an inconsistent impact of pegbovigrastim on the rate of clinical mastitis during the first 30 d after calving and no treatment effect on SCC in the subsequent lactation (Canning et al., 2017; Zinicola et al., 2018).

SUMMARY

Mastitis is a disease that affects cow welfare, increases AMU, and consequently reduces profitability on dairy farms. Understanding key factors that influence total AMU on farm is important to help implement management practices to reduce risk of mastitis and AMU on farm.

In dairy herds, reduction of AMU could be achieved by targeting current management practices adopted for treatment or prevention of mastitis. Reports that easily demonstrate the financial benefit from implementing these practices to dairy farmers could motivate the implementation of these management practices, helping reduce AMU on farm.

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CHAPTER 2. QUANTIFICATION OF ANTIMICROBIAL USAGE IN ADULT COWS AND PRE-WEANED CALVES ON 40 LARGE WISCONSIN DAIRY FARMS USING DOSE-BASED AND MASS-BASED METRICS

ABSTRACT

Use of antimicrobials in animal agriculture is under increasing scrutiny, but the quantity of antimicrobials used on large U.S. dairy farms has not been evaluated using data from large farms and different metrics. This study investigated total antimicrobial usage **(AMU)** in adult dairy cows and pre-weaned calves **(PWC)** and contrasted two metrics used for measurement of AMU. Wisconsin dairy farms were eligible if they had >250 lactating cows, maintained computerized animal health records, and were willing to allow researchers access to treatment records. Animal health data for a one-year period was retrospectively collected from computerized records, and a farm visit was performed to verify case definitions and recording accuracy. Both dose-based (animal daily doses; **ADD)** and mass-based (total mg of antimicrobials per kg of body weight; **BW**) metrics were calculated at the herd, cow, and PWC levels. Descriptive statistics for AMU were examined for both age groups. Mean AMU was compared among active ingredients and route of usage using ANOVA models that included farm as a random variable. At enrollment, farms (n = 40) contained approximately 52,639 cows ($\bar{x}$ 1,316 ± 169; 95% CI 975, 1657) and 6,281 PWC ($\bar{x}$ 180 ± 33; 95% CI 112, 247). When estimated using ADD, total herd AMU was 17.2 ADD per 1,000 animal-d (95% CI 14.9, 19.5) with 83% of total herd-level AMU in adult cows. When estimated using mass-based metric, total herd AMU was 13.6 mg of antimicrobial per kg of animal BW (95% CI 10.3, 17.0), with 86% of total AMU used in adult cows. For cows, 78% of total ADD (15.8 ADD per 1,000 cow-d) was administered as intramammary **(IMM)** preparations. In contrast, when AMU was estimated using a mass-based metric, IMM preparations represented only 24% of total AMU (12.1 mg per kg of cow BW). For cows, ceftiofur was the primary antimicrobial used and accounted for 53% of total ADD, with 80% attributed to IMM and 20% attributed to injectable treatments. When

estimated using a mass-based metric, ampicillin was the predominant antimicrobial used in cows and accounted for 33% of total antimicrobial mass per kg body weight. When AMU was estimated for PWC using ADD, injectable antimicrobials represented 79% of total usage (28.3 ADD per 1,000 PWC-d). In contrast, when AMU was estimated for PWC using a mass-based metric, injectable products represented 42% of total AMU, even though more farms administered antimicrobials using this route. When AMU in PWC was summarized using ADD, penicillin represented 32% of AMU, and there were no significant differences in ADD among ampicillin, oxytetracycline or enrofloxacin. When a mass-based metric was used to estimate AMU in PWC, oral products (sulfadimethoxine and trimethoprim-sulfa) represented more than half of the total AMU given to this group. Overall, these results show that choice of metric and inclusion of different age groups can substantially influence interpretation of AMU on dairy farms.

INTRODUCTION

The discovery of antimicrobials revolutionized medicine by providing an effective method of treatment for many bacterial diseases in both humans and animals (Aminov, 2010; Davies and Davies, 2010). Antimicrobials are used in animal agriculture to treat bacterial diseases that reduce animal welfare and production efficiency and many benefits of antimicrobial usage **(AMU)** have been recognized. Reduced morbidity and mortality from infectious bacterial diseases have resulted in more efficient production of animal-based protein (Johnston, 1998; Saini et al., 2012a; Hao et al., 2014). Improvement in animal welfare by reducing discomfort and pain in infected animals is another important benefit.

In the U.S., antimicrobial classes including aminoglycosides, lincosamides, macrolides, β-lactams, sulfonamides, and tetracyclines are used to treat dairy cows, and cephalosporins are the primary antimicrobial administered to adult dairy cows (USDA, 2008; USDA–APHIS–VS–

CEAH, 2008; USDA–APHIS–VS–CEAH–NAHMS, 2014). For calves, a greater variety of AMU has been reported, including tetracyclines, cephalosporins, sulfonamides, macrolides, amphenicols, and penicillins (USDA–APHIS–VS–CEAH–NAHMS, 2018). Importantly, many classes of antimicrobials are used in both animals and humans for treatment of bacterial diseases, and efforts to maintain responsible use of these antimicrobials are crucial (CDC, 2017).

Gathering quantitative data about the scope and scale of AMU on farms is an important step in understanding associations between AMU and development of antimicrobial resistance **(AMR)** (Grave et al., 1999; Pol and Ruegg, 2007; Saini et al., 2012a; CDC, 2014; MacFadden et al., 2016). Increased quantitative information about AMU on individual farms would help veterinarians and government agencies better understand relationships between AMU and development and transmission of AMR. Attempts to quantify AMU began in the mid-1960s in Europe with the goal of comparing AMU among countries and regions (Wade., 1984; (WHO, 2003). Quantification of AMU is typically based on standardized metrics such as animal daily dose **(ADD)** and/or total mg of antimicrobial per kg of animal body weight (**BW**) (Hyde et al., 2017; Mills et al., 2018).

Several studies have quantified AMU on small or midsized dairy farms in the United States (Pol and Ruegg, 2007; Redding et al., 2019), Europe (Stevens et al., 2016), Argentina (Pereyra et al., 2015), and Canada (Saini et al., 2012a). These studies quantified AMU using ADD with adult cows as the denominator and estimates of usage ranged from approximately 14 to 20 ADD/1,000 cow-d. Fewer researchers have reported AMU in pre-weaned dairy calves **(PWC)** (Pereyra et al., 2015; Redding et al., 2019). Descriptions of AMU are useful for understanding variation in AMU among antimicrobial classes, animal categories, and diseases. However, data are lacking for AMU on larger dairy farms (>250 cows) that produce the majority

of milk in the U.S. (MacDonald and Newton, 2014) and we are not aware of studies that have compared dose-based and mass-based metrics measuring AMU on U.S. dairy farms. The objective of this study was to quantify AMU for cows and PWC on large dairy farms in Wisconsin contrasting dose-based (Animal Defined Dose) and mass-based (mg of antimicrobial per kg of body weight) metrics. We hypothesized that AMU would vary among farms and that use of different metrics would change the interpretation of AMU.

MATERIAL AND METHODS

Recruitment, Eligibility, and Selection of Herds

Conventional Wisconsin dairy herds were eligible for this retrospective, observational study if the farm had ≥250 lactating dairy cows when they were initially contacted, used antimicrobials to treat or prevent at least one event in the previous year, maintained computerized records of antimicrobial treatments, and would allow researchers access to their dairy management records.

A sampling frame of conventional dairy farms that met herd-size criteria was compiled from a list of dairy herds enrolled in previous studies (Rowbotham and Ruegg, 2015); the list had originally been compiled from dairy farm permit data obtained from Wisconsin Department of Trade and Consumer Protection. The list was cross checked and supplemented from a publicly available list of Wisconsin herds classified as concentrated animal feeding operations (https://dnr.wi.gov/topic/AgBusiness/CAFO/StatsMap.html), resulting in a total of 413 potentially eligible farms. Following Institutional Review Board **(**University of Wisconsin-Madison, 2017-1333-CR002) approval, a postcard and a recruitment letter were mailed in June 2017. Farmers who returned a postcard indicating that were willing to participate in the study were contacted by phone and were questioned about farm size, antimicrobial usage, and

availability of records. Based on logistical and budgetary considerations as well as needs of a companion study, we sought to enroll 40 eligible farms and conduct and conclude farm visits during September to December 2017.

Data Collection and Questionnaire

The majority of treatment records were extracted from dairy management software, but a small amount of data was retrieved from customized spreadsheets or was based on farmer recall during the onsite survey. Among dairy management software used by enrolled herds, 37 herds used Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), 2 herds used DairyQuest (ProfitSource, Merril, WI), and 1 farm used Afimilk (Afimilk, Fitchburg, WI).

Most of the farms that used Dairy Comp 305 sent their computerized animal health records to researchers prior to the farm visit. These records were reviewed to familiarize researchers with protocols and recording systems. Computerized animal health records for farms that did not use Dairy Comp 305 were obtained during farm visits. All animal health data (including information about diseases that were not treated) for adult (lactating and dry cows) and PWC (up to 60 days of age) were reviewed to evaluate disease definitions, detection and recording intensity and to understand codes used for recording disease events. When using Dairy Comp 305 to obtain animal health records, a gap of 0 (all events regardless of time between episodes) was defined under ALTER\9 command to ensure that the total number of cases treated were acquired and the EVENTS\5O FOR LACT >0 command was used to obtain adult cow files (Wenz and Giebel, 2012) and EVENTS\5O FOR LACT =0 command was used to obtain PWC files. Farms were visit only once, and during a farm visit, animal health records were reviewed, and a survey was used to collect additional information from owners or farm workers who were responsible for animal care. Treatment data for post-weaning heifers were not collected because

many farmers sent these animals to other locations and disease recording systems were not considered reliable for these age groups. While few farms retained bull calves, antimicrobial treatment of PWC could have included bulls as AMU was quantified for all animals under 60 days of life. Farmers received a $100 incentive for their participation. All questions referred to the 1-year period preceding the day of the farm visit.

Additional data were obtained using a survey instrument that contained 137 questions (available in supplemental materials). The survey was adapted from a previous study (Pol and Ruegg, 2007) and was administered by a single individual (JLC) during the farm visit. Farm owners or herd-health managers were questioned about farm structure and demographics (17 questions), inventory (5 questions), replacement management (5 questions), antimicrobial treatment records for lactating cows (4 questions) and calves (2 questions), disease treatment or preventive practices in adult cows (80 questions) and calves (14 questions), veterinary feed directive (5 questions), veterinary involvement (3 questions), and drug purchase (2 question). To aid in the identification of antimicrobials and to confirm label information, laminated pages containing full color pictures of commercially available veterinary antimicrobial drugs for oral, systemic, and IMM use were shown to interviewees.

Estimation of Antimicrobial Usage

Antimicrobial usage was quantified using a standard unit referred to as animal daily doses (**ADD**) (Jensen et al., 2004), following a methodology described in previous studies (Pol and Ruegg, 2007; Saini et al., 2012a; Mills et al., 2018). For each active ingredient, a standard ADD was calculated (Table 1) and defined as the maximum antimicrobial dose per day that an animal would receive using the Food and Drug Administration (FDA) approved label dosages. Holsteins were the predominant breed, but some farms contained some Brown Swiss, Jerseys, or

crossbred animals (Table 2). As the average estimated BW of adult cows was 678 kg ± 9.9 among farms, to estimate the standard ADD, body weight (BW) of 680 kg were used for adult cows (Pol and Ruegg, 2007) and a BW of 64 kg for PWC (Jones and Heinrichs, 2016). Approved dosages for licensed animal drugs were obtained from the U. S. National Library of Medicine-DailyMed (https://dailymed.nlm.nih.gov/dailymed/index.cfm), while dosages for antimicrobials not approved were estimated using dosages from reputable veterinary manuals (Aiello, S. E.,Moses, M. A., & Allen, D. G., 2016), or for one antimicrobial (trimethoprim-sulfa) from personal communication with a faculty member at the University of Wisconsin-Madison School of Veterinary Medicine.

Animal Defined Doses for intramammary (IMM) AMU were calculated using the following formula:

$$ADD_{IMMA} = \frac{(\#\ of\ quarters\ treated)x\ \left(\#\ of\ \frac{tubes}{application}\right)x\ \left(\frac{applications}{day}\right)x\ (\#\ days)}{ADD_{IMMstandard}}$$

where ADD_{IMMA} is the final ADD for intramammary antimicrobial "A"; # of quarters treated is the number of quarters treated with antimicrobial "A"; # of tubes per application is the number of tubes used per treatment; applications per day is the frequency that antimicrobial "A" is administered per day; # days is the total number of days that antimicrobial "A" was treated; and $ADD_{IMMstandard}$ is the standard ADD for antimicrobial "A".

Animal Defined Doses for systemic and oral antimicrobial treatments, were calculated using the following formula:

$$ADD_{SYS/ORALA}$$

$$= \frac{(Antimicrobial\ concentration)x\ (Maximal\ dose)x\ \left(\frac{applications}{day}\right)x\ (\#\ days)}{ADD_{SYS/ORALstandard}}$$

where $ADD_{SYS\backslash ORALA}$ is the final systemic or oral ADD for antimicrobial "A"; antimicrobial concentration is the mg or IU per mL of antimicrobial "A"; maximal dose is the maximal dosage of antimicrobial "A"; applications per day is the frequency that antimicrobial "A" is administered per day; # days is the total number of days that antimicrobial "A" was treated; and $ADD_{SYS/ORALstandard}$ is the standard ADD for antimicrobial "A".

Similar to (Saini et al., 2012) and (Grave et al., 1999), the ADD of Trimethoprim sulfamethoxazole combination was based on the principle active ingredient (trimethoprim) dosage. Similar to Sani et al., (2012), IU of the IMM combination compounds (Novobiocin sodium & penicillin G procaine; Dihydrostreptomycin sulfate; penicillin G procaine) were converted to milligrams using a conversion of 1,000 IU of penicillin G procaine equals 0.6 mg. When the labeled dose included a range, an average dose was calculated using the initial and subsequent doses values to calculate an average dose according to the maximum treatment days described on label. Each dry cow therapy (**DCT)** tube was defined as 1 ADD, resulting in a total of 4 ADD for most cows administered DCT (Scherpenzeel et al., 2014; Stevens et al., 2016). Due to challenges in adequately measuring AMU in foot baths, wound sprays, and intraocular sprays, these antimicrobials were not included in the analysis. While we collected information about usage of ionophores, we did not include them in analysis as they are not considered medically important.

Dose-based estimates (ADD) were estimated at herd-level, for cows and for PWC. Herd-level AMU per animal was defined as the sum of the ADD used on a farm divided by the total animals at risk during the 365-d period ((Adult cows + PWC) *365 days * 1,000). For cows AMU was defined as the sum of the ADD used in adult cows divided by the average adult cows at risk during the 365-d period (Average adult cows *365 d * 1,000). Dose-based AMU in PWC

was estimated as sum of the ADD used in PWC divided by the average PWC at risk during the 365-d period (PWC *365 d*1,000). Animals at risk were estimated based on number of adult cows or PWC as indicated in the dairy management software during the month of visit.

Mass-based estimates (total mg of antimicrobial per kg of animal weight) were calculated following methodology as described by (Mills et al., 2018). In brief, total mg of antimicrobials for IMM compounds were calculated using the following formula:

$$\left(\frac{Total\ mg}{kg}\right)_{IMMA} = \frac{(\#\ of\ quarters\ treated)x\ \left(\frac{applications}{day}\right)x\ (\#\ days)x\ (Antimicrobial\ concentration)}{\#\ of\ animals\ at\ risk\ x\ BW}$$

where Total mg/kg $_{IMMA}$ is the final mg for intramammary antimicrobial "A" per kg of BW; # of quarters treated is the number of quarters treated with antimicrobial "A"; applications per day is the frequency that antimicrobial "A" is administered per day; # days is the total number of days that antimicrobial "A" was treated; antimicrobial concentration is the mg per mL of antimicrobial "A"; and # of animals at risk x BW is the number of animals at risk times the standard body weight defined for each animal class.

Total mg of antimicrobials for systemic and oral compounds were calculated using the following formula:

$$Total\ mg_{SYS/ORALA} = \frac{(Antimicrobial\ concentration)x\ (Maximal\ dose)x\ \left(\frac{applications}{day}\right)x\ (\#\ days)}{\#\ of\ animals\ at\ risk\ x\ BW}$$

where Total mg/kg $_{SYS/ORALA}$ is the final mg for systemic or oral antimicrobial "A" per kg of BW; antimicrobial concentration is the mg per mL of antimicrobial "A"; "; maximal dose is the

maximal dosage of antimicrobial "A"; applications per day is the frequency that antimicrobial "A" is administered per day; # days is the total number of days that antimicrobial "A" was treated; and # of animals at risk x BW is the number of animals at risk times the standard body weight defined for each animal class.

Mass-based calculations were estimated at the herd level, for cows and for PWC. For cows, AMU was defined as the sum of the mg of antimicrobial used divided by the number of adult cows multiplied by a standard BW. Antimicrobial usage in PWC was defined as the sum of the mg of antimicrobial used divided by the number of PWC multiplied by a standard BW. A standard body weight of 680kg was used for lactating cows (Pol and Ruegg, 2007) and 64kg for PWC (Jones and Heinrichs, 2020). At the herd-level, AMU was defined as the sum of the total mg of antimicrobial used divided by animal body weight (cow weight + PWC weight).

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS version 9.4 (SAS Institute, 2018). Descriptive statistics were performed using PROC MEANS and used to characterize participating herds and summarize AMU by active ingredient, route, and animal class. ANOVA was performed using GLIMMIX with dependent variable of ADD or Total mg/kg of BW and independent variables were active ingredients or route of administration:

$$Y_i = \mu + \tau_i + e_i$$

Where Yi = the dependent variable, μ + τ_i is the effect of active ingredient or route, and e = the residual error. Farm was the experimental unit. Route of usage (oral, injectable, IMM), and active ingredient (Table 1), were defined as categorical variables, while values for ADD and mass-based estimates were defined as continuous variables. Normality of the data was evaluated using normal probability and box plots using PROC UNIVARIATE and normality of residuals

was evaluated based on plots of residuals versus predicted values. A natural log-transformation was used for ADD and mass estimates to normalize the distributions. Statistical analyses were performed only for antimicrobials used on ≥5 herds. The null hypothesis was that AMU did not vary by route or active ingredient and statistical difference was considered when $P < 0.05$.

RESULTS

Characteristics of Herds

Of farms that received invitation letters (n = 413), 109 (26%) responded, with 80 affirmative and 29 negative responses. After phone interviews of affirmative responders, 43 farmers remained interested in participating, whereas 16 farmers could not be contacted or were not eligible, and 21 declined participation. From the 43 eligible farms, a convenience sample of 40 herds were enrolled based on ease of scheduling and the need to identify qualifying herds for enrollment in a broader study that includes additional objectives related to antimicrobial resistance.

Enrolled farms were distributed across Wisconsin. Based on cow numbers found in dairy management records collected at enrollment they contained 52,639 ($\bar{x}$ = 1,316 ± 169) adult cows and 6,281 PWC ($\bar{x}$ = 180 ± 33) (Table 2). Cows were housed in freestalls containing fresh sand (n = 22), recycled sand (n = 9), manure solids (n= 4), wood products (n = 2), or mixed bedding materials (n = 3). The rolling herd average RHA was 13,295 ± 164.4 kg/cow/year and ranged from 10,829 to 15,059 kg/cow/year. Average bulk tank somatic cell count was 143,600 ± 7,600 cells/mL and ranged from 60,000 to 320,000 cells/ml. Among parity groups, the greatest proportion of adult animals was in first lactation (Table 2). Of enrolled farms, 35 raised PWC on the farm while 5 sent PWC to other specialized locations.

Treatment records obtained from computerized records

Enrolled farms included 96,431 treatment remarks, in which 76,239 remarks were related to treatments given to adult cows and the remaining 20,192 to treatments given to PWC. Of total treatment remarks for adult cows, 78% (59,213) were for IMM treatments, (36,161 remarks for DCT, 23,042 remarks for treatment of lactating cows, 10 remarks for intraocular treatment using IMM product), 22% (16,913) were for systemic treatment, and the remaining 0.2% (113) were for oral treatments. When events from dairy management software included a treatment protocol that did not specify the actual number of days for which the treatment was administered, treatment days were obtained from survey data for that farm. Records for PWC contained 20,192 remarks, of which 90% (18,100) were for systemic treatments and the remaining 10% (2,092) were for oral treatments.

Of the total remarks related to adult cows, about 99% (76,171) were extracted from computerized records, with approximately 94% (71,839) of adult cow health records obtained from electronic records and the remaining 6% (4,332) also obtained from electronic records but had to be manually entered because researchers did not have access to the software used by three farms. Of farms using IMM antimicrobials to treat lactating cows (n=39), almost every farm used a dairy management program as their only mechanism for recording IMM antimicrobial treatment of lactating dairy cows, with the exception of 2 farms that also kept paper files of treatments, such that only 36 IMM treatment events had to be manually entered. All IMM dry-off treatments and records of oral treatments were obtained from dairy management software, and less than 1% (32 treatments) of systemic treatments were obtained from paper records or based on recall. Approximately 87% (17,584) of PWC animal health records were acquired from computerized records, with 16,277 (93%) remarks obtained from electronic records and the

remaining 1,307 (7%) remarks also obtained from electronic records but had to be manually entered because researchers did not have access to the software used by three farms. Of the remaining 13% (2,608) of the animal health records, about 6% (159 treatments) were oral records and 94% (2,449) of the systemic treatments were obtained from paper records or based on recall and had to be entered manually.

Overall AMU in adult cows and PWC

In accordance with enrollment criteria, all farms reported AMU for treatment or prevention in adult cows, while 35 farms reported AMU in PWC. At herd-level and across animal classes, 24 active ingredients were reported. Ceftiofur was the only antimicrobial used on all farms, followed by ampicillin (n =36) and tulathromycin (n = 30). Three antimicrobials were used only on a single farm (Table 3).

For cows across all routes of administration, 18 different active ingredients were used for treatment of adult dairy cows (Table 4). Ceftiofur and cephapirin were most commonly used and all farms reported use of ceftiofur. Ceftiofur was administered as an IMM preparation at dry-off (23 farms), for treatment of mastitis during lactation (36 farms), or as an injectable product (40 farms). Of all antimicrobial treatments given to adult cows, 5 products were used by just one farm. Four products used in lactating cows (florfenicol, tylosin, lincomyin, and lincomycin-spectinomycin combination) were not approved for use in this class of animals but can be used under guidance of a veterinarian based on extra-label usage guidelines. Two products given to adult cows (tulathromycin and tilmicosin) are not permitted for use in that class of animals under any conditions. All farms reported administration of antimicrobials using both IMM and systemic routes, and 4 reported administration of oral antimicrobials (Table 5). Use of IMM antimicrobials at dry-off was reported by all farms, while use of IMM antimicrobials for

treatment of lactating cows was reported by 39 farms. Intramammary tubes were used for intraocular treatment of a few cases of infectious bovine keratoconjunctivitis (pink eye) in adult cows on one farm.

Fifteen different active ingredients were used for treatment of PWC (Table 6). All farms that had PWC on site reported usage of injectable antimicrobials for treatment of PWC, while 8 farms reported usage of antimicrobials administered orally (Table 5). Ceftiofur, enrofloxacin, florfenicol, penicillin G, and tulathromycin were reported to be used by at least half of the farms (Table 6). All antimicrobials given to PWC, were either approved for use in this class of animal or permitted for use under extra-label usage guidelines.

Use of ionophores was reported by 38 farms, with 11 reporting usage for both adult cows and PWC, 25 only for adult cows, and 2 farms reporting usage for growing heifers.

Quantification of AMU as estimated using a dose-based metric

By Farm and Animal Class. Mean herd-level ADD (denominator included adult cows and PWC) was 17.2 ± 1.1 ADD per 1,000 animal-d per farm and ranged from 6.1 to 42.6 ADD per 1,000 animal-d (Figure 1-A). For all 40 enrolled farms, combined herd-level ADD totaled 687.6 ADD per 1,000 animal-d. Proportionally, ceftiofur (46%), cephapirin (13%), penicillin G (9%), ampicillin (6%), and dihydrostreptomycin-penicillin (5%) totaled 79% of total herd-level ADD per 1,000 animal-d (Table 3). The remaining 21% of ADD was contributed by 19 antimicrobials. Antimicrobial usage varied among active ingredients ($P < 0.001$; Table 3). For active ingredients used on >5 farms, herd-level ADD (back transformed LSM) were greatest for ceftiofur, cephapirin, dihydrostreptomycin, and cloxacillin (Table 3) and there was a tendency for greater herd-level ADD for ceftiofur as compared to cephapirin (5.33 versus 1.22 ADD per 1,000 animal-d; $P = 0.07$). Among farms that contained both PWC and adult cows (n = 35), AMU in

adult cows represented 83% (95% CI 78 – 88) of the combined herd-level ADD and ranged from 31% - 99.9% of the total AMU.

Mean ADD for cows (denominator is adult cows) was 15.8 ± 0.9 ADD per 1,000 cow-d per farm and ranged from 6.1 to 29.8 ADD per 1,000 cow-d. The combined ADD for cows in the entire 40 herds was 633.4 ADD per 1,000 cow-d. Among antimicrobials, ceftiofur represented 53% of all ADD administered in adult cows. The second antimicrobial with the greatest proportion of ADD was cephapirin representing 16% of all ADD given to adult cows.

Mean ADD for PWC (denominator is PWC) was 28.3 ± 5.4 ADD per 1,000 PWC-d per farm and ranged from 0.3 to 135.4 ADD per 1,000 PWC-d. The ADD for PWC on all 35 farms that contained PWC totaled 990.9 ADD per 1,000 PWC-d per farm. For PWC, penicillin G was the antimicrobial that accounted for the greatest proportion of AMU, representing 32% of all ADD in PWC, while proportions of ceftiofur, enrofloxacin, sulfadimethoxine, trimethoprim-sulfa and tulathromycin varied from 9% to 11% of ADD administered to PWC (Table 6).

Adult Cows - By Route of Administration. Intramammary, systemic and oral routes were used to administer antimicrobials to adult cows (Table 5). When estimated using ADD, IMM administration was the primary route that antimicrobials were administered (Table 5). For adult cows, ADD of IMM products were almost 5 times greater than ADD of antimicrobials administered systemically (P <0.001; Table 5) and represented 78% of total ADD given to adult cows. Intramammary treatment included antimicrobials given at dry-off (63% of IMM ADD in adult cows) and for treatment of lactating cows (37% of IMM ADD in adult cows).

Mean ADD used for IMM treatment of lactating cows was 4.7 ± 0.6 ADD per1,000 cow-d per farm (Figure 2-A) and ranged from 0.2 to 14.6 ADD per 1,000 cow-d. Total ADD used for IMM treatment of lactating cows in herds that used IMM antimicrobials (n = 39) summed to

184.1 per 1,000 cow-d. Among IMM antimicrobials used in lactating cows, ceftiofur (75% of ADD) and cephapirin (7% of ADD) accounted for almost 82% of ADD.

All farms reported use of IMM antimicrobials for drying off cows. Mean ADD used for IMM treatment at dry-off was 7.7 ± 0.2 ADD per 1,000 cow-d per farm (Figure 2- A) and ranged from 3.5 to 11.3 ADD per 1,000 cow-d. Total ADD used for IMM treatment at dry-off totaled 309.6 ADD per 1,000 cow-d. Three β-lactam antimicrobials and 2 combination products were given at dry-off (Table 4) but no differences in LSM ADD were observed among active ingredients (P = 0.38). Cephalosporin antimicrobials represented about 70% of ADD used at dry-off (Table 4).

All farms reported use of injectable antimicrobials in adult cows (Table 5). Mean ADD administered systemically to adult cows were 3.4 ± 0.5 ADD per 1,000 cow-d per farm (Figure 2-A) and ranged from 0.1 to 14.1 ADD per 1,000 cow-d. Total ADD used for systemic treatment of adult cows on all farms totaled 137.4 ADD per 1,000 cow-d. Of 11 antimicrobials used systemically, 98% were accounted for by ceftiofur, ampicillin, penicillin G, and oxytetracycline (Table 4). Among active ingredients, ceftiofur accounted for most ADD (Table 4), but LSM ADD did not vary among ceftiofur, ampicillin, and penicillin G (P= 0.89; Table 4).

Only 4 herds reported oral administration of antimicrobials in adult cows (Table 5). For adult cows 0.6 ADD ± 0.3 per 1,000 cow-d per farm were administered orally (Figure 2-A). Total ADD given orally totaled 2.3 ADD per 1,000 cow-d (Table 5).

PWC - By Route of Administration. Both injectable and oral antimicrobials were used in calves but the number of ADD did not vary by route (Table 5; P= 0.75). For PWC, 22.4 ± 3.9 ADD per 1,000 PWC-d per farm were given using an injectable route (Figure 3-A) and ranged from 0.3 to 91.3 ADD per 1,000 PWC-d. The combined ADD given to PWC using injections on

all 35 farms that contained PWC summed to 783.7 ADD per 1,000 PWC-d and represented 79% of all ADD given to PWC. For active ingredients used in greater than 5 herds using an injectable route, the adjusted mean ADD varied on active ingredient (Table 7; $P = 0.003$). Gamithromycin accounted for the fewest ADD while penicillin G accounted for the greatest (Table 7). No difference in mean ADD was observed among ampicillin, enrofloxacin, oxytetracycline (Table 7; $P = 0.64$).

For PWC,25.9 ± 10.5 ADD per 1,000 PWC-d were given orally (Figure 3-A). The combined ADD of all antimicrobials given orally to PWC summed to 207.1 ADD per 1,000 PWC-d and represented the remaining 21% of all AMU used in PWC. Among active ingredients given orally, the proportion of ADD were 5%, 47% and 48%, for oxytetracycline, sulfadimethoxine and TMP-sulfa, respectively (Table 7).

Quantification of AMU as estimated using a mass-based metric

By Farm and Animal Class. Mean herd-level antimicrobial mass density was 13.6 ± 1.7 mg of antimicrobials per kg of animal BW (combined weight of adult cows and PWC) per farm and ranged from 2.5 to 46.7 mg per kg of animal BW (Figure 1-B). Herd-level mass density of antimicrobial summed for all herds was 545.2 mg per kg BW. Ampicillin (30%) and ceftiofur (26%) accounted for more than half of combined antimicrobial mass density followed by sulfadimethoxine (12%) (Table3). Mass density of antimicrobials varied among active ingredients ($P < 0.001$; Table 3). When estimated using mass-density, dihydrostreptomycin, ceftiofur, and ampicillin contributed the greatest mass, but did not differ from each other (Table 3; $P > 0.99$). Of farms that contained both PWC and adult cows, antimicrobials administered to adult cows represented 86% (95% CI 80 – 92) of the herd-level mass-density of AMU and ranged from 36% - 99.9%.

For adult cows, mean mass density of antimicrobials was 12.1 ± 1.6 mg per kg of cow BW per farm and ranged from 1.6 to 36.3 mg/kg of cow BW. Mass-density of antimicrobials used in cows in enrolled herds summed to 485.2 mg of antimicrobial per kg of adult cow BW. Among antimicrobials used in adult cows across routes, ampicillin and ceftiofur accounted for the greatest proportion of mass and represented 33% and 29% of antimicrobial mass given to adult cows, respectively.

For PWC, mean mass density of antimicrobials was 146 ± 40.6 mg per kg of PWC BW per farm and ranged from 1.0 to 1,075.1 mg/kg of PWC BW. Combined mass-density for PWC in enrolled herds was 5,113.2 mg of antimicrobials per kg of PWC BW. Among antimicrobials given to PWC, greater proportion of mass was observed for sulfadimethoxine (28%) and Trimethoprim-sulfa (29%) (Table 6).

By route of Administration in Adult Cows. When AMU was quantified using a mass-based metric, the greatest mass was contributed by antimicrobials given by an injectable route as compared to IMM (Table 5). The LSM mass density of antimicrobials given via an injectable route was 2 times greater than the mass density of IMM products (Table 5; P <0.001). Proportionally, for adult cows, antimicrobials given by injection totaled 71% of antimicrobial mass density, while IMM (24%) and oral (6%) accounted for the remaining 29%.

Mean mass used for IMM treatment of adult cows was 0.4 ± 0.05 mg/kg of cow BW per farm (Figure 2-B) and ranged from 0.01 to 1.37 mg/kg. Combined mass of antimicrobials used for IMM treatment of lactating cows for enrolled herds summed to 16.1 mg per kg of adult cow BW. The LSM mass density of ceftiofur, cephapirin, and amoxicillin did not differ (*P*= 0.99), but greater proportion of mass was accounted for by ceftiofur (58% of all lactating cows IMM mg/kg of cow BW) and cephapirin (34% of all lactating cows IMM mg/kg of cow BW) (Table 4).

The mean mass density used for IMM treatment at dry-off was 2.5 ± 0.3 mg/kg of cow BW per farm (Figure 2-B) and ranged from 0.9 to 7.2 mg/kg of cow BW. The combined mass density of antimicrobials used for IMM treatment at dry-off totaled 98.3 mg/kg of cow BW. No difference in LSM mass density was found among IMM antimicrobials given at dry-off (*P*= 0.37), but the proportion of mass was mainly contributed by ceftiofur (36% of all IMM DCT mass) and dihydrostreptomycin (36% of all IMM DCT mass) (Table 4).

Mean mass density for antimicrobials given systemically was 8.6 ± 1.4 mg of antimicrobials per kg of cow BW per farm (Figure 2-B) and ranged from 0.2 to 32.8 mg/kg of cow BW. Combined mass density of antimicrobials administered systemically totaled 343.5 mg/kg of cow BW. Differences in mass were observed among antimicrobials used systemically (Table 6; *P* <0.001), with ceftiofur representing the greatest mass. Among the 4 farms that used oral antimicrobials, a total of 27.5 mg/kg of cow BW was quantified ($\bar{x}$= 6.9 ± 3.3 mg/kg of cow BW).

By Route of Administration in PWC. When AMU was quantified for PWC using mass density, there was a large difference in LSM mass based on route of administration, but this difference was not statistically significant due to large variation in usage and a relatively small sample size (Table 5; *P*= 0.30).

Mean mass density of antimicrobials given by an injectable route was 61 ± 10.5 mg /kg of PWC BW (Figure 3-B) and ranged from 1.0 to 272.8 mg/kg of PWC BW. The combined mass density of injectable antimicrobials totaled 2,139.4 mg/kg PWC BW (Table 7). The mass of antimicrobials given to PWC varied among active ingredients (Table 7, *P*< 0.001). Florfenicol, penicillin G, and ampicillin had the greatest mass density and did not differ among each other (*P* > 0.99).

Mean mass density for antimicrobials given orally was 371.7 ± 126.4 mg/kg of PWC BW per farm (Figure 3-B) and ranged from 3.9 to 1,060.3 mg/kg of PWC BW. The combined mass density of oral antimicrobials used for these herds totaled 2,973.7 mg/kg of PWC BW (Table 7). Three antimicrobials (Oxytetracycline, sulfadimethoxine, and Trimethoprim-sulfa) were used for oral administration, with 95% of the usage attributed to sulfadimethoxine (46%) and Trimethoprim-sulfa (49%).

DISCUSSION

Antimicrobial usage on US dairy farms has previously been quantified on smaller farms in Wisconsin (Pol and Ruegg, 2007), Pennsylvania (Redding et al., 2019), and in countries such as Canada (Saini et al., 2012a), Belgium (Stevens et al., 2016), Austria (Firth et al., 2017), The United Kingdom (Hyde et al., 2017), and Argentina (Pereyra et al., 2015). All previous studies have enrolled small or midsized farms and none have focused on large farms. While we included only 40 herds, our farms contained >52,000 cows, thus providing a large number of animals who would be potentially susceptible to bacterial diseases that may benefit from antimicrobial therapy. Inclusion of large farms add valuable perspective about AMU on dairy farms that produce the majority of milk in the U.S. (MacDonald and Newton, 2014). In addition, our use of a dose-based metric allowed comparison to previous studies while quantification using a mass-based metric provides important new information that illustrates how choice of metric can influence interpretation of AMU.

Enrolled dairy farms represented about 10% of all Wisconsin dairy farms with >250 dairy cows and were representative of this demographic as they used typical management practices for larger herds in this region (USDA–APHIS–VS–CEAH–NAHMS, 2014; Rowbotham and Ruegg, 2015). Farms were recruited based on herd size and availability of treatments records and they

likely represent dairy herds that have better recording systems than the overall population of WI dairy herds (Hoe and Ruegg, 2006). This trend was previously observed by (USDA, 2007) who reported that adoption of computerized dairy management records increased from about 9% of small farms (<100 cows) to 38% of the medium farms (100 – 499 cows) and 83% of large farms (>500 cows). Although selection criteria included questions about availability of records, some data were missing for some herds, including name of drug, number of days treated, or dosage administered, as has been noted in previous publications (Wenz and Giebel, 2012). The most popular dairy management software provides only 8 characters to describe an event which limits opportunities for full recording of some treatments. In the U.S., dairy farm records are private and in contrast to some other countries (Espetvedt et al., 2013), there is no centralized registry for treatment records of agricultural animals. In our study, all farms recorded AMU data in computerized recording systems and farmers were interviewed to verify disease definitions and treatment protocols. The one-year retrospective data collection period allowed us to capture seasonal effects that may influence AMU (Mills et al., 2018). Review of computerized health records was useful as we were able to assess the number of treatments per disease as well as the number of days each treatment was administered. A minority of disease events were not entered in electronic records and these events and the proportion of non-recorded cases receiving antimicrobials were estimated by farm owners during the survey. The possibility of information bias cannot be excluded because in some instances, we interviewed farm owners who did not personally administer antimicrobials, and there may have been errors in administering treatment protocols. Even though >90% of data was obtained from electronic dairy management records, it is possible that recall or recording bias could have influenced our results.

Ceftiofur and cephapirin were the most common antimicrobials used on these farms and ceftiofur was the only antimicrobial used on all farms. Several products and routes are used to administer ceftiofur and this antimicrobial accounted for almost half of herd-level ADD, more than half of cow-level ADD, and 9% of ADD used in PWC. Ceftiofur has previously been reported as the most frequent antimicrobial used for treatment of diseases other than mastitis, with 85% of smaller farms using ceftiofur to treat respiratory problems and metritis and 65% of smaller farms using ceftiofur to treat foot infections (Pol and Ruegg, 2007). Use of ceftiofur as an IMM antimicrobial is approved in the U.S., but some non-approved IMM use of ceftiofur to treat mastitis was reported even before approval of the IMM ceftiofur product (FDA, 2005; Pol and Ruegg, 2007). In the U.S., several formulations of ceftiofur are approved for use in dairy cattle but extra-label usage of this compound is not allowed. In Canada, ceftiofur represented 15% of total ADD used at national-level and usage increased with increasing herd size (Saini et al., 2012a). Multiple formulations of ceftiofur are approved for treatment of dairy cattle in the U.S. and none of the systemically administered products require a milk withholding period when used according to label indications, dosage, and route. Administration of ceftiofur outside of approved label instructions for dose, frequency, duration or route is not permitted by FDA regulations. The approved IMM ceftiofur product is very popular and has been used to treat about half of all cases of clinical mastitis in the U.S. (USDA–APHIS–VS–CEAH, 2008; USDA–APHIS–VS–CEAH–NAHMS, 2014). The popularity of this product is probably based on several favorable characteristics, including broad-spectrum activity, a dosing schedule that includes once daily administration and a flexible dosing interval of 2 - 8 days

Of 7 IMM antimicrobials approved for administration to dry cows, five are β-lactams and the other 2 products include the only antimicrobial combination products licensed for use in U.S.

dairy cows. Only 2 classes of antimicrobial (6 β-lactam products and 1 lincosamide) are approved for IMM treatment of lactating cows in the US and all but one of those products were used by farms enrolled in our study. Similar to previous studies (Pol and Ruegg, 2007; Redding et al., 2019) first and third generation cephalosporins were used most frequently and accounted for the greatest proportion of ADD and of mass for IMM treatment of lactating cows. Use of ceftiofur is concerning because the World Health Organization has classified third generation cephalosporins as a critically important class for human health (WHO, 2018a) and responsible usage guidelines encourage use of narrow spectrum antimicrobials when appropriate.

Among the 18 active ingredients reported for injectable usage in adult cows, 2 antimicrobials are not approved for usage in adult cows and accounted for a small proportion of total AMU in adult cows. For PWC, the main classes used were macrolides (gamithromycin, tildipirosin, tilmicosin, tulathromycin, and tylosin), fluoroquilones (danofloxacin and enroxoflacin), penicillins (ampicillin and penicillin G), amphenicols (florfenicol), cephalosporins (ceftiofur), and tetracyclines (oxytetracycline). Our estimates are comparable with USDA survey data that indicated that macrolides and amphenicols were the primary antimicrobials used for treatment of respiratory diseases in PWC, and reinforced that tetracyclines, ceftiofur, and trimethoprim-sulfa are the primary antimicrobials used for treatment of digestive diseases (USDA–APHIS–VS–CEAH–NAHMS, 2018).

Several metrics have been used for measurement of AMU, and the choice of metric should be based on the purpose for measuring AMU (Mills et al., 2018). Our data demonstrated that interpretation of AMU can be altered depending on the metric that is used. None of the metrics are ideal for all situations, but measurement of AMU is essential to evaluate interventions used to reduce AMU and for research about potential associations of AMU with

measures of AMR. Regardless of metric, accuracy and consistency of the health records are crucial for analysis of AMU (Wenz and Giebel, 2012). When quantifying AMU, animal weight is an important component (Mills et al., 2018) as it may vary among farms and breeds, thus altering calculations. For example, in our study, four breeds were reported, but Holsteins represented 99% of total cows. During the farm visit, a question regarding animal weight was asked but owners/managers estimated weights were very similar among farms, thus we used a standard BW for calculating the mass-based metric for all farms.

We used ADD as our dose-based metric since this standardized method is widely adopted for quantification of AMU in both humans and animals (Jensen et al., 2004; CDC, 2014; WHO, 2018b). In some countries, ADD has been used to measure changes in AMU as the result of national legislative efforts, but comparisons among countries is complicated by differences in how the metric is calculated (Taverne et al., 2015). The formula used to calculate ADD can be altered to demonstrate larger or smaller reductions in AMU. For example, in dairy herds, some studies have considered 4 IMM dry cow antimicrobial tubes as 4 doses (Stevens et al., 2016) whereas others have considered the 4 doses to be a single ADD (Pol and Ruegg, 2007; Redding et al., 2019). When the goal is to reduce ADD on dairy farms, selective DCT programs are often pursued (Scherpenzeel et al., 2016) and use of a single (rather than 4), IMM tube to define ADD will have the effect of magnifying apparent reductions in AMU. The impact of using 4 versus 1 IMM tubes as an ADD is apparent when comparing results from (Pol and Ruegg, 2007)) where IMM treatment of clinical mastitis accounted for more AMU than DCT versus our current study where we observed that DCT accounted for the majority of IMM AMU. Most previous studies that measured AMU on dairy farms have used ADD, although denominators have varied (Pol and Ruegg, 2007; Saini et al., 2012a; Stevens et al., 2016; Redding et al., 2019). Use of ADD

allows comparisons among different antimicrobials, without regard for potency, concentration, units or route (WHO, 2018b). One disadvantage of ADD is that it does not account for multiple administrations per day and among countries ADD may vary depending on the approved dosing schedule. When using ADD to quantify antimicrobial usage, variation in BW among herds should be clearly specified, as well as the minimum, mean, or maximum dose rate chosen to be the defined daily dose since these choices significantly impact final assessments of AMU (Mills et al., 2018).

Although farms enrolled in our study were in the same state and shared many management characteristics, considerable variation in AMU was observed among herds. Use of different metrics influenced overall ranking of AMU, but higher consuming herds remained in the upper quartiles, regardless of metric. Compared to the herd that used the least ADD, the herd that was ranked highest used about 7 times more ADD per 1,000 animal-d. Both of the herds that recorded the greatest number of ADD used considerable antimicrobials to treat PWC. This study was not designed to investigate management practices risk factors associated with AMU and AMR, but wide variation in AMU among herds demonstrates that there is considerable opportunity for reductions in AMU based on adoption of management practices that are already used by herds consuming fewer doses.

Herd-level comparisons of ADD among studies are difficult because of differences in approved product labels among countries and variation in denominators. Most previous research has quantified AMU only for adult cows (Pol and Ruegg, 2007; Stevens et al., 2016; Hyde et al., 2017) but few have included calves (Pereyra et al., 2015; Redding et al., 2019). When ADD is calculated using only PWC in the denominator, the smaller number of calf-days results in about 1.5 times greater ADD per calf-day as compared to ADD density measured in cows. This is

expected as adult dairy cows are typically at risk for diseases that are treated with antimicrobials for a relatively short proportion of the typical 365d lactation cycle and the need to discard milk results in an economic disincentive for treatment of lactating cows. In contrast, PWC are vulnerable to infectious bacterial diseases for most of the period prior to weaning, and they are expected to remain in the herd for years, thus meat and withholding periods are not as great of a concern.

Some studies have quantified AMU using ADD per cow per year (Pol and Ruegg, 2007; Pereyra et al., 2015) and ADD density (ADD per 1,000 cow- d) can be converted to this value by dividing by 2.74 (1,000 cow-days /365 days). When our data are converted (15.8 ADD per 1000 cow-d/2.74 = 5.8 ADD per cow per year), AMU as measured by ADD is remarkedly similar to previous estimates for smaller herds in Wisconsin (5.4 ADD per cow per year; (Pol and Ruegg, 2007) and Canada (5.2 ADD per cow per year; (Saini et al., 2012a). As reported in previous studies (Pol and Ruegg, 2007; Pereyra et al., 2015; Hyde et al., 2017; Redding et al., 2019) when measured using ADD, IMM administration has a considerable impact on estimates of AMU. In our study, use of IMM antimicrobials accounted for 78% of ADD given to adult cows. Mastitis is the most frequent bacterial disease occurring on dairy farms, and until recently, antimicrobials were generally administered based on clinical signs, regardless of etiology (Ruegg, 2017) and blanket DCT has been routinely recommended as part of mastitis control program (NMC, 2020). As selective dry cow and lactating cow therapy programs for clinical mastitis are increasingly adopted (Ruegg, 2018), it is likely that AMU attributable to IMM therapy on dairy farms will decline.

As compared to ADD, the mass-based estimate was easier to calculate, but this metric does not account for variation in potency among active ingredients and thus favors

antimicrobials with lower dosing concentrations (e.g., ceftiofur). One previous study compared mass-based and dose-based metrics (Hyde et al., 2017; Redding et al., 2019), and similar to our study, they demonstrated that the predominant route and antimicrobials responsible for the greatest proportion of usage are altered by use of different metrics. Depending on metric, dramatic differences in usage are inferred based on route. When ADD is used, IMM administration accounts for about 78% of doses but when mass density is used, the proportions are reversed and systemic administration totals 71% of mass. The significance of selecting a metric relative to studying associations between AMU and development of AMR are unknown and should be explored in future studies.

As compared to estimating AMU in adult cows, quantification of AMU in replacement animals is more difficult. Issues for estimating AMU in replacements include larger variation in BW among youngstock ((Pereyra et al., 2015), transfer of replacements animals to remote facilities (rather than remaining on site) and maintenance of fewer treatment records for this group of animals (Zwald et al., 2004). It is apparent from our data, that some farms use considerable quantities of antimicrobials for treatment of PWC and use of oral antimicrobials accounted for 21% of ADD and 58% of mass. Products given via oral administration are often given in greater dosages thus increasing the total exposure to active ingredients. Reduced resistance of commensal *Escherichia coli* to some antimicrobials has been documented with reduced antimicrobial usage in calves (Afema et al., 2019) and increased education of farmers relative to AMU in this class of animals is needed.

CONCLUSION

Antimicrobial usage in large WI dairy farms was estimated using two different metrics. When estimated using ADD, AMU in adult cows was similar to previous reports that included

smaller farms and different countries. Regardless of metric, AMU varied substantially among farms, and future studies should identify risk factors associated with greater consumption of antimicrobials. Ceftiofur was used in multiple formulations and accounted for a large proportion of AMU. At herd level, adult cows represented the greatest proportion of AMU due to the greater number of animals in this category, but greater mass-density of AMU was observed for PWC. At the cow level, IMM administration was the primary route of AMU when ADD was used for estimation, but systemic administration accounted for most antimicrobial usage when mass-density was used for estimation. Among antimicrobials used on adult cows, cephalosporins were most frequently administered and represented a significant proportion of AMU, regardless of metric. Among antimicrobials administered to PWC, β-lactams and macrolides were the primary classes of antimicrobials. Overall, these results show that choice of metric and consideration of route of administration can substantially influence estimates of AMU on large dairy farms.

APPENDICES

Table 2.1. Animal daily doses (ADD) used for estimating antimicrobial usage in adult cows and pre-weaned calves on 40 Wisconsin dairy farms.[1]

Active ingredient	Product Type[2]	Route[3]	Concentration	Times per day	Dosage	ADD	
						Adult cows[4]	Pre-weaned calves[4]
Amoxicillin	RX	IMM	62.5 mg/tube	2	1 tube	2 tube (s)	–
Ceftiofur	RX	IMM	125 mg/tube	1	1 tube	1 tube (s)	–
Cephapirin	OTC	IMM	200 mg/tube	2	1 tube	2 tube (s)	–
Hetacillin	RX	IMM	62.5 mg/tube	1	1 tube	1 tube (s)	–
Pirlimycin	RX	IMM	50 mg/tube	1	1 tube	1 tube (s)	–
Cloxacillin	RX	IMM	200 mg/tube	2	1 tube	2 tube (s)	–
Ceftiofur	RX	IMM	500 mg/tube	1	1 tube	1 tube	–
Cephapirin	OTC	IMM	300 mg/tube	1	1 tube	1 tube	–
Cloxacillin	RX	IMM	500 mg/tube	1	1 tube	1 tube	–
Cloxacillin	RX	IMM	500 mg/tube	1	1 tube	1 tube	–
Novobiocin; penicillin G	OTC	IMM	400; 200,000 mg; IU/tube	1	1 tube	1 tube	–
Dihydrostreptomycin sulfate; pen G	RX	IMM	1,000; 1,000,000 mg; IU/tube	1	1 tube	1 tube	–
Ampicillin	RX	IM	250 mg/mL	1	11 mg/kg	7,480 mg	704 mg
Ceftiofur	RX	SQ	200 mg/mL	1	6.6 mg/kg	4,488 mg	422.4 mg
Ceftiofur	RX	IM, SQ	50 mg/mL	1	2.2 mg/kg	1,496 mg	140.8 mg
Danofloxacin	RX	SQ	180 mg/mL	1	6 mg/kg	–	384 mg
Enrofloxacin (single dose)	RX	SQ	100 mg/mL	1	12.5 mg/kg	–	800 mg
Enrofloxacin (multiple doses)	RX	SQ	100 mg/mL	1	5 mg/kg	–	320 mg
Florfenicol (single dose)	RX	IM	300 mg/mL	1	40 mg/kg	–	2,560 mg
Florfenicol (multiple doses)	RX	SQ	300 mg/mL	1	20 mg/kg	13,600 mg	1,280 mg
Florfenicol	RX	SQ	300 mg/mL		40 mg/kg	–	2,560 mg
Gamithromycin	RX	SQ	150 mg/mL	1	6 mg/kg	–	384 mg
Gentamicin	RX	IM	100 mg/mL	1	12 mg/kg	–	768 mg
Oxytetracycline (single dose)	OTC	SQ	200 mg/mL	1	19.8 mg/kg	13,464 mg	1,267 mg
Oxytetracycline (multiple doses)	OTC	SQ, IV	200 mg/mL	1	11 mg/kg	7,480 mg	704 mg

Table 2.1. (cont'd).

Oxytetracycline (single dose)	OTC	IM, SQ	200 mg/mL	1	19.8 mg/kg	13,464 mg	1,267 mg
Oxytetracycline (multiple doses)	OTC	IM, IV, SQ	200 mg/mL	1	11 mg/kg	7,480 mg	704 mg
Oxytetracycline	OTC	IV	100 mg/mL	1	11 mg/kg	7,480 mg	–
Penicillin G procaine	OTC	IM	300,000 IU/mL	1	6,614 IU/kg	4,497,520 mg	423,296 mg
Spectinomycin	RX	SQ	100 mg/mL	1	15 mg/kg	10,200 mg	–
Sulfadimethoxine	OTC	IV	400 mg/mL	1	33 mg/kg	22,440 mg	2,112 mg
Tildipirosin	RX	SQ	180 mg/mL	1	4 mg/kg	–	256 mg
Tilmicosin	RX	SQ	300 mg/mL	1	10 mg/kg	6,800 mg	640 mg
Tulathromycin	RX	SQ	100 mg/mL	1	2.5 mg/kg	1,700 mg	160 mg
Tylosin	OTC	IM	200 mg/mL	1	17.6 mg/kg	11,968 mg	1,126.4 mg
Lincomycin; spectinomycin	RX	IM	–	–	10 mg/kg	6,800 mg	–
Sulfadimethoxine	OTC	ORAL	5,000; 15,000 mg/bolus	1	33.1 mg/kg	22,508 mg	2,118.4 mg
Trimethoprim sulfamethoxazole	RX	ORAL	960 mg/bolus	1	20 mg/kg	–	1,280 mg
Oxytetracycline	OTC	ORAL	500 mg/bolus	2	22 mg/kg	–	1,408 mg
Sulfadimethoxine	OTC	ORAL	5,000; 15,000 mg/bolus	1	33.1 mg/kg	22,508 mg	2,118.4 mg

[1]Data were obtained using the current label listed in the U.S. National library of medicine, veterinary manuals (Gentamicin and Lincomycin- spectinomycin; Aiello, S. E., Moses, M. A., & Allen, D. G., 2016), or veterinary school recommendations Wisconsin sick calf protocols (Trimethoprim- sulfamethoxazole; McGuirk).

[2]RX= prescription animal drug, OTC= over the counter.

[3]IMM= intramammary infusion, IM= intramuscular, IV= intravenous, SQ= subcutaneous.

[4]A body weight of 680 kg used for lactating dairy cows and 64 kg for pre-weaned calves.

Table 2.2. Characteristics of Wisconsin dairy herds (n= 40) enrolled in a study about antimicrobial usage in Sept. to Dec. 2017.

Farm	RHA (kg/cow/yr)[1]	BTCC (cells per mL)[2]	Adult cows (n)[3]	Pre-weaned calves (n)[4]	Parity (%)			Adult cows body weight[5] kg	Holstein cows (%)[6]
					1st	2nd	≥3rd		
1	13,141	122,000	2,781	337	40	29	30	726	100
2	14,878	80,000	469	65	44	28	28	721	95
3	13,313	175,000	598	65	34	27	39	499	100
4	13,608	110,000	1,559	201	38	30	33	656	99
5	14,389	78,000	332	49	41	30	30	726	100
6	13,103	120,000	816	92	38	27	34	634	99
7	12,928	86,000	393	53	37	28	35	590	100
8	13,140	187,000	2,357	314	37	30	33	679	97
9	10,829	195,000	2,382	320	45	30	25	724	99
10	14,061	105,000	623	95	46	30	25	726	100
11	12,505	181,000	1,197	178	35	30	35	680	100
12	13,298	121,000	475	64	35	30	35	612	99
13	14,075	188,000	2,152	283	47	30	23	703	100
14	13,381	126,000	454	46	38	31	31	725	99
15	14,334	155,000	734	—	44	23	33	787	90
16	14,375	146,000	604	97	37	26	37	612	100
17	12,993	118,000	2,031	333	43	32	24	680	100
18	13,608	140,000	1,016	130	35	28	37	635	100
19	14,742	162,000	762	99	38	27	35	649	95
20	14,061	115,000	583	67	39	29	32	590	100
21	13,154	120,000	3,070	—	35	27	38	634	99
22	10,905	128,000	5,005	861	40	30	30	741	98
23	10,925	256,000	1,615	—	37	29	34	791	99
24	13,337	142,000	1,160	182	36	29	34	631	77
25	12,353	133,000	887	107	32	35	33	647	86
26	13,117	150,000	676	109	39	30	31	629	96
27	13,517	105,000	581	86	39	30	31	658	100
28	13,154	110,000	592	50	40	21	39	702	100
29	12,775	60,000	441	37	45	29	26	629	88
30	15,059	174,000	586	70	41	25	34	771	100
31	13,517	320,000	954	126	41	31	28	748	99
32	14,288	160,000	598	64	0	0	0	680	100
33	14,601	188,000	443	49	37	24	39	658	100

Table 2.2. (cont'd).

34	13,608	164,000	1,415	184	39	27	35	680	100
35	13,081	165,000	954	160	34	37	28	680	99
36	13,381	148,000	1,448	262	45	34	21	701	98
37	13,245	77,000	3,444	–	36	36	28	771	97
38	13,608	129,000	1,189	157	38	25	38	771	100
39	12,530	137,000	1,527	–	46	27	26	622	99
40	10,886	126,000	3,736	889	41	33	26	633	98
Mean	13,295	142,600	1.316	180	38	28	31	678	99

[1]Rolling herd average.
[2]Bulk tank somatic cell count from the month preceding farm visit.
[3]Total lactating and dry cows.
[4]< 60 d.
[5]Estimated body weight.
[6]Weighted by proportion of predominant breeds.

Table 2.3. Total antimicrobial usage administered to pre-weaned calves and adult cows on 40 Wisconsin dairy farms for a 1-year period estimated using animal daily doses (ADD per 1,000 animal-d) or mass-density (total mg of antimicrobial per kg animal weight).

		Animal daily dose per 1,000 animal-days					Mass-based (mg of drug per kg body weight)				
Active ingredient[1]	Farms (n)	LSM[2,3]	SEM	Median	Total	(%)	LSM[2,3]	SEM	Median	Total	(%)
Amoxicillin	6	0.17[bcd]	2.05	0.18	2.89	0.42	0.02[cdef]	2.07	0.03	0.43	0.08
Ampicillin	36	0.62[bcd]	1.35	0.72	43.46	6.32	2.07[a]	1.35	3.12	161.41	29.60
Ceftiofur	40	5.33[a]	1.33	6.56	315.39	45.86	2.21[a]	1.33	2.34	140.68	25.80
Cephapirin	24	1.22[abc]	1.44	1.62	86.85	12.63	0.35[abc]	1.44	0.63	19.11	3.51
Cloxacillin	8	1.57[abc]	1.87	4.80	31.18	4.53	0.44[abc]	1.88	1.38	9.15	1.68
Danofloxacin	1	—	—	0.07	0.07	0.01	—	—	0.01	0.01	<0.01
Dihydro-streptomycin	9	2.76[ab]	1.80	3.86	36.14	5.26	2.73[a]	1.82	3.75	34.87	6.40
Enrofloxacin	27	0.16[cd]	1.41	0.21	12.01	1.75	0.05[cde]	1.42	0.06	3.59	0.66
Florfenicol	21	0.06[d]	1.47	0.06	4.90	0.71	0.09[dc]	1.48	0.09	9.46	1.74
Gamithromycin	11	0.05[d]	1.70	0.10	1.89	0.27	0.01[def]	1.72	0.02	0.49	0.09
Gentamicin	2	—	—	0.01	0.02	<0.01	—	—	0.01	0.01	<0.01
Hetacillin	12	0.38[bcd]	1.66	0.47	7.74	1.13	0.01[def]	1.68	0.02	0.29	0.05
Linco-spectinomycin[4]	1	—	—	0.14	0.14	0.02	—	—	0.02	0.02	<0.01
Oxytetracycline	25	0.10[d]	1.43	0.09	9.54	1.39	0.33[abc]	1.43	0.40	32.94	6.04
Penicillin G	25	0.59[bcd]	1.43	0.88	60.45	8.79	0.20[bcd]	1.43	0.30	35.26	6.47
Penicillin novobiocin	6	0.76[abcd]	2.05	2.37	14.27	2.08	0.24[abcd]	2.07	0.74	4.52	0.83
Pirlimycin	19	0.17[cd]	1.50	0.18	19.16	2.79	0.01	1.51	<0.01	0.57	0.10
Spectinomycin	1	—	—	0.04	0.04	0.01	—	—	0.22	0.22	0.04
Sulfadimethoxine	13	0.12[cd]	1.63	0.11	15.39	2.24	1.15[ab]	1.64	1.42	65.80	12.07
Tildipirosin	4	—	—	0.02	0.09	0.01	—	—	<0.01	0.01	<0.01
Tilmicosin	5	—	—	0.05	0.32	0.05	—	—	0.06	0.81	0.15
TMP-sulfa[5]	5	—	—	3.24	13.77	2.00	—	—	4.99	21.80	4.00
Tulathromycin	30	0.09[d]	1.38	0.08	11.65	1.69	0.01[ef]	1.39	0.01	1.67	0.31
Tylosin	2			0.15	0.29	0.04	—	—	1.05	2.10	0.39

[a-f]Mean values within the same column with different superscripts differ from each other ($P < 0.001$).

Table 2.3. (cont'd).

[1]Active ingredients used on farm in either adult cows or pre-weaned calves or both.
[2]Active ingredients used on $\leq$5 farms were not used in analysis among means.
[3]Statistical analyzes were performed on natural logs, data is presented as back transformed LSM.
[4]Lincomycin-spectinomycin combination.
[5]Trimethoprim (TMP)- sulfamethoxazole combination.

Table 2.4. Comparison between dose- (Animal Daily Doses per 1,000 cow-d) or mass-based (Total mg antimicrobial per adult cow body weight) metrics used to estimate antimicrobial usage on 40 WI dairy farms for a 1 year-period.

		Animal daily dose per 1,000 cow-days					Mass-based (mg of drug per kg body weight)				
Active ingredient	Farm (n)	LSM[1,2]	SEM	Median	Total	(%)[3]	LSM[1,2]	SEM	Median	Total	(%)[3]
Intramammary dry cow products											
Ceftiofur	23	3.26	1.37	7.36	131.55	42.49	0.89	1.36	1.98	35.31	35.94
Cephapirin	14	4.51	1.50	7.54	86.04	27.79	0.95	1.51	1.23	13.81	14.05
Cloxacillin	7	3.05	1.78	6.81	34.43	11.12	0.81	1.75	1.83	9.24	9.40
Penicillin	9	2.79	1.66	4.43	41.13	13.29	2.41	1.64	3.81	35.32	35.95
Dihydrostreptomycin Penicillin novobiocin	6	0.95	1.86	2.67	16.42	5.30	0.27	1.83	0.75	4.58	4.66
Intramammary lactating cow products											
Amoxicillin	6	0.18[b]	1.93	0.20	3.23	1.75	0.02[ab]	1.93	0.03	0.43	2.68
Ceftiofur	36	2.32[a]	1.31	3.02	137.79	74.83	0.16[a]	1.31	0.20	9.24	57.57
Cephapirin	17	0.30[b]	1.48	0.49	12.78	6.94	0.13[a]	1.48	0.21	5.49	34.21
Cloxacillin	1	—	—	0.01	0.01	0.01	—	—	0.01	0.01	0.06
Hetacillin	12	0.40[b]	1.59	0.55	8.80	4.78	0.01[b]	1.59	0.02	0.30	1.87
Pirlimycin	19	0.19[b]	1.45	0.18	21.52	11.69	0.01[b]	1.45	<0.01	0.58	3.61
Oral products											
Sulfadimethoxine	4	—	—	0.44	2.27	100.00	—	—	5.34	27.45	100.00
Injectable products											
Ampicillin	34	0.63[a]	1.31	1.29	39.82	28.99	2.49[a]	1.31	3.26	159.89	46.55
Ceftiofur	40	0.85[a]	1.28	2.36	68.76	50.05	1.12[ab]	1.29	1.04	96.37	28.06
Florfenicol	1	—	—	0.43	0.43	0.31	—	—	3.16	3.16	0.92
Lincomycin-spectinomycin[4]	1	—	—	0.15	0.15	0.11	—	—	0.02	0.02	0.01
Oxytetracycline	23	0.07[bc]	1.38	0.31	6.17	4.49	0.33[c]	1.39	0.32	29.89	8.70
Penicillin G	14	0.24[ab]	1.51	1.47	19.73	14.36	0.34[bc]	1.51	0.40	28.81	8.39
Spectinomycin	1	—	—	0.04	0.04	0.03	—	—	0.22	0.22	0.06
Sulfadimethoxine	9	0.04[c]	1.67	0.26	1.86	1.35	0.40[bc]	1.67	0.70	22.36	6.51
Tilmicosin	2	—	—	0.07	0.09	0.07	—	—	0.31	0.63	0.18
Tulathromycin	1	—	—	<0.01	<0.01	<0.01	—	—	0.01	0.01	<0.01
Tylosin	1	—	—	0.33	0.33	0.24	—	—	2.12	2.12	0.62

[a-b]Mean values within the same product type and column with different superscripts differ from each other ($P < 0.001$).

[1]Active ingredients containing 5 or less farms were not used in the comparison among means.

[2]Statistical analyses were performed using natural logs, data presented as back transformed LSM.

[3]Proportion within route of antimicrobial usage.

Table 2.4. (cont'd).

[4]Lincomycin-spectinomycin combination.

Table 2.5. Estimated antimicrobial usage in adult cows and pre-weaned calves on 40 Wisconsin dairy farms by route of administration for a 1-year period using dose-based (animal daily doses; ADD) and mass-based (total mg antimicrobial per kg animal weight).

		Animal daily dose (ADD/1,000 cow-d; ADD per 1,000 PWC-d)					Mass-based (mg of drug per kg body weight)				
Route	Farms (n)	LSM[1,2]	SEM	Median	Total	(%)[3]	LSM[1,2]	SEM	Median	Total	(%)[3]
Adult cows											
Injectable	40	2.22[b]	1.13	2.48	137.38	21.69	5.12[a]	1.15	5.79	343.47	70.78
Intramammary[4]	40	11.69[a]	1.13	11.40	493.72	77.95	2.56[b]	1.15	2.32	114.37	23.56
Oral	4	—	—	0.44	2.27	0.36	—	—	5.34	27.45	5.66
Pre-weaned calves											
Injectable	35	11.22	1.30	19.40	783.74	79.10	5.39	3.60	38.85	2139.42	41.84
Oral	8	9.22	1.72	17.00	207.11	20.90	148.32	14.55	321.37	2973.74	58.16

[a-b]Mean values within the same column with different superscripts differ from each other ($P < 0.001$).
[1]differences among routes were not estimated if ≤5 farms reported use of compound.
[2]Statistical analyses were performed using natural logs, data presented are back transformed LSM.
[3]Proportion within class of animal.
[4]Intramammary animal daily doses include antimicrobials used mastitis treatment, dry cow therapy, and intraocular treatment.

Table 2.6. Comparison of antimicrobial usage in pre-weaned calves by active ingredient estimated using dose based (Animal daily doses per 1,000 pre-weaned calf-d) and mass-based (Total mg antimicrobial per kg of pre-weaned calf body weight) metric on 35 Wisconsin dairy farms.

		Animal daily dose per 1,000 pre-weaned calf-d					Mass-based (mg of drug per kg body weight)				
Active ingredient	Farms (n)	LSM[1,2]	SEM	Median	Total	(%)	LSM[1,2]	SEM	Median	Total	(%)
Ampicillin	16	1.32[ab]	1.61	2.46	67.11	6.77	5.24[a]	1.61	9.89	269.46	5.27
Ceftiofur	20	0.56[b]	1.54	0.52	90.95	9.18	0.65[b]	1.53	0.53	83.21	1.63
Danofloxacin	1	–	–	0.86	0.86	0.09	–	–	1.89	1.89	0.04
Enrofloxacin	27	1.34[ab]	1.45	1.74	106.24	10.72	3.93[a]	1.45	4.70	270.12	5.28
Florfenicol	21	0.51[b]	1.52	0.53	42.96	4.34	6.82[a]	1.52	6.96	576.17	11.27
Gamithromycin	11	0.44[b]	1.77	0.71	14.97	1.51	1.07[ab]	1.76	1.68	35.27	0.69
Gentamicin	2	–	–	0.09	0.17	0.02	–	–	0.38	0.77	0.02
Oxytetracycline	11	0.60[ab]	1.77	2.17	44.07	4.45	3.85[ab]	1.76	11.83	365.68	7.15
Penicillin G	24	3.75[a]	1.49	3.09	315.32	31.82	5.53[a]	1.48	4.52	460.36	9.00
Sulfadimethoxine	3	–	–	9.30	102.86	10.38	–	–	292.12	1422.35	27.82
Tildipirosin	4	–	–	0.20	0.99	0.10	–	–	0.29	1.45	0.03
Tilmicosin	5	–	–	0.41	2.16	0.22	–	–	2.98	15.79	0.31
TMP-sulfa[3]	5	–	–	24.01	100.06	10.10	–	–	350.62	1460.88	28.57
Tulathromycin	30	0.80[ab]	1.43	0.70	102.05	10.30	0.79[b]	1.42	0.67	149.44	2.92
Tylosin	1	–	–	0.05	0.05	0.01	–	–	0.33	0.33	0.01

[a-b]Mean values within the same column with different superscripts differ from each other ($P < 0.05$).

[1]Active ingredients containing 5 or less farms were not used in the comparison among means.

[2]Statistical analyzes were performed on natural logs for farms using the active ingredient, data presented are back transformed LSM.

[3]Trimethoprim (TMP)- sulfamethoxazole combination.

Table 2.7. Animal daily doses (ADD) administered in pre-weaned calves (per 1,000 pre-weaned calf-d) via oral and injectable routes on 35 Wisconsin dairy farms.

		ADD per 1,000 pre-weaned calf-d					Mass-based (mg of drug per kg body weight)				
Active ingredient	Farm (n)	LSM[1,2]	SEM	Median	Total	(%)[3]	LSM[1,2]	SEM	Median	Total	(%)[3]
Oral											
Oxytetracycline	1	–	–	9.99	9.99	4.82	–	–	160.47	160.47	5.40
Sulfadimethoxine	2	–	–	48.53	97.06	46.86	–	–	676.19	1352.39	45.48
TMP-sulfa[4]	5	–	–	24.01	100.06	48.31	–	–	350.62	1460.88	49.13
Injectable											
Ampicillin	16	1.32[ab]	1.61	2.46	67.11	8.56	5.20[a]	1.60	9.89	269.46	12.60
Ceftiofur	20	0.57[ab]	1.54	0.52	90.95	11.60	0.66[b]	1.53	0.53	83.21	3.89
Danofloxacin	1	–	–	0.86	0.86	0.11	–	–	1.89	1.89	0.09
Enrofloxacin	27	1.34[ab]	1.45	1.74	106.24	13.56	3.94[a]	1.45	4.70	270.12	12.63
Florfenicol	21	0.51[ab]	1.52	0.53	42.96	5.48	6.75[a]	1.51	6.96	576.17	26.93
Gamithromycin	11	0.44[b]	1.77	0.71	14.97	1.91	1.05[ab]	1.76	1.68	35.27	1.65
Gentamicin	2	–	–	0.09	0.17	0.02	–	–	0.38	0.77	0.04
Oxytetracycline	10	0.48[ab]	1.82	1.48	34.08	4.35	2.85[ab]	1.80	8.75	205.20	9.59
Penicillin G	24	3.76[a]	1.48	3.09	315.32	40.23	5.54[a]	1.48	4.52	460.36	21.52
Sulfadimethoxine	1	–	–	5.81	5.81	0.74	–	–	69.96	69.96	3.27
Tildipirosin	4	–	–	0.2	0.99	0.13	–	–	0.29	1.45	0.07
Tilmicosin	5	–	–	0.41	2.16	0.28	–	–	2.98	15.79	0.74
Tulathromycin	30	0.80[ab]	1.43	0.7	102.05	13.02	0.80[b]	1.42	0.67	149.44	6.99
Tylosin	1	–	–	0.05	0.05	0.01	–	–	0.33	0.33	0.02

[a-b] Mean values within the same column with different superscripts differ from each other ($P < 0.05$).
[1]Active ingredients containing 5 or less farms were not used in the comparison among means.
[2]Statistical analyzes were performed on natural logs for farms using the active ingredient, data presented are back transformed LSM.
[3]Proportion within route of antimicrobial usage.
[4]Trimethoprim (TMP)- sulfamethoxazole combination.

Figure 2.1. Herd-level antimicrobial usage for 40 Wisconsin dairy farms by animal category estimated using dose-based (A) and mass-based (B) metrics.

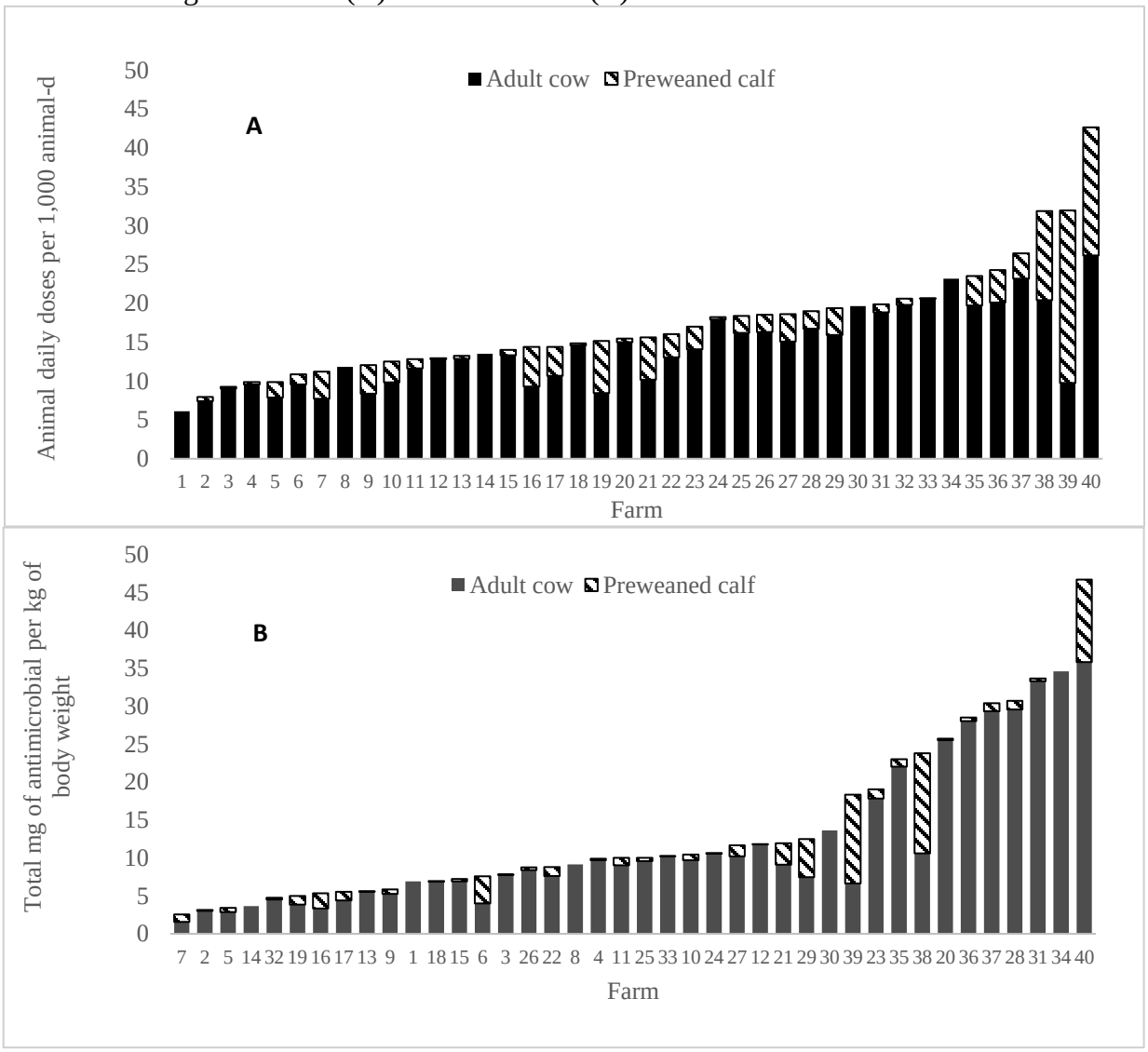


Figure 2.2. Antimicrobial usage in adult cows by route estimated using dose-based (A) and mass based (B) metrics. Bars represent SEM.

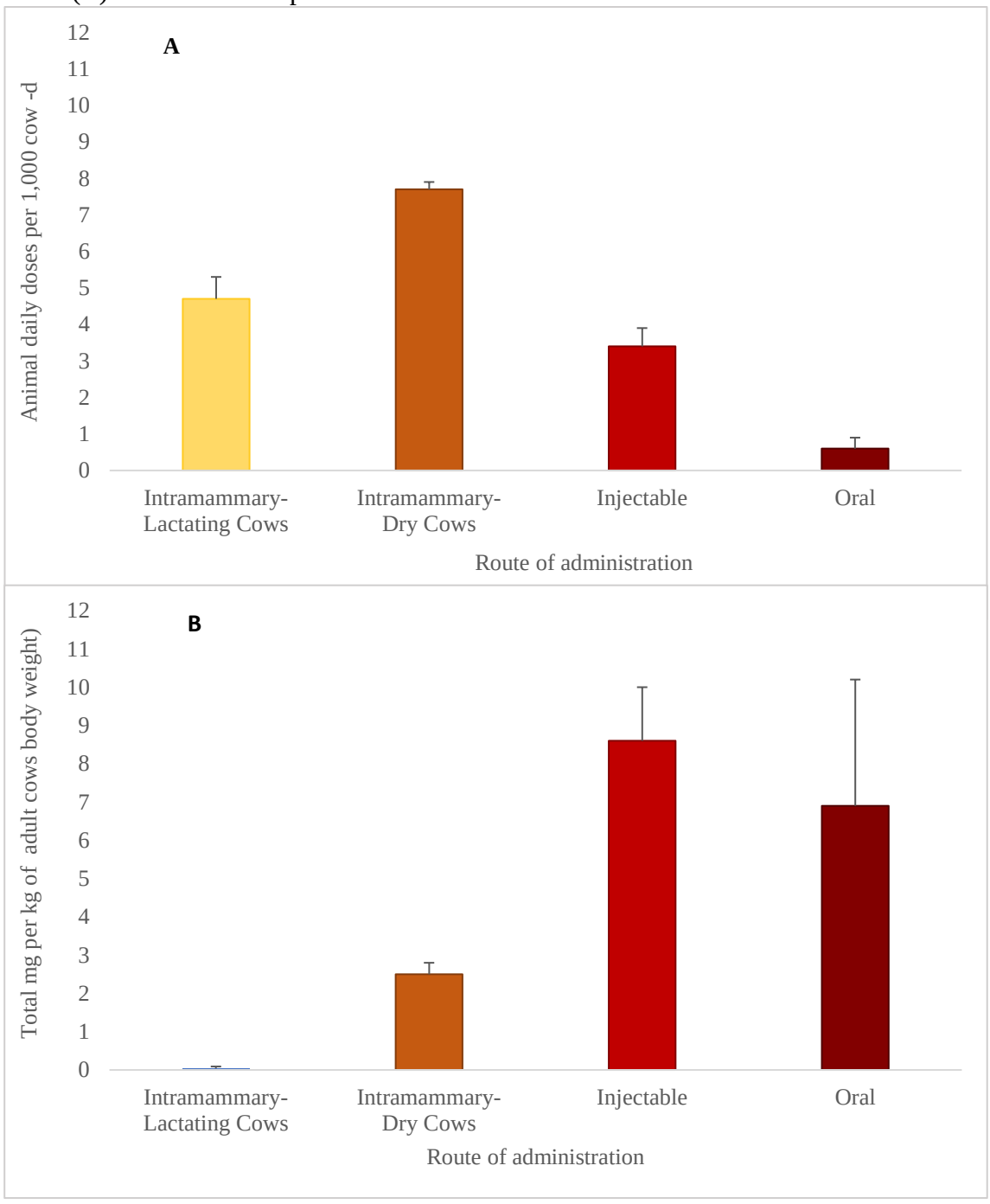
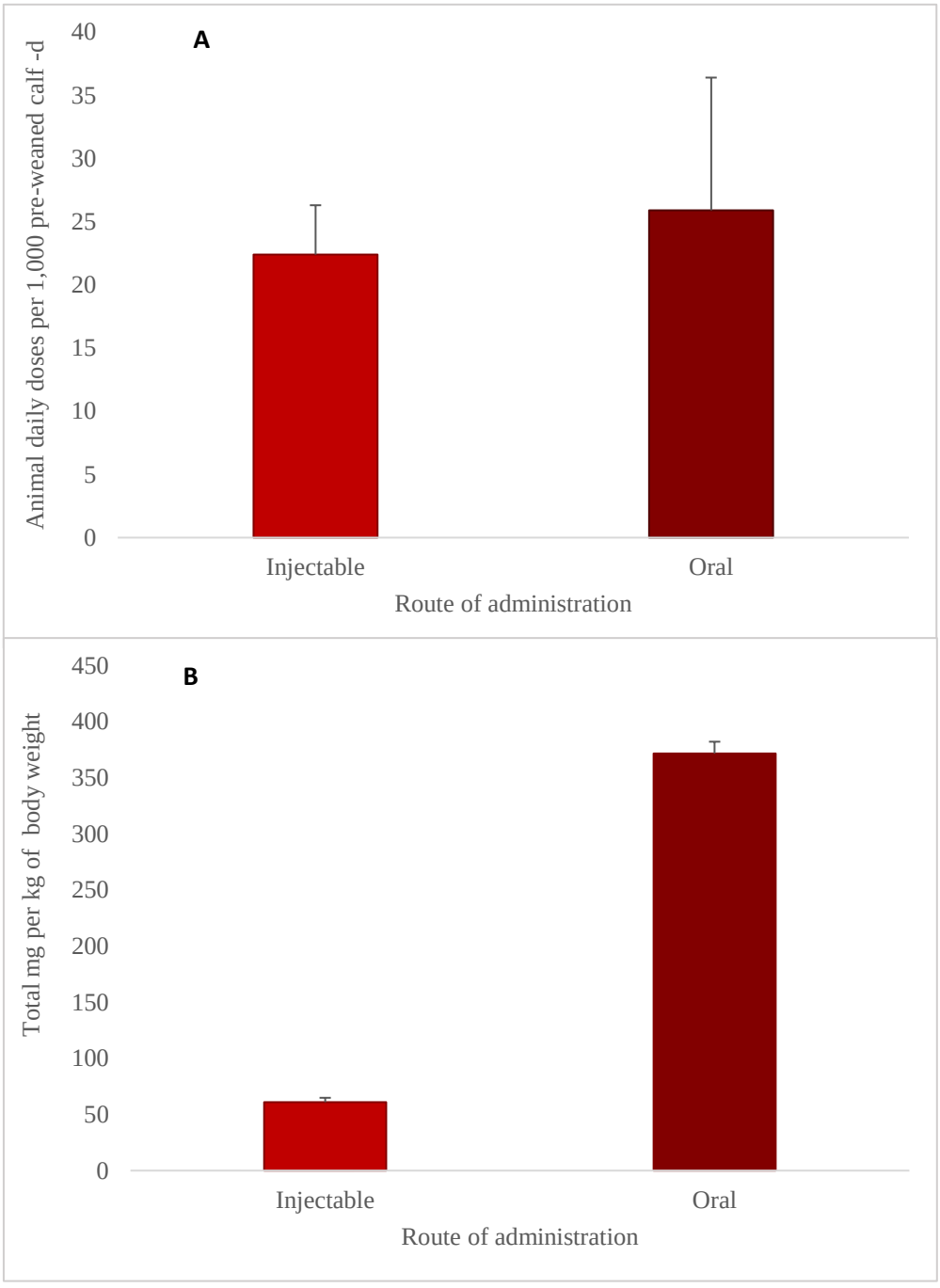


Figure 2.3. Antimicrobial usage in pre-weaned calves by administration route as estimated using dose-based (A) and mass-based (B) metrics. Bars represent SEM**.**



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CHAPTER 3. VARIATION IN DIRECT COSTS OF TREATING CLINICAL MASTITIS AMONG 37 WISCONSIN DAIRY FARMS

ABSTRACT

The objective of this study was to describe variation in direct costs of clinical mastitis **(CM)** treatments among 37 dairy herds using data obtained from herd management records. Animal health and drug purchase records were retrospectively collected from 37 Wisconsin dairy herds for a period of 1 yr. A farm visit was performed to verify case definitions and detection criteria. Descriptive statistics were used to summarize drug costs and milk discard costs. Differences in costs among protocols, intramammary products, parities, DIM, and recurrence were analyzed using ANOVA. Of 20,625 cases of CM, 31% did not receive antimicrobial treatment. The average cost of drugs and milk discard (including cases treated or not treated using antimicrobials) was $192.36 ± 8.90 per case and ranged from $118.13 to $337.25. For cases treated with IMM antimicrobials, milk discard costs accounted for 87% of total costs and was dependent on duration of therapy. Differences in costs were observed among parities, recurrence, and stage of lactation at case detection ($P < 0.001$). Eight different treatment protocols were observed, but treatment using only IMM antimicrobials accounted for 64% of cases. Treatment costs per case varied among protocols ($P< 0.01$) and cases treated using both intramammary and injectable antimicrobials as well as supportive therapy were treated for the longest duration and had the greatest costs. Ceftiofur was used for treatment of 82% of all cases treated using intramammary antimicrobials while ampicillin was used for 51% of all cases treated using injectable antimicrobials. As compared to observed costs of cases treated using only intramammary therapy, estimated costs were reduced by $65.20 per case when the minimum labeled duration of the products were used ($P= 0.01$). Overall, direct costs per case varied among herds, cow factors, and treatment protocols and were highly dependent on duration of therapy which influenced the amount of milk discarded.

INTRODUCTION

Dairy farm productivity and animal welfare are impaired by the occurrence of disease and avoiding unnecessary costs associated with treatment is crucial to maintain profitability. Among diseases, clinical mastitis **(CM)** occurs in about 25% of lactating cows each year (USDA–APHIS–VS–CEAH–NAHMS, 2018) and is detected on almost every dairy farm. Mastitis is an expensive disease and costs of CM include direct and indirect costs of treatment such as costs of milk discard, diagnosis, and labor as well as losses due to reduced milk production, reduced value of milk, increased risk of culling (Pinzón-Sánchez et al., 2011), and reduced fertility (Fuenzalida et al., 2015).

The overall economic impact of CM has been evaluated (Morse et al., 1987; Halasa et al., 2007) and total losses due to CM are often estimated by developing models using assumptions obtained from scientific literature. In these studies, estimated total costs of CM have ranged from approximately $40 to $500 per case depending on the type of model, variables used to estimate costs, types of treatment decisions, and the duration of treatment (Pinzón-Sánchez et al., 2011; Rollin et al., 2015; Liang et al., 2017). Of variables used to estimate costs of CM, expenses related to purchase of drugs, milk discard, labor, diagnosis, and culling are often included (Seegers et al., 2003; Huijps et al., 2008). Costs associated with milk discard are an important determinant of CM costs. Abnormal milk may not be sold for human consumption and milk from cows receiving antibiotics must be discarded for prescribed withdrawal periods. Thus, increased monetary losses are associated with longer durations of treatment (Pinzon-Sanchez et al., 2011). For example, expected monetary losses of CM were estimated to increase from approximately $65 to $187 per case as treatment days increased from 2 to 8 days.

Antimicrobials are usually used for treatment of CM and account for 30% to 37% of total antimicrobial doses used on dairy farms (Pol and Ruegg, 2007; Stevens et al., 2016; Leite de Campos et al., 2021). Consumers have expressed considerable concern about usage of antimicrobials on dairy farms (Wemette et al., 2021) and reductions in antimicrobial usage must address use of antibiotics for treatment and prevention of mastitis. Not all cases of CM require antimicrobial therapy to achieve successful outcomes (Lago et al., 2011a; b; Fuenzalida and Ruegg, 2019; Ruegg, 2021). Reducing unnecessary usage of antimicrobials by identifying cows with CM that will not benefit from antimicrobial treatment as well as reducing treatment duration (when feasible) will reduce costs of treatment as well as the number of antimicrobial doses used on dairy farms (Ruegg, 2021). A better understanding of direct costs associated with treatment of CM will help dairy farmers and their advisors make informed treatment decisions about the impact of longer duration therapy on profitability. The objective of this study was to describe drug and milk discard costs associated with treatment of CM on 37 dairy herds using data obtained from herd management records and drug purchase receipts and compare variation in components of treatment cost and treatment protocols among herds.

MATERIAL AND METHODS

Herd Selection and Data Collection

Costs of treatment of CM associated with milk discard (due to abnormal milk and the milk withholding period when drugs are administered) and drugs were considered direct costs of treatment and were used in this study. These costs were estimated using computerized herd records obtained from 37 large dairy herds in Wisconsin. Herd selection and data collection have been previously described (Leite de Campos et al., 2021). In brief, 40 dairy herds were selected based on herd size (≥ 250 lactating cows) and availability of drug purchase, computerized animal

health, and treatment records. Treatment records were retrospectively collected for a period of one year and a single farm visit was performed to verify treatment records and case definitions. During our farm visit, animal health managers were surveyed about herd characteristics, detection procedures, and treatment protocols. For this analysis, 37 of the original 40 herds were selected based on usage of a common dairy management program (Dairy Comp 305; Valley Agricultural Software, Tulare, CA). Data on each case of CM and monthly DHIA milk production per cow were obtained from herd records. Data entered into herd records was verified during the herd visit and when electronic records did not list specific antimicrobials used or the duration of treatment, that information was imputed based on responses to survey questions. Of 37 herds, electronic records for 21 included the duration of IMM antimicrobial treatment for almost all (> 96%) CM events, electronic records for 2 herds included duration of IMM antimicrobial treatment for the majority (>57%) of CM events, while electronic records for 14 herds did not include information on duration of treatment. Overall, duration of IMM antimicrobial treatments was recorded for 59% of CM events and duration of treatment for the remaining 41% of IMM treatments were imputed by using the average number of treatment days described by interviewing animal health managers on each farm.

Direct treatment costs of CM were calculated for all cases, including those treated using antimicrobials as well as cases that received supportive therapy or no treatment. For cases that did not receive antimicrobials, direct costs of each case of CM were estimated based on costs of milk discard and supportive treatment (when given). For cases that received antimicrobial treatment, costs of intramammary **(IMM)** treatment (if any), injectable **(INJ)** treatment (if any), supportive treatment (if any), and milk discard were used.

Each case of CM was defined at cow-level and included all treatments that occurred within 14 d from the initial date that the case was recorded. When multiple treatments were given within the 14-d period, milk discard was determined based on the beginning of the first treatment to longest withholding period for any drug given. Recurrent cases of CM were defined when a case occurred in the same cow > 14 d after the first CM event.

Cost of Drugs

Costs of drugs used for treatment of mastitis were estimated using purchase records obtained during the farm visit. To standardize purchase prices among farms, prices were summarized per product and the overall average price was used to calculate the costs across all herds in the study. Costs of IMM treatments were estimated using this formula:

$$IMM\ Cost = quarters\ treated\ \times days\ of\ treatment\ \times applications\ per\ day \times\ price\ per\ intrammmary\ antibiotic\ tube$$

Costs of INJ and oral treatments were estimated based on the dose given and calculated using the following formula:

$$INJ\ Cost = maximum\ dose\ \times days\ of\ treatment\ \times applications\ per\ day \times price\ per\ mL$$

Milk discard

Monthly test day milk production for each cow were obtained from electronic records by creating items and using the following command: EVENTS\2S365I ID BDAT LACT ITEM1 ITEM2 ITEM3 FOR LACT>0.

The value of the test day milk production closest to the event of CM was used to estimate the amount of milk discarded. Values within a range of -14 d before a case of CM to + 30 d after the event were used. If a cow had more than one test day during this period, the value from the

closest test date after CM was selected. For cases that did not have a value for milk within this range, herd average milk production was used to estimate milk discard. Across all herds, milk discard values for 90% (n= 18,609) of CM cases were within the specified range while average milk production was used to estimate milk discard for the remaining 10% (n= 2,016) of cases.

For animals treated with any medication that required milk discard, total days of milk discard was defined as the sum of treatment days plus withholding days. Withholding period was determined using the current label listed in the US National Library of Medicine (DailyMed nih.gov). For animals that were not given any medication that required milk discard, we estimated a milk discard period of 5 d based on expected period of abnormal milk (Oliveira and Ruegg, 2014). A 5-d milk discard after treatment was completed was used for systemic treatments using florfenicol and spectinomycin as they do not have a labeled milk withholding period.

For cows treated using both IMM and INJ routes, the totals days of milk discard was based on the withholding period for the product used for the longest duration. Milk price per kg of milk was estimated based on the monthly records from USDA-NASS, (2022) and were: $0.40 (Sep. 2016), $0.37 (Oct. 2016), $0.41 (Nov. 2016), $0.43 (Dec. 2016), $0.43 (Jan. 2017), $0.42 (Feb. 2017), $0.40 (Mar. 2017), $0.38 (Apr. 2017), $0.38 (May 2017), $0.39 (Jun. 2017), $0.38 (Jul. 2017), $0.40 (Aug. 2017), $0.40 (Sep. 2017), and $0.41 (Oct. 2017). No costs of farm labor or veterinary inputs were included in these estimates as these costs were not accessible in dairy records and would have been evenly distributed among cases.

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Descriptive characteristics were summarized using PROC FREQ. The cost of CM treatment per case was summarized for each farm using PROC MEANS. Costs were estimated on a per-case basis and summarized for cases that received solely IMM treatments, solely INJ treatments, solely supportive treatments, combination of IMM and INJ treatments, combination of IMM and supportive therapy, combination of IMM, INJ, and Supportive therapy, combination of INJ and Supportive therapy, or cases that did not receive any therapies.

For CM cases that did not receive antimicrobials, costs per case were calculated by summing the total cost of milk discard and supportive treatments (if any) and dividing by the sum of cases that received supportive therapy only and cases that did not received any antimicrobials. Direct costs of treatment of CM cases that received antimicrobials were calculated by summing the cost of milk discard and treatments and dividing by the number of cases that received antimicrobials. At the farm level, overall treatment costs were calculated by summing the milk discard and drug costs of treated and non-treated cases and dividing by the total number of CM cases occurring on each farm.

Herd-size and CM rate were classified based on median values and associations between herd-level cost per case and these variables were analyzed using ANOVA. Differences in observed direct costs per case were compared to estimated treatment costs if IMM treatments had been used according to minimal approved label durations. Label instructions for IMM products were obtained from the US National Library of Medicine (Table 3) and a minimum of 2 d duration of treatment was chosen for ceftiofur hydrochloride and pirlimycin hydrochloride products (each have an approved range treatment duration of 2 to 8 d). Difference in means between observed cost per case and costs of treatment using label durations were analyzed using a paired TTEST.

PROC UNIVARIATE was used to assess normality of data and differences in direct costs among products and protocols were evaluated using a model that used cost per case as the dependent variable and products or treatment protocols as independent variables. Difference in costs per case based on parity groups (Parity 1, 2, ≥ 3), recurrence (1^{st}; 2^{nd}; ≥ 3^{rd}), or stage of lactation at case detection (0-30 DIM; 31-60 DIM; 61-90 DIM; 91-120 DIM; 121-150 DIM; 151-180 DIM; 211-240 DIM; 241-270 DIM; >270 DIM) were analyzed using PROC GLIMMIX in models that used cost per case as the dependent variable and parity, recurrence, or stage of lactation as independent variables. For all models, farm was included as a random effect and differences among least square means were adjusted by Tukey. Statistical analyses were performed only for products or treatment protocols used on ≥ 5 herds. The null hypothesis was that cost per case did not vary among products and statistical difference was considered when $P < 0.05$.

RESULTS

Herd description

Descriptive statistics of the original 40 herds have been previously reported (de Campos et al., 2021). Herds (n = 37) included in this study contained an average of 1,351 ± 180 lactating cows per farm (ranging from 332 to 5,005) and were primarily composed of Holstein cows (98% ± 0.76). Bulk tank SCC among herds was 143,000 cells/mL ± 7,230 (ranging from 77,000 to 320,000 cells/mL) and rolling herd average for milk was 13,377 ± 164 (ranging from 10,829 to 15,059). All herds housed lactating cows in freestalls and the primary bedding type was fresh sand (n=20), followed by recycled sand (n=9), manure solids (n= 3), wood products (n= 2), or mixed bedding materials (n= 3). Average milk production of affected cows prior to occurrence of CM was 43 kg ± 0.71(Table 1). Of 20,625 cases of CM, 6,473 (31%) did not receive

antimicrobial treatments while 14,152 (69%) were treated using antimicrobials (Table 1). Except for one farm that recorded very few cases of CM and only used supportive products, all farms used IMM antimicrobials for treatment of at least one case of CM. Injectable antimicrobials were used for treatment of at least one case of CM in 24 herds (65%) and one farm used an oral antimicrobial for treatment of two cases of CM. Supportive treatment was used by 15 farms (41% of farms).

Overall cost per case of clinical mastitis

Across all farms, the average direct cost of treatment per case was $192.36 ± 8.90 and ranged from $118.13 to $337.25 (Figure 1). For herds (n=36) that reported administration of antimicrobials (either IMM or INJ) for treatment of CM, the average direct costs per treated case were $209 ± 9.65 and ranged from $119.95 to $372.26. For herds (n=25) that reported CM cases not treated with antimicrobials, the average direct costs per non-treated case were $87.44 ± 4.34 and ranged from $7.99 to $119.59 (Table 1).

Overall direct costs of treatment per case (including cases treated with antimicrobials and not treated cases) were not associated with herd size ($186.0 ± 9.74 for herds with ≤ 954 cows; $199.80 ± 15.77 for herds with > 954 cows; P = 0.44) or CM rate ($179.50 ± 10.99 for herds with a CM rate ≤ 31%; $207.50 ± 13.89 for herds with CM rate > 31%; P= 0.52).

Cow-factors influencing cost of treatment

Cases were distributed among cows in first lactation (n = 3,973 cases; 19%), second lactation (n = 6,711; 33%) and ≥ third lactation (n = 9,941, 48%). Treatment costs varied among parities, and cost per case was greater for cows with ≥3 lactations (196.02 ± 8.56 per case) than for cows in second ($185.57 ± 8.56 per case) or first lactation ($163.35 ± 8.56 per case; P < 0.001).

Of incident cases, a total of 28.5% of the cases experienced a recurrence, 19.3% (n= 3,981) of first cases presented a second recurrence and 9.2% (n= 1,899) of the first cases presented a third recurrence. Treatment costs per case varied among recurrence cases and were greater for first cases ($193.03 ± 8.99) as compared second ($178.49 ± 9.09) or third cases ($154.36 ± 9.29; P < 0.001).

The average DIM at occurrence of CM was 162 ± 0.77 and average milk production during this period was 41 kg ± 0.09. Days in milk at occurrence of a case ranged from 0 to 948 and 25 % of CM cases occurred within 78 d after calving, 50% of CM cases occurred within 147 d after calving, and 75% of the CM cases occurred within 232 d after calving. Treatment costs per case varied across DIM, and cost per treatment was greater at 91 to 120 DIM ($203.49 ± 9.21) than when DIM was > 270 ($151.32 ± 9.24; P< 0.001; Figure 2).

Comparisons among Treatment Protocols

The most common treatment protocol was administration of only IMM antimicrobials which was reported by 35 farms and accounted for 64 % of cases (Table 2). The average days of discard (duration of treatment plus the withholding period) was 9.3 ± 0.35 d and costs per case were $184.93 ± 7.76, ranging from $126.97 to $320.12 (Table 2). For these cases, costs were distributed as 13% for IMM antimicrobials ($23.91 ± 1.58) and 87% for milk discard ($161.50 ± 6.54).

Cases that did not receive any antimicrobial or supportive therapies were reported by 22 farms and accounted for 30% of all CM cases. The average cost per non-treated case was $79.01 ± 9.47 ranging from $61.73 to $110.54 (Table 2). The entire direct cost of these cases was based on milk discard.

Combined treatments using both IMM and INJ antimicrobials were reported by 22 farms and accounted for 3% of all CM cases. The average days of discard was 9.1 ± 0.58 d and cost per case was $206.70 ± 9.47, ranging from $96.54 to $400.85 (Table 2). In this scenario, costs were distributed as 31% for IMM and INJ antimicrobials ($63.37 ± 9.16) and 69% for milk discard ($139.64 ± 9.78).

Cases that were treated using both IMM antibiotics and supportive therapies were reported by 10 farms and accounted for 1% of all CM cases. The average days of discard was 10.2 ± 1.24 d and cost per case was $198.51 ± 13.47, ranging from $180.28. to $388.74 (Table 2). In this scenario, costs were distributed as 11% for IMM antimicrobials ($22.58 ± 1.83), 7% for supportive products ($13.90 ± 4.90, and 83% for milk discard ($173.50 ± 20.53).

Cases treated only with supportive products were reported by 12 farms and accounted for 1% of all CM cases. The average days of discard was 4.9 ± 0.23 d and cost per case was $82.89 ± 12.41, ranging from $7.99 to $130.39 (Table 2). In this scenario, costs of supportive products were 14% ($13.66 ± 4.43) while costs of milk discard accounted for 86% of total costs ($82.56 ± 8.72).

Cases treated only using INJ antimicrobials were reported by 9 farms and accounted for less than 1% of all CM cases. The average days of discard was 5.9 ± 0.43 d and cost per case of $130.25 ± 14.17, ranging from $82.63 to $193.09 (Table 2). In this scenario, costs of INJ antimicrobials accounted for 21% of the total ($26.78 ± 11.11) while costs of milk discard accounted for 79% of the total ($103.71 ± 11.30).

Treatment using IMM and INJ antimicrobials, in combination with supportive therapies were reported by 3 farms and accounted for less than 1% of all CM cases. The average days of discard was 11.5 ± 2.48 d and costs per case were $240.03 ± 30.73, ranging from $207.75 to

$301.47 (Table 2). In this scenario, costs were distributed as 47% for antimicrobial and supportive products ($43.31 ± 10.38 for IMM antimicrobials, $56.04 ± 29.04 for INJ antimicrobials $13.18 ± 4.66 for supportive products) and 53% for milk discard ($127.50 ± 12.86).

Only 15 CM cases from one herd were treated solely with INJ antimicrobials and supportive products. The average days of discard was 6.6 d and cost per case was $162.67. In this scenario, costs of INJ antimicrobials accounted for 16% of the total ($26), supportive products accounted for 9% of the total cost ($14.04), and costs of milk discard accounted for 75% of the total ($122.63) (Table 2).

Intramammary products and estimated cost of intramammary treatment when following label directives

Among herds that used IMM antimicrobials for treatment of CM (n= 36 herds), 6 different IMM products were reported (Table 3). Ceftiofur was the most commonly used antimicrobial, and was administered in 32 herds, representing 82% of the IMM treatments (Table 2). Other IMM products included, cephapirin (used in 13 herds for 9% of treatments), hetacillin (9 herds, 3 of the treatments), pirlimycin (12 herds, 4% of the treatments), amoxicillin (6 herds, 1% of the treatments), and cloxacillin (1 herd, < 1% of the treatments). For cases that received only IMM treatments, there were significant differences in the observed costs per case based on active ingredient (P= 0.06; Table 2).

For first cases treated solely using IMM products, costs per case were less for observed cases ($158.83 ± 4.76) than as compared to costs estimated when using the minimal label duration of treatment ($93.63 ± 1.34; P< 0.01). Among products, the difference in estimated costs using minimal labeled duration versus observed costs based on duration administered per

case were $-73.65, $-62.96, $-64.06, $-12.52, $-12.62, and $-51.41 for cases treated using amoxicillin, ceftiofur, cephapirin, cloxacillin, hetacillin, and pirlimycin, respectively.

Products used for injectable or supportive treatment

Among herds that used INJ antimicrobial products either solely or combined with other products (n= 24 herds), 8 different antimicrobials were used. Ampicillin was used by 50% (n= 12 herds) and represented 51% of the treatments. Injectable ceftiofur products and oxytetracycline were used by 10 herds each and represented 44% of INJ treatments, while florfenicol, spectinomycin, and sulfadimethoxine were each used by 1 herd and together represented approximately 5% of the treatments.

A total of 10 products were included in electronic records as supportive therapy of CM. Among products, hypertonic saline (n= 8 herds; 317 cases) and flunixin meglumine (n= 7 herds; 122 cases) were most commonly used. Additional supportive products include dexamethasone (n= 4 herds; 26 cases), aspirin (n= 2 herds; 15 cases), and others such as oxytocin, vitamin B, and oral calcium supplements that were used on as treatments for 64 cases on <5 herds each.

DISCUSSION

Bulk tank somatic cell count has decreased in U.S. dairy herds indicating control of subclinical mastitis and continued improvements in milk quality (APHIS, 2019). In contrast, the proportion of cows affected with CM appears to be stable or increasing, probably as a result of exposure to environmental pathogens that tend to cause larger inflammatory responses resulting in non-severe CM. Mastitis is an expensive disease as IMM infection reduces productivity and results in discarded milk that reduces farm income. With net returns often negative (USDA ERS - Milk Cost of Production Estimates), maintaining control of costs associated with disease treatment is crucial to maximize productivity. Reducing antimicrobial usage to that necessary to

maintain animal health is an important aspect of antimicrobial stewardship and demonstration of economic advantages based on reductions in duration or usage of antimicrobials may motivate examination of treatment protocols for mastitis. By calculating the average cost per case based on differences in treatment protocols and among herds, we were able to estimate difference in cost among protocols that may inform future treatment decision and contribute to judicious usage of antimicrobials.

Similar to previous studies, great variation in cost was observed among herds (Rodrigues et al., 2005; Aghamohammadi et al., 2018). Herds with the greatest costs spent almost three times more per case compared to herds with the least costs, and this difference is associated with differences among treatment protocols, including differences in duration of therapy and the proportion of cases that are not treated using antimicrobials. Our cost estimates are partial costs and do not include diagnostic costs used to guide selective therapy programs such as use of culture or other diagnostic tests. However, based on bulk tank SCC and other characteristics of these herds, these farms likely had minimal prevalence of mastitis caused by pathogens with very low expected spontaneous cure rates (such as *Staphylococcus aureus*) and thus may benefit from selective use of antimicrobials or shorter duration therapies that result in less discarded milk.

Treatment costs for CM have varied among studies and are highly dependent on the variables and assumptions included in analyses. Direct costs often include costs such as diagnostics, therapeutics, milk discard, veterinary service, and labor. Cost components such as labor and cost of diagnosis are important in determining total cost of CM, but on a practical basis, when comparing costs, those components are often standardized and vary little among cases (Pinzón-Sánchez et al., 2011). As our objective was to determine variation among farms using actual data included in herd records, we chose not to include those costs in our calculation.

Variation in direct costs of treatment can be attributed to differences in duration of treatment, milk yield of affected cows, milk price, severity, and choice of therapies. While most of these variables are not under the control of the farmer, duration of treatment can be controlled. Expected monetary losses of a case of clinical mastitis have previously been shown to be strongly associated with number of treatment days (Pinzón-Sánchez et al., 2011). Of 6 IMM products used on these farms, 4 have labeled durations of treatment of <3 days but the observed duration of treatment for all these products exceeded label recommendations. Two products have flexible labels with recommended durations of 2 to 8 days but were administered for about 5 days each. For most etiological agents, there is very limited evidence to support improvement in clinical outcomes based on longer duration therapy (Ruegg, 2021) and use of shorter duration therapy (when appropriate) is an obvious way to reduce treatment costs. Others have demonstrated that reduced milk production and cost of discarding milk often represents the largest proportion of total cost of treatment (Seegers et al., 2003; Halasa et al., 2007). Two critical elements in estimating costs are the milk price and the average milk production produced by affected cows. When milk price is higher, or when more milk is discarded, losses associated with treatment increase. In previous studies that calculated direct treatment cost (drug, milk discard, labor) of CM, cows produced approximately 30 to 37 kg of milk per day and milk discard represented about 70% of the total cost of CM (Rodrigues and Ruegg, 2005; Pinzón-Sánchez et al., 2011). In our study, the treatment protocol most used was administration of IMM products only, and milk discard for these cases represented about 90% of the total cost of treatment. Costs of treating mastitis were greatest during periods of highest milk production and each day of treatment beyond label recommended durations results in additional cost with questionable impacts on efficacy. Our results demonstrated that the costs of treatment for cows

producing around 25 kg of milk per day was approximately $62 while for cows producing about 50kg of milk per day the cost per treatment was $174. To make informed treatment decisions, it is important for veterinarians and farmers to understand the impact of extended durations on costs of therapy.

As compared to a national level report (USDA–APHIS–VS–CEAH–NAHMS, 2018) and Oliveira and Ruegg, (2014), the proportion of cases of CM treated with antimicrobials was less than previously observed. Most cases were treated only with IMM antimicrobials (Oliveira and Ruegg, 2014; Aghamohammadiet al., 2018), and we may have underestimated the proportion of non-antimicrobial treated cases as 14 herds recorded 100% of their cases as treated and may not have recorded CM cases that did not receive treatments. In the U.S., only IMM antimicrobials are approved for treatment of mastitis, although extra-label usage of some systemically administered drugs are allowed under veterinary supervision (Ruegg, 2021). While about 60% of the herds enrolled in our study reported usage of INJ antimicrobials, only 4% of cases were treated using this route, and it is likely that these cases were more severe. Very few farms recorded severity in the electronic records, and we were unable to determine severity of most cases based on treatment protocol. Use of INJ antimicrobials for treatment of CM are often associated with severe cases (Oliveira and Ruegg, 2014), and although our data does not include severity score, it is likely that INJ treatments are linked with severe cases. Among IMM and INJ products reported in this study, ceftiofur was the primary IMM antimicrobial used and ampicillin was the primary INJ antimicrobial (Oliveira and Ruegg, 2014). Clinical mastitis cases had similar characteristics as previously described (Oliveira et al., (2013) and about half of the cases occurred during early to middle lactation. Older cows (parity ≥ 3) accounted for most of the cases, and as expected, based on greater milk yield in older cows, costs were approximately $40

and $30 greater for cows in parity ≥ 3rd as compared to cows in 1st and 2nd parity, respectively. Surprisingly, we also observed a reduction in cost per case based on recurrence of CM, and cases that recurred for a second and third time or more were approximately $20 and $42 cheaper than the cost of first case. Similar to difference in cost among parities, difference in cost based on recurrence could be explained by difference in milk production across DIM and milk production loss caused by CM.

Treatment decisions for CM should be performed based on etiology and take into consideration cow factors such as stage of lactation, parity, and history of previous clinical and subclinical mastitis (Ruegg, 2021). While duration of treatment is sometimes altered based on pathogen, there is little evidence in difference of efficacy among different IMM products or for most etiologies evidence to demonstrate benefit from extended duration of treatment (Ruegg, 2021; Kolar et al., 2021). We used the treatment duration indicated on product labels to compare differences in costs from that we observed. Treatments with IMM cephapirin had the greatest difference between label duration and usage observed in our study, and this result agrees with results from Oliveira and Ruegg, (2014), that shows that label compliance for duration of treatment was only followed in approximately 30% of mild and 6% of moderate CM cases treated with cephapirin.

CONCLUSION

Our results demonstrate variation in cost of treatment of CM among herds as well as differences in the proportion of cases treated with antimicrobials. Great variation in cost was associated with parity, recurrence of CM cases, DIM at occurrence of the case, and treatment protocol. Milk discard represented the greatest proportion of cost and CM cases treated for longer duration were associated with greater costs. Reducing the duration of treatment to the

approved label directives could results in both reduced antimicrobial usage and less losses associated with treatment of CM.

APPENDICES

Table 3.1. Characterization of clinical mastitis treatment on 37 dairy herds in Wisconsin. Data was collected from September to December 2017 and included all cases of clinical mastitis recorded in computerized records.

Farm	Cows (n)	Cases (n)	CM rate[1]	Milk withhold (days)[2]	Milk (kg/d)[3]	Injectable usage (%)[4]	Antimicrobial usage (%)[5]	Supportive usage (%)[6]	Cost per case (USD) Not Treated[7]	Treated[8]	Overall[9]
F01	2,781	1,967	71%	8.2	43	2%	43%	0%	$94.05	$311.09	$250.25
F02	469	180	38%	7.7	45	94%	96%	51%	$90.37	$220.76	$218.04
F03	598	11	2%	7.5	29	100%	100%	0%	–	$119.95	$119.95
F04	1,559	826	53%	8.6	41	–	100%	0%	–	$153.53	$153.53
F05	332	191	58%	5.9	50	10%	13%	7%	$110.79	$212.24	$151.71
F06	816	168	21%	6.8	44	1%	98%	0%	$61.73	$205.75	$204.71
F07	393	43	11%	8.5	40	–	100%	0%	–	$160.18	$160.18
F08	2,357	685	29%	8.3	40	1%	100%	0%	–	$197.18	$197.18
F09	2,382	733	31%	7.6	41	–	93%	0%	$77.96	$144.54	$141.04
F10	623	191	31%	6.0	47	–	42%	1%	$97.13	$153.38	$130.54
F11	1,197	429	36%	10.2	43	–	97%	0%	$62.28	$203.05	$199.60
F12	475	80	17%	8.7	43	–	100%	0%	–	$171.75	$171.75
F13	2,152	1,082	50%	8.6	42	8%	47%	1%	$108.81	$372.26	$312.24
F14	454	68	15%	9.8	43	4%	100%	0%	–	$195.67	$195.67
F15	734	147	20%	5.9	47	1%	37%	14%	$96.74	$156.94	$132.17
F16	604	53	9%	7.6	46	4%	100%	0%	–	$159.65	$159.65
F17	2,031	1,559	77%	5.4	36	< 1%	3%	0%	$76.01	$176.37	$118.13
F18	1,016	8	1%	5.0	45	–	0%	100%	$119.59		$119.59
F19	762	15	2%	9.6	54	–	100%	0%	–	$237.61	$237.61
F20	583	222	38%	9.0	46	–	95%	0%	$96.19	$202.96	$199.38
F21	3,070	1,950	64%	8.7	47	1%	92%	0%	$84.01	$224.18	$217.20
F22	5,005	2,331	47%	11.5	37	4%	100%	< 1%	$7.99	$225.41	$229.24
F23	1,160	430	37%	8.2	40	10%	100%	8%	–	$195.52	$195.52
F24	887	117	13%	8.2	39	2%	87%	35%	$86.78	$194.98	$187.03
F25	676	197	29%	9.0	38	28%	100%	0%	–	$183.96	$183.96
F26	581	133	23%	6.3	43	47%	95%	0%	$92.83	$170.11	$169.44
F27	586	230	39%	6.8	46	–	100%	0%	–	$178.39	$178.39
F28	954	740	78%	7.3	40	2%	100%	1%	$101.28	$256.66	$256.48
F29	598	168	28%	9.6	46	9%	100%	0%	–	$215.06	$215.06
F30	443	53	12%	10.7	39	26%	98%	0%	$73.61	$295.41	$293.58
F31	1,415	505	36%	10.7	44	1%	99%	< 1%	$97.39	$328.49	$337.25

Table 3.1. (cont'd).

F32	954	359	38%	6.8	39	21%	94%	0%	$80.25	$156.99	$155.17
F33	1,448	176	12%	6.8	43	1%	81%	0%	$88.51	$150.02	$143.63
F34	3,444	330	10%	7.3	43	—	55%	1%	$89.86	$175.62	$146.14
F35	1,189	260	22%	12.8	46	—	81%	1%	$88.40	$315.81	$281.29
F36	1,527	995	65%	8.2	41	7%	41%	< 1%	$94.00	$237.62	$173.88
F37	3,736	2,993	80%	8.9	37	—	40%	9%	$109.47	$264.79	$181.03
Mean	1,351	557	33%	8.2	43	4%	69%	2%	$87.44	$209.00	$192.36

[1]Clinical mastitis (CM) rate was calculated as the number of cases divided by the number of adult cows (dry and lactating cows). A CM case was defined as all cases that occurred within 14 d from the first event.

[2]Average days of milk out of tank and it was calculated as the sum of treatment days plus withholding days.

[3]Average milk production (kg) of CM cases obtained from monthly DHIA in period of 14 d before CM to 30 d after.

[4] Proportion of CM cases treated with injectable antimicrobials.

[5]Proportion of CM cases treated with any antimicrobial.

[6]Proportion of CM cases treated with supportive therapy.

[7]Average cost per case for CM events that did not receive antimicrobial treatment. Average cost per case was calculated by dividing the total cost with milk discard for a period of 5 d and supportive treatment (if any) by the frequency of non-treated cases.

[8]Average cost per case for CM events treated with antimicrobials (intramammary; injectable; or intramammary + injectable). Average cost per case was calculated by dividing the total cost with milk discard and any treatment (intramammary or injectable antimicrobials; supportive products) by the frequency of treated cases.

[9] Average cost per case for all CM cases. Average cost per case was calculated by dividing the total cost of cases treated and not treated with antimicrobials by the frequency all CM cases on farm.

Table 3.2. Characterization of the treatment practices of cases of clinical mastitis (n=20,625) obtained from 37 dairy herds in Wisconsin from September 2017 to December 2017.

		Cases		Milk Withhold (days)				Cost per case (USD)			
Treatment options	Herds (n)	n	%	Mean	SE	Minimum	Maximum	LSM[1]	SEM	Minimum	Maximum
Intramammary only	35	13,155	64%	9.3	0.35	6.5	14.7	$184.93[a]	$7.76	$126.97	$320.12
Injectable only	9	77	< 1%	5.9	0.43	5.0	8.8	$130.25[b]	$14.17	$82.63	$193.09
Supportive only	12	241	1%	4.9	0.23	2.5	5.5	$82.89	$12.41	$7.99	$130.39
Intramammary & Injectable	22	650	3%	9.1	0.58	6.1	18.5	$206.70	$9.47	$96.54	$400.85
Intramammary & Supportive	10	184	1%	10.2	1.24	2.5	16.1	$198.51[a]	$13.47	$180.28	$388.74
Injectable & Supportive	1	15	< 1%	6.6	–	6.6	6.6	$162.67	–	$162.67	$162.67
Intramammary, Injectable, & Supportive	3	71	< 1%	11.5	2.48	7.4	16.0	$240.03	$30.73	$207.75	$301.47
Not treated	22	6,232	30%	5.3	0.14	5.0	8.0	$79.01	$9.47	$61.73	$110.54

[a,b] Least square mean values within the same column with different superscripts differ from each other ($P < 0.01$).

[1]Treatment practices that occurred in ≤5 farms were not included in the analysis among means.

Table 3.3. Cost of treatment (USD) for first cases of clinical mastitis treated only with IMM products (n= 8,630) based on label directives of approved intramammary antimicrobials for clinical mastitis in the U.S. and estimated cost of treatment based on data records obtained from computerized records from 37 dairy farms in Wisconsin from September to December 2017.

								Milk withholds (days)[2]		Cost per case (USD)			
		Cases			Label indication[1]			Observed		Label		Observed[3]	
Product	Herds (n)	(n)	%	Tube price	Treatment days	Daily frequency	Days of discard	Mean	SEM	Mean	SEM	LSM	SEM
Amoxicillin	6	88	1%	$3.15	1.5	2	2.5	6.9	0.92	$77.50	$4.21	$151.15	$28.77
Ceftiofur hydrochloride	32	7368	82%	$3.77	2-8	1	3	8.0	0.24	$93.56	$1.22	$156.52	$4.61
Cephapirin sodium	13	846	9%	$2.78	1	2	4	8.1	0.40	$87.72	$3.21	$151.78	$11.05
Cloxacillin sodium	1	1	< 1%	$4.99	1.5	2	2	4.0	—	$77.61	—	$90.13	—
Hetacillin potassium	9	273	3%	$2.76	3	1	3	6.6	0.25	$120.25	$3.95	$132.87	$6.63
Pirlimycin hydrochloride	12	363	4%	$3.90	2-8	1	1.5	6.1	0.68	$66.93	$4.82	$118.34	$13.57

[1]Label indications were obtained from the U.S. National Library of Medicine (DailyMed (nih.gov) and a minimum of 2 d of treatment was used for ceftiofur and pirlimycin products.

[2]Milk withhold days is the sum of the number of days actually treated plus the label directed withholding period (for example, amoxicillin was treated for 4.5 days and withheld for 2.5).

[3]Statistical analysis to assess difference in cost per treatment among intramammary products was performed only in products that were used in ≥ 5 farms and no difference in least square means was observed (P= 0.12). Figure 1. Average cost of milk discard and drugs per case (USD) for clinical mastitis cases by farm (n = 37). Clinical mastitis cases (n = Wisconsin dairy farms from September 2017 to December 2017.

Figure 3.1. Average cost per case (USD) for clinical mastitis cases treated and not with antimicrobials. Clinical mastitis cases were obtained from 37 Wisconsin dairy farms from September 2017 to December 2017.

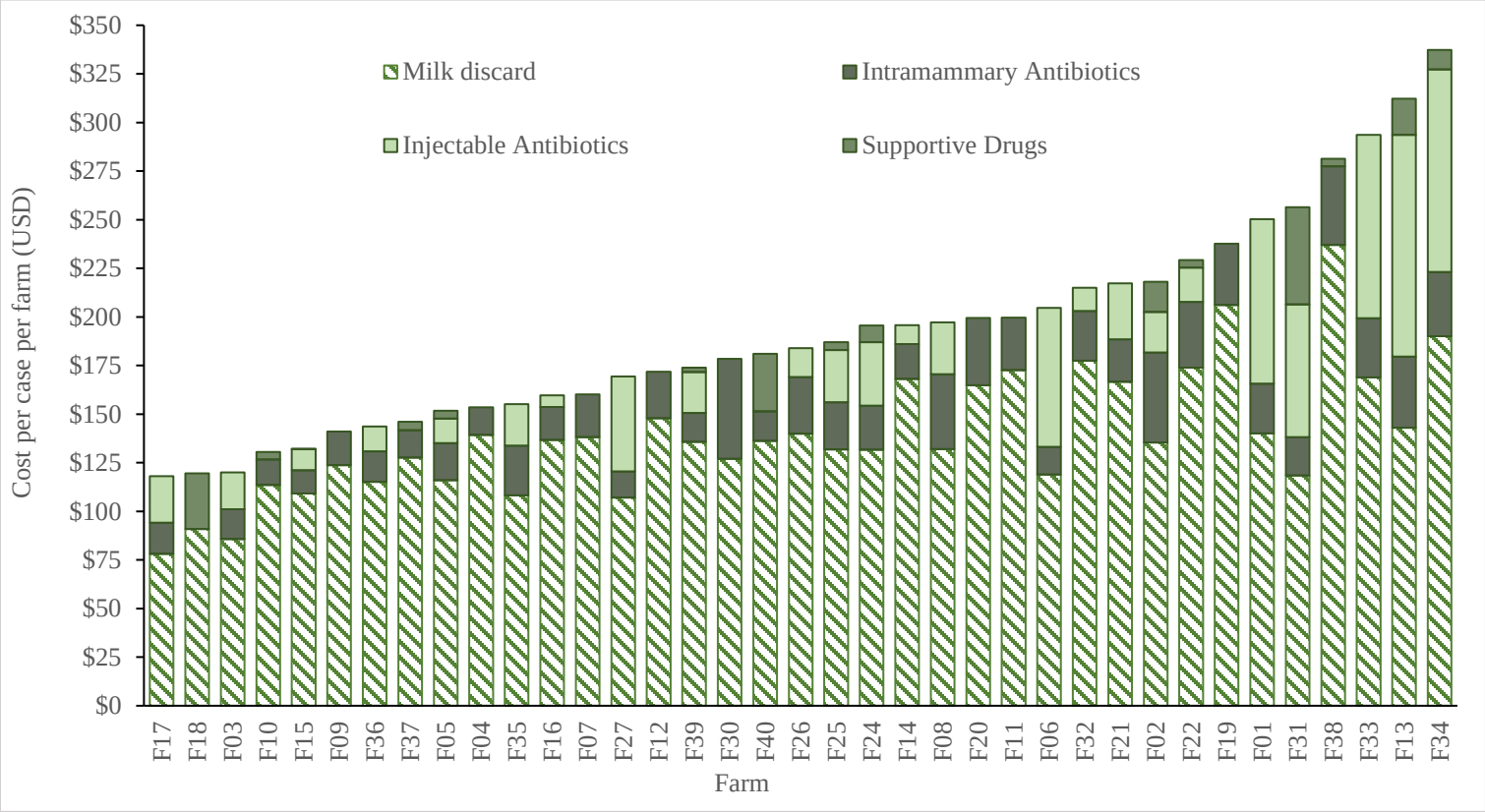
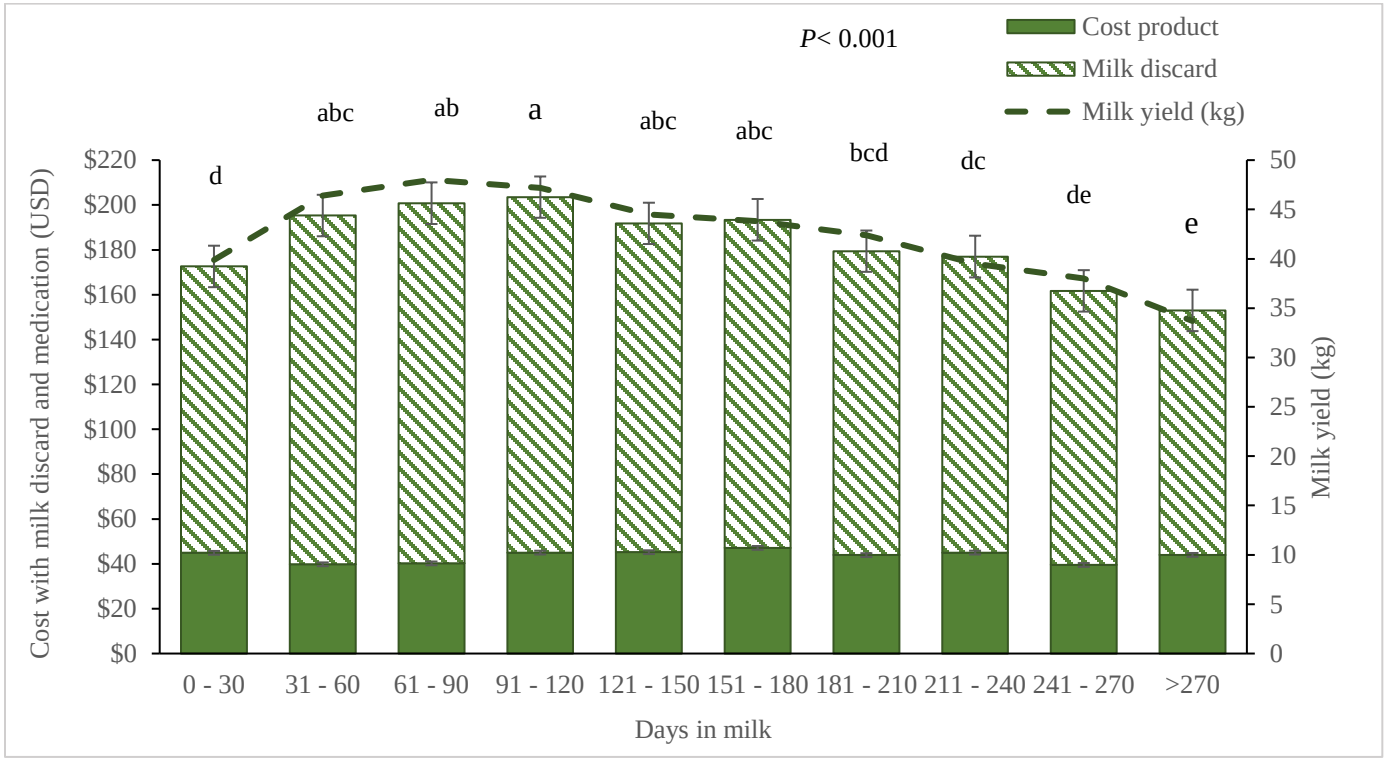


Figure 3.2. Difference in cost (USD) with milk discard and medication for treatment of clinical mastitis by milk yield (kg) across DIM.



[a,b] Least square mean values within DIM with different superscripts differ from each other (P < 0.001)

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CHAPTER 4. SHORT COMMUNICATION: VARIATION IN DIRECT COSTS OF DRY COW THERAPY ON 37 LARGE DAIRY HERDS

ABSTRACT

The objective of this study was to estimate direct treatment costs of dry cow therapy **(DCT)** and estimate potential monetary savings and reduction in antimicrobial usage if selective DCT was used on 37 large dairy herds in Wisconsin. Direct costs DCT were calculated for each herd based on actual costs associated with intramammary **(IMM)** antimicrobials and teat sealant. Data was retrospectively collected on 37 large dairy farms for a period of 1 year and included the total number of cows dried-off, milk yield, clinical mastitis history, monthly somatic cell count **(SCC)**, usage and purchase records of antimicrobials. An absence of clinical mastitis **(CM)** and SCC history <200,000 cells/mL for the lactation prior to dry-off were used to identify cows that would have been eligible to be treated only with internal sealant rather than IMM antibiotics at dry-off. Descriptive statistics were performed using PROC MEANS to summarize continuous herd and cow characteristics. Differences in costs of DCT among IMM products and eligibility to not receive antibiotics by parity were analyzed using ANOVA. Differences in milk yield at the last test date and based on DIM at dry-off were analyzed using PROC MIXED. A total of 35,691 cows were dried-off and all herds used IMM antimicrobials for DCT for most cows, except for one farm that used only internal sealant in 58% of cows. Teat sealant was used at dry-off in 34 of 37 herds. Of enrolled farms, 30 herds used antimicrobial DCT in all quarters of all cows and 7 herds used selective DCT to dry-off between 0.8% to 58% of the cows within herd. The average costs of DCT per dried cow was $19.57 ± 0.64 and ranged from $8.72 to $24.04. Cows dried-off with high-cost products had higher cost per dried cow ($23.45 ± 0.38) as compared to herds that used low-cost products ($16.64 ± 0.40). Eligibility to receive only internal sealant ranged from 27.3% to 93.3% within-herds and varied by parity, milk yield at last test day, and DIM at dry-off (P< 0.001). We estimated that based on a simple history-based algorithm, if selective DCT were

used in eligible cows on all farms, an average reduction of $5.37 would be expected per cow at dry-off. Likewise, use of selective DCT would reduce antimicrobial usage approximately 51%. Variations in costs at dry-off were observed among herds based on treatment protocols. Usage of selective DCT based on cow history could be used as an integral approach to reduce antimicrobial usage on herds and would reduce costs of mastitis control.

INTRODUCTION

Use of intramammary **(IMM)** antimicrobials at dry-off is a highly adopted udder health practice on U.S. dairy farms and is the standard protocol for treatment of subclinical intramammary infections **(IMI)** that have been acquired during the lactating period as well as prevention of new IMI during the early dry period (USDA, 2014). Blanket dry cow therapy (BDCT) refers to administration of IMM antimicrobials in all quarters of all cows at dry-off and has been an integral practice used to improve productivity and welfare of cows in subsequent lactation. The need for BDCT has been debated for years (Ruegg, 2017) based on emphasis on reducing antimicrobial usage to avoid accelerating antimicrobial resistance (CDC, 2017). Blanket dry cow therapy was adopted about 50 years ago due to high prevalence of IMI and lack of a screening tests to identify cows that would benefit from use of IMI antibiotics (Natzke, 1971). With improvements in diagnostics and udder health management practices, as well as widespread usage of internal teat sealants, selective dry cow therapy **(SDCT)** programs that emphasize usage of antibiotics only to treat existing IMI, while using internal teat sealants to prevent new IMI have been increasingly implemented with the overall objective of promoting conscious usage of antimicrobials in the dairy industry.

Recent clinical trials evaluating effects of SDCT on antimicrobial usage and udder health across the dry period and subsequent lactation have demonstrated promising outcomes (Rowe et

al., 2020; McDougall et al., 2022). Stochastic modeling of economic impacts of SDCT have shown positive outcomes for dairy farmers. Rowe et al., 2020 evaluated use of a simple history-based algorithm to select cows for administration of antibiotics (Rowe et al., 2020). Cows that had at least 1 case of clinical mastitis **(CM)** or at least 1 SCC >200,000 cells/mL were given IMM antimicrobials at dry-off while cows that did not meet those criteria received only internal sealants. Use of those criteria resulted in a 55% reduction in antimicrobial usage without negatively affecting udder health in subsequent lactation. Economic benefits of reduced antimicrobial usage were also estimated to be approximately $7.85 per cow at dry-off (Rowe et al., 2021).

Most antimicrobial usage on dairy farms is related to IMM products given at dry-off or for treatment of CM (Leite de Campos et al., 2021). On many herds, use of SDCT programs can result in reduced AMU and potential economic benefits. The purpose of this study was to estimate direct costs of antimicrobials given at dry-off for 37 WI dairy herds and estimate potential monetary savings and reduced antimicrobial usage if a simple SDCT program was adopted. We hypothesized that in this population of well managed dairy herds, considerable monetary savings and reductions in AMU could be achieved by implementing SDCT.

MATERIAL AND METHODS

Direct cost of BDCT and SDCT were calculated at cow-level and were estimated in a retrospective, observational study performed using data from 37 large dairy farms in Wisconsin. Direct costs of DCT were estimated based on animal health records obtained from a computerized herd management software (Dairy Comp 305; Valley Agricultural Software, Tulare, CA). Costs of drugs were estimated based on herd drug purchase records for IMM antimicrobials (if any) and teat sealant product (if any). Labor costs were not included as these

costs are not accessible in dairy records and would have been evenly distributed among cases and herds. Herds were selected based on size (≥ 250 lactating cows), availability of animal health records, and usage of a common dairy management software (Leite de Campos et al., 2021, Leite de Campos et al., 2022). Farms were visited once from September 2017 to December 2017 and animal health managers were surveyed about herd characteristics and treatment protocols used at dry-off. Data exported from Dairy Comp 305 included the number of cows dried-off, treatment records, monthly DHIA milk yield and SCC, and CM history for a period of one year. Items were created in Dairy Comp 305 to obtain SCC and milk yield results from test days and exported using the following command: EVENTS\2S365I ID BDAT LACT ITEM1 ITEM2 ITEM3 FOR LACT>0. When records did not specify the protocol used, that information was estimated based on the response obtained from the survey.

Costs of IMM products were estimated for each product and were calculated based on the number of IMM infusions used per cow. Costs of each product were calculated based on an average of actual costs obtained using drug purchase records from enrolled farms. Among farms, 6 different IMM products approved for use at dry-off, one IMM antimicrobial approved for lactating cows, and one internal teat sealant were reported to be used. The average price per IMM infusion for DCT was $3.86 for ceftiofur product, $2.18 for cephapirin product, $2.18 for cloxacillin, $2.50 for Pen G-dihydrostreptomycin combination product, $3.19 for Pen G-novobiocin combination product, $2.78 for cephapirin approved for use in lactating cows, and $2.15 for IMM infusion of internal teat sealant.

Potential monetary savings and differences in total antimicrobial usage among farms were estimated based on adoption of SDCT at dry-off. Cows were classified as eligible to receive only internal teat sealant (no IMM antibiotics) when they did not have CM or any test day SCC ≥

200,000 cells/mL across the entire lactation before dry-off. Antimicrobial usage was calculated using Animal Daily Doses **(ADD)** and detailed description can be found in (Leite de Campos et al., 2021). In brief, a standard ADD was calculated for each IMM antimicrobial product and each DCT tube was defined as 1 ADD (Stevens et al., 2016).

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). PROC MEANS was used to summarize characteristics of cows at dry-off (number of cows, DIM, SCC, and milk yield), costs based on treatment protocols and IMM products used at dry-off and describe difference in AMU based on adoption of SDCT program. PROC FREQ was used to summarize differences in eligibility to not receive antimicrobials for parities (1, 2, and ≥ 3) distribution.

Normality of outcome variables was assessed using PROC UNIVARIATE. Differences in proportions of cows eligible for internal teat sealants only based on parity were analyzed using ANOVA. Differences in DIM based on eligibility (yes or no) were analyzed using PROC MIXED. Differences in on milk yield at last test date before dry-off based on eligibility were analyzed using PROC MIXED and included parity as a covariate. Differences in costs per dried cow based on IMM products and among herds that used BDCT solely and herds that utilized SDCT were analyzed using ANOVA. Farm was included as random effect in all models and differences among least square means were adjusted using Tukey. Statistical analyses among herds were performed only for products used on ≥ 5 herds. Statistical difference was considered when $P < 0.05$.

RESULTS AND DISCUSSION

Selective DCT has been adopted as an option to reduce AMU on farms by identifying cows with IMI at quarter level or based on SCC records (Østerås et al., 1999). As part of the objective of this study, we aimed to compare potential monetary savings and reduction in AMU using animal-health records to identify cows that did not require use of antimicrobials at dry-off and received only internal teat sealants. Herds enrolled in this study had similar characteristics to herds evaluated by Rowe et al., (2020) and are representative of large conventional dairy herds in the Upper Midwest. Herd characteristics have been previously described (Leite de Campos et al., 2021). Enrolled farms (n= 37 herds) included 35,691 cows (mean = 965 ± 131 cows) and were dried-off with an average of 338 ± 2 DIM. Daily milk yield and SCC at the last DHI test day before dry-off were 31.5 ± 0.71 kg of milk per day and 226,110 ± 14,640 cells/mL, respectively (Table 1). Blanket DCT is commonly used by dairy farm in the U.S., and the proportion of herds using BDCT has been reported to be greater for large dairy herds (96.4%) as compared small and medium sized farms (about 82%) (USDA–APHIS–VS–CEAH–NAHMS, 2014). In our study, IMM antimicrobial DCT was used on all farms and the most common treatment protocol used at dry-off was administration of IMM DCT and teat sealant in all quarter of all cows (n= 27 herds), followed by usage of SDCT and teat sealant (n= 7 herds) and BDCT without internal teat sealant (n= 3 herds). Across farms, 89.6% (n= 31,994 on 34 farms) of all cows were dried off using IMM DCT and teat sealant, 6.3% (n= 2,239 cows on 7 farms) were dried-off using teat sealant solely, and 4.1% (n = 1,458 cows on 3 farms) of the cows were dried-off using solely IMM antibiotics.

Costs per dried cow treated with IMM antibiotics and teat sealant were $20.64 ± 0.50 and ranged from $17.32 to $24.04 per cow depending on the IMM product used. Costs per cow dried

treated using IMM antibiotics only were $12.11 ± 2.78 and ranged from $8.72 to $17.62 depending on the IMM product used. All herds used the same internal teat sealant and cost for cows treated with teat sealant only were $8.60 per cow. Costs per dried cow treated using IMM antibiotics and internal teat sealants were similar to estimates in previous studies that used fixed prices for IMM products (Rowe et al., 2020; Hommels et al., 2021). Different from these studies, we used purchase records to estimate costs related to dry-off and excluded labor costs as it was not recorded in electronic records and likely varied little among herds.

The overall observed costs per dried cow was $19.57 ± 0.64 and ranged from $8.72 to $24.04 (Table 1). The average cost per dried cow was $19.48 ± 0.72 for herds (n = 30) that used BDCT and $19.98 ± 1.56 for herds (n = 7) that used SDCT, and these costs did not differ (P= 0.76).

However, differences in costs per dried cow were found based on usage of different IMM antibiotics (P< 0.001). The costs per cow were greater for cows treated using IMM ceftiofur hydrochloride DCT ($23.45 ± 0.38) as compared to cows treated with Pen G- novobiocin combination DCT ($20.70 ± 0.42), Pen G-dihydrostreptomycin combination DCT $17.54 ± 0.41), cloxacillin DCT ($16.78 ± 0.48), or cephapirin DCT ($16.64 ± 0.40). Costs observed for herds using SDCT were slightly greater than costs observed for herds that used BDCT because those herds all used the highest cost DCT product (IMM ceftiofur) and internal teat sealants. In addition, there was a great variation in the percentage of cows dried without antimicrobials among SDCT herds. Herds with ≥20% of cows dried without antimicrobial DCT, the cost per dried cow was $17.59 ± 2.69, whereas herds with <20% dried without antimicrobial DCT, the cost per dried cow was $21.77 ± 1.55.

The average price per IMM antibiotic tube in BDCT herds was $2.92 ± 0.13, while the average price per IMM antibiotic tube on herds that used SDCT was $3.33 ± 0.30. Different

prices of IMM antibiotic were observed based on differing selection of antibiotics used by herd manager. If a standard product with an average price per IMM antibiotic tube ($2.72) was used for all IMM products, the costs per dried cow would have been $17.69 ± 0.88 for herds that used SDCT and $18.68 ± 0.44 for herds that used blanket DCT. Variation in costs were associated with different prices for higher and lower-cost products as well as prices associated with teat sealants given at dry-off. For example, using data obtained in this study, the approximate costs for a farm drying off 1,000 cows/yr using blanket DCT (all cows and all quarters) as well as a teat sealant would be $24,040 for herds using the highest cost product and $17,320 for herds using the lowest cost product.

We estimated that a similar proportion of cows would be eligible to not receive antibiotics as previously reported (Rowe et al., 2020). Based on an algorithm that used history of CM and SCC across lactation, 51% (n= 18,318) of cows included in this dataset would have been eligible to be treated using only internal sealants. The remaining 49% (n= 17,373) either had a CM event (n= 2,211 cows), or at least 1 monthly SCC ≥ 200,000 cell/mL (n= 9,929 cows), or both (n= 5,233 cows). For cows that were eligible to be dried off using only internal sealants the distribution of parities was 56.7% (1^{st}), 26.1%, (2^{nd}) and 17.9% (≥ 3^{rd}) lactation. Among cows that would need to receive IMM antibiotics in all quarters, the distribution of parities was 32.4% (1^{st}), 31.3% (2^{nd}), and 36.3% (3^{rd}) lactation. More 1^{st} parity cows were qualified to receive sealants only as compared to cows in second and ≥3^{rd} lactation (P< 0.001; Table 2). Cows that were eligible for internal sealant only, produced more milk at the last test day (33.7± 0.69 and 29.5 kg of milk per day) as compared to cows that required antibiotic therapy (29.5 ± 0.69 kg of milk per day) (P< 0.001; Table 2). Cows that did not require antibiotics were dried off slightly earlier in lactation (334 ± 2.2 DIM) as compared to cows that required IMM antibiotic DCT (343 ± 2.2 DIM; P<

0.001; Table 2). Previous studies have reported that cows in ≥ 3rd lactation often represents the greatest proportion of cows affected with CM (Green et al., 2002; Oliveira et al., 2013). Occurrence of clinical or subclinical mastitis has been associated with losses in pregnancy (Fuenzalida et al., 2015), therefore, increasing DIM. Likewise, lower milk yield should be expected from cows in the "not eligible" group as this group is composed of animals that either had clinical or subclinical mastitis (Huijps et al., 2008).

Antimicrobials are given to dairy cows for treatment or prevention of bacterial diseases and studies quantifying antimicrobial usage have reported similar amounts of antimicrobials administered for treatment of lactating cows, in small and large herds, across a gap of almost 15 years (Pol and Ruegg, 2007; Leite de Campos et al., 2021). When using a dose-based metric to quantify AMU, the largest share of doses are related to mastitis treatments or prevention. Overall, a total of 280.02 ADD (mean= 7.6 ± 0.16 ADD per 1,000-cow-d) were used for IMM DCT in these 37 herds. Based on the algorithm we used, if a SDCT program was implemented and only internal sealants were given to eligible cows, the number of ADD would have dropped to 139.44 ADD (mean= 3.8 ± 0.16 ADD per 1,000-cow-d) a 51% reduction. If selective DCT were implemented in these herds, the estimated costs per dried cow would be $14.20 ± 0.40 and range from $8.63 to $19.82 (among herds).

Costs could be reduced at dry-off by using accessible udder health records to select cows selecting cows eligible to selective DCT. Selective DCT would not only benefit dairy farmers with reduction in costs as would help dairy farmers complain with demands to reduce AMU as well.

APPENDICES

Table 4.1. Description of animal records (somatic cell count (cells/mL), milk yield (kg), days in milk) and total direct costs (USD) for cows before dry-off on 37 large dairy herds.

Farm	Last test day value before dry-off			Selective dry cow therapy usage (% of cows per herd)		Total costs per farm (USD)		Total cost per dry cow (USD)	
	SCC (cells/mL)[1]	Milk yield (kg)[1]	Average DIM at dry-off	Observed	Eligible[3]	Observed	Eligible	Observed	Eligible
F01	166,647	28.7	337	25.3%	52.6%	$35,985.12	$27,890.70	$19.60	$15.19
F02	118,477	35.6	347	0%	60.5%	$7,827.56	$4,814.84	$23.79	$14.63
F03	464,476	27.3	361	0%	36.0%	$7,464.92	$6,113.32	$17.32	$14.18
F04	194,613	32.0	351	2.2%	46.3%	$18,037.40	$13,834.36	$17.13	$13.14
F05	131,418	36.7	331	0%	55.4%	$2,490.00	$2,296.80	$10.00	$9.22
F06	194,216	31.4	338	0%	53.1%	$10,341.60	$7,391.60	$18.60	$13.29
F07	104,771	32.0	312	0%	71.8%	$7,764.92	$4,182.84	$24.04	$12.95
F08	340,672	28.5	338	0%	40.3%	$28,803.16	$22,952.04	$17.32	$13.80
F09	376,065	20.7	341	0.8%	31.1%	$38,714.56	$30,993.36	$23.46	$18.78
F10	294,489	36.2	340	0%	49.6%	$8,829.64	$6,198.48	$21.69	$15.23
F11	305,299	31.2	346	0%	44.4%	$19,970.40	$14,288.48	$24.00	$17.17
F12	186,260	34.1	329	0%	61.2%	$6,777.60	$4,547.60	$18.52	$12.43
F13	329,024	33.7	341	3.7%	40.9%	$31,754.12	$23,462.84	$23.47	$17.34
F14	213,469	32.5	360	0%	52.1%	$5,057.44	$3,732.00	$17.32	$12.78
F15	181,835	35.3	339	0%	58.2%	$12,463.88	$7,879.08	$23.38	$14.78
F16	210,933	32.3	354	0%	58.9%	$7,719.84	$5,502.80	$17.16	$12.23
F17	169,715	29.1	322	0%	47.6%	$24,958.12	$18,976.20	$17.32	$13.17
F18	170,829	44.5	327	0%	72.0%	$6,819.04	$6,751.48	$8.72	$8.63
F19	168,580	34.6	351	20.0%	59.8%	$11,491.44	$7,436.72	$20.89	$13.52
F20	160,508	33.4	322	0%	49.4%	$7,572.44	$5,647.88	$17.57	$13.10
F21	155,853	28.5	308	0%	50.8%	$57,864.28	$38,996.60	$24.04	$16.20
F22	224,833	25.6	338	0%	47.3%	$68,494.16	$51,279.44	$18.40	$13.78
F24	284,942	29.8	336	0%	47.8%	$16,284.16	$12,461.20	$18.26	$13.97
F25	195,743	30.4	346	1.5%	55.2%	$15,366.72	$10,046.96	$23.00	$15.04
F26	328,956	24.9	322	0%	40.4%	$8,679.60	$6,909.44	$17.29	$13.76
F27	125,745	31.0	355	0%	55.2%	$8,818.40	$6,117.60	$21.00	$14.57
F30	292,653	32.1	336	0%	38.8%	$6,287.16	$5,057.64	$17.32	$13.93
F31	343,615	28.6	343	0%	27.3%	$16,539.52	$13,636.80	$24.04	$19.82
F32	215,087	38.0	336	0%	47.6%	$9,904.48	$6,878.24	$24.04	$16.69
F33	291,684	32.1	363	0%	46.6%	$5,832.72	$4,310.92	$19.84	$14.66
F34	299,056	34.2	335	0%	36.0%	$23,751.52	$18,254.88	$24.04	$18.48
F35	202,928	28.3	331	0%	39.1%	$11,617.20	$9,330.52	$17.34	$13.93
F36	222,675	37.4	341	0%	66.7%	$21,137.00	$12,755.12	$21.20	$12.79
F37	46,375	29.0	323	58.0%	93.3%	$32,236.40	$23,263.52	$12.27	$8.85
F38	332,555	28.1	347	0%	45.2%	$18,529.24	$14,057.76	$19.53	$14.81
F39	127,027	29.0	325	0%	55.6%	$25,331.32	$16,283.48	$24.01	$15.43
F40	194,198	27.8	337	0%	46.7%	$43,455.88	$33,244.76	$17.32	$13.25
Mean	226,114	31.5	338	15.9%	50.8%	$18,674.94	$13,723.74	$19.57	$14.20

[1]SCC: Somatic cell count (cells/mL) obtained from the last test day before dry-off.
[2]Milk yield (kg) obtained from the last test day before dry-off.
[3]Refers to percent of cows that have no history of clinical mastitis and all monthly SCC tests <200,000 cells/mL in the previous lactation.

Table 4.2. Characteristics of eligible and not eligible cows to dry-off using selective dry cow therapy.

			Percentile				
Variables	LSM	Minimum	25th	50th	75th	Maximum	*P*-value
Days in milk at dry-off							< 0.001
Eligible	333.5	304.4	325.3	331.6	338.7	366.5	
Not eligible	342.7	310.7	335.2	342.7	353.1	365.8	
Parity							< 0.001
1 (eligible)	56.0%	42.3%	51.0%	54.2%	59.1%	77.6%	
2 (eligible)	26.1%	17.7%	22.9%	25.6%	29.1%	35.1%	
≥3 (eligible)	17.9%	4.70%	14.1%	17.9%	23.0%	27.5%	
1 (not eligible)	32.4%	20.4%	25.8%	33.3%	35.9%	49.7%	
2 (not eligible)	31.3%	18.7%	28.8%	31.3%	35.9%	40.9%	
≥3 (not eligible)	36.3%	22.0%	30.9%	35.7%	40.8%	52.7%	
Milk yield at last test day (kg per day)							< 0.001
Eligible	33.7	24.8	31.4	33.3	35.7	44.4	
Not eligible	29.6	18.9	26.7	29.1	32.5	44.8	
Somatic cell count at last test day (cells/mL)							< 0.001
Eligible	59,400	22,600	53,900	58,700	65,00	79,200	
Not eligible	383,600	86,000	292,500	365,300	498,500	681,800	

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CHAPTER 5. A PILOT RANDOMIZED CLINICAL TRIAL EVALUATING CLINICAL EFFECTS OF PEGBOVIGRASTIM ADMINISTERED TO DAIRY COWS AT DRY-OFF

ABSTRACT

The objective of this randomized clinical trial was to evaluate effects of an alternative dosing schedule for pegbovigrastim (**PEG**; Imrestor®, Elanco Animal Health, Greenfield, IN) on mammary gland health, rear udder width, milk leakage, and milk production of healthy dairy cows. Pregnant late lactation cows were randomly assigned to receive treatment with 15 mg of PEG (n = 10 cows) or a sham injection with saline (n = 10 cows) administered 7 d before dry-off and again on the day of dry-off **(DRY).** No antimicrobial therapy was administered at DRY. Quarter milk samples were collected at 8 periods (7 and 2 d before DRY, at DRY, 7 and 14 d after DRY, and 5, 10, and 14 d after calving) and used for bacteriological culture and to enumerate somatic cells **(SCC).** Mammary gland width was assessed at 9 periods (7, 1, and 2 d before DRY, at DRY, 1, 2, 4, 7, and 14 d after DRY). Milk leakage was observed from day 1 to 7 after DRY and again at day 14. Daily milk yields in the subsequent lactation were evaluated on days 5, 10, 14, 30, 60, and 120. Chi-square analysis was used to assess effects of treatment on incidence of intramammary infection **(IMI)** and milk leakage. Multivariable modeling was used to determine effects of treatment on SCC, milk yield, and mammary gland width. The incidence of IMI was greater for quarters of cows in the control group as compared to quarters of cows that received PEG (X^2= 6.1; P = 0.01). Compared to cows receiving PEG, the odds of new IMI were 5 times greater (95% CI:1.3, 19.7) for quarters of cows in the control group. While the overall effect of treatment on SCC was not significant (P = 0.23), significant effects of period and treatment by period interaction were found at day 2 before DRY and at DRY (P = 0.01). Similar to SCC, no effect of treatment was found for milk production (P = 0.20), but an effect of period and an interaction of treatment by period (P < 0.001) were identified at 30 DIM, 60 DIM, and 120 DIM. Rear udder width after dry-off was not affected by treatment, but an effect of period

was found ($P < 0.001$), with an increase in mammary gland width after DRY followed by reduction at day14. Milk leakage was observed for 4 cows in each treatment group ($P = 0.99$). In this pilot study, cows treated with PEG using an alternative dosing schedule had reduced incidence of IMI and an interaction of treatment by sampling period was observed for milk yield and further studies using this schedule are warranted.

INTRODUCTION

The dry period marks the beginning of a critical transition for dairy cows and is marked by several physiological and metabolic changes that help determine future productivity, profitability, and longevity of dairy cows (Drackley, 2004). Involution of the mammary gland subsequent to cessation of milking is an important physiological transition that consists of distinct periods defined by active and steady involution, formation of colostrum and initiation of lactation (Oliver and Sordillo, 1989). Increased risk of intramammary infection **(IMI)** at dry-off, occurs due to mammary glands producing considerable quantities of milk (Oliver and Sordillo, 1989), that might lead to milk leakage (Oliver and Sordillo, 1989; Rajala-Schultz et al., 2005; Silanikove et al., 2013). Within 70 h after cessation of milking, the mammary gland accumulates about 70 to 80% of typical daily milk production (Oliver and Sordillo, 1989).

In many dairy herds, 10 to 50% of mammary gland quarters have developed IMI by the end of a lactation (Green et al., 2005; Pantoja et al., 2009) and these infections are usually treated with antibiotics at dry-off . Blanket dry cow therapy (DCT; administration of antimicrobials to all quarters of all cows at dry-off) is used for treatment of infected quarters as well as prevention of new infections during the critical early dry-off period (Ruegg, 2017) and has been routinely recommended as part of a mastitis control program (Neave et al., 1969; NMC, 2020). Blanket DCT is widely adopted by U.S. dairy farmers (USDA–APHIS–VS–CEAH–NAHMS, 2014), but

contributes to increased use of antimicrobials on farms, often accounting for 11% to 48% of total antimicrobial usage (Pol and Ruegg, 2007; Saini et al., 2012; Stevens et al., 2016; Leite de Campos et al., 2021). As demonstrated by the continued reduction in bulk tank SCC observed in U.S. dairy herds, (APHIS (2019), the prevalence of subclinical mastitis has steadily declined, decreasing the need for blanket DCT and favoring the usage of non-antibiotic alternatives to prevent mastitis at dry-off.

Imrestor© (Elanco Animal Health, Greenfield, IN) is a commercially marketed two dose immunomodulator which is labeled to be given 7 d prior to calving and again at calving to enhance the immune system of dairy cows, with the overall objective of reducing the incidence of clinical mastitis during early postpartum period. Pegbovigrastim **(PEG)** is the active ingredient, which is a pegylated recombinant form of granulocyte colony stimulating factor which is a naturally occurring bovine cytokine, (Canning et al., 2017). This cytokine stimulates production and differentiation of neutrophils by progenitor cells in bone marrow (Canning et al., 2017). Neutrophils are the primary defense against IMI , but while an increase in circulating neutrophils has been consistently reported for animals treated with PEG (Canning et al., 2017; Ruiz et al., 2017; Zinicola et al., 2018), the apparent impact of this product on reducing clinical mastitis was not sufficient to maintain commercial sales in the U.S. and the product is not currently marketed. During the early dry period, polymorphonuclear cells and macrophages are the primary leukocytes in the mammary gland responsible for resorption of milk components and removal of degenerated epithelial cells, and to facilitate involution (Sordillo et al., 1987; Zhao et al., 2019). Altering the dosing schedule of this product to coincide with dry-off (rather than calving) may result in faster involution and reduced need for antimicrobials. The modified administration schedule of PEG at dry-off is an innovative approach that could reduce

antimicrobial usage while maintaining mammary gland health. We hypothesize that PEG may be an accelerator of mammary gland involution, and administration of this product at dry-off may decrease the risk of IMI in subsequent lactation. The objective of this pilot project was to generate preliminary data on the effects of an altered administration schedule of PEG on selected clinical measurements of mammary gland health in healthy cows during the early dry and subsequent post-partum periods.

MATERIAL AND METHODS

Study design and herd characteristics

A negatively controlled, randomized clinical trial was conducted at the Michigan State University Dairy Cattle Teaching and Research Center (East Lansing, MI) from November 2019 to April 2020. The dairy farm milked approximately 200 lactating cows that were housed in tie stall bedded with sawdust. Cows transitioned to maternity pen before calving and remained there until calving. Cows were milked twice daily in a milking parlor that included automated daily milk yield recording. Animal health records were recorded in computerized dairy software (DairyComp 305, Valley Agricultural Software, Tulare, CA). Gram-negative core antigen vaccines (Bovilis J-5, Merck, Kenilworth, NJ) were given to cows. Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (#202100019)

Enrollment criteria

Pregnant late-lactation Holstein dairy cows (n = 20) that were finishing their 1^{st} to 4^{th} lactations and had body condition scores between 3.0 - 4.0 were enrolled based on absence of clinical mastitis 30 days prior to enrollment and current composite SCC <200,000 cells/mL. Due

to the high prevalence of bovine leukemia virus in this herd, seropositive cows were enrolled only if a blood count demonstrated < 10,000 lymphocytes/mL at the time of enrollment.

Intervention

Cows were blocked by parity (1, 2, and ≥ 3), BLV status (seronegative, seropositive), and milk yield (Low ≤ Median ME305; High > median ME305) using PROC PLAN in SAS (SAS Institute Inc., Cary, NC). High producing cows were defined based on milk yield greater than median milk production of all cows enrolled in the study. Treatments were administered in the morning after collection of milk samples. Cows assigned to the treatment group received 15 mg of PEG (Imrestor©) 7 days before dry-off and a second dose on the day of dry-off. Cows assigned to the control group received a sham injection of saline using the same schedule. All treatments were administered by one researcher (JG) and other researchers responsible for sample collection and analysis were blinded until initial data analysis was completed. Cows were dried off using only an iodine post-dip, and no intramammary antimicrobials or internal teat sealants were given at dry-off (to allow for sampling). An external teat sealant was applied to all quarters after the last dry period milk sample was collected 14 days after dry-off.

Collection of clinical data

Rear udder width & Milk leakage. Physical changes in mammary gland dimensions were assessed once after morning milking by measuring the distance between marks made on the rear left and right mammary gland similar to Larsen et al. (2021). Quarters were marked with a permanent marker on the center of each rear quarter and paints were reinforced as necessary. Manual measurements were taken from the middle rear right quarter to the middle rear left quarter following the curve of the udder (Figure 1). Data were collected at defined intervals before dry-off, at dry-off and after dry-off (Figure 2). After dry-off, mammary glands were

observed for milk leakage twice daily (morning and afternoon) with approximately 12 h between observations by two researchers (JLC and JS; Figure 2).

Milk samples. Quarter milk samples were collected in the morning on days 7 and 2 before dry-off **(BDRY)**, at dry-off **(DRY)**, at days 7 and 14 after drying off **(ADRY)**, and days 5, 10, and 14 after calving **(ACALV)** and used for microbiological culture and determination of SCC (Figure 2). Milk samples were collected either during the first milking of the day (7 BDRY, 2 BDRY, at DRY, 5 ACALV, 10 ACALV, and 14 ACALV) or at the dry cows pen (7 ADRY, 14 ADRY). Milk samples for microbiological analysis were aseptically collected following NMC guidelines (NMC, 2004) and immediately stored on ice until they were transported to the laboratory by study personnel. After each milk sampling during the dry period, an iodine post-dip was applied to all quarters and at day 14 ADRY, an external teat sealant was administered to all quarters. Milk samples were immediately frozen at -20C upon arrival at the laboratory.

Microbiological Analysis

Initial microbiological analysis was performed at Michigan State University following National Mastitis Council guidelines (NMC, 2017). Milk samples were thawed at room temperature and 10 μL of milk were plated onto one quarter of a trypticase soy agar plate that contained 5% blood agar and 0.1% esculin (Thermo Fisher Scientific, Kalamazoo, MI). An additional 10 μL of milk was inoculated onto one-quarter of a MacConkey agar plate (Laboratory for Udder Health, University of Minnesota, St. Paul, MN). Plates were incubated at 37ºC for 48 h. Microbiological diagnosis was defined at the quarter level and an IMI was defined as the isolation of $\geq$ 300 cfu/mL of identical colonies. Milk samples were considered contaminated when 3 or more different colony types were isolated from the same sample. After growth on primary agar, species level identification of colonies was performed using matrix-

assisted lased desorption/ionization time-of-flight mass spectrometry at the Michigan State University Veterinary Diagnostic Laboratory. A new IMI was defined as microbiologic identification of a different pathogen in the same quarter within 14 d or the first sample collected after calving. Recurrent IMI was defined as identification of the same pathogen in milk from the same quarter within 14 d or the first sample collected after calving. Clinical mastitis was defined as the presence of abnormal milk regardless of other clinical signs. Somatic cell count of quarter-milk samples was determined using a Direct Cell Counter (Delaval, Kansas City- Missouri).

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Somatic cell count was Log 10 transformed to achieve normality and PROC MEANS was used to generate descriptive statistics for parity, DIM, number of days dry, Log 10 SCC, and milk yield (kg). Univariate analyses were performed using PROC GLIMMIX to assess potential differences between treatment groups and parity, DIM, days dry, Log 10 SCC, and milk yield. Outcome variables were defined as incidence of IMI, Log 10 SCC, Udder width, and milk production. Fisher's Exact test and odds ratio were performed using PROC FREQ to assess difference in IMI between treatment groups. Quarters with IMI at day 7 BDRY were removed from the incidence calculation.

Quarter-level SCC values were averaged for each cow and assessed at the cow-level. The effect of treatment on SCC was determined using PROC MIXED with SCC as the dependent variable in a repeated measure analysis. Independent variables included treatment (control, PEG), time (2 d BDRY, at DRY, 7 and 14 d ADRY, and 5, 10, and 14 d ACAVL) and the interaction of treatment and time, cow (treatment) was included as a random effect. Somatic cell count values for day 7 BDRY was included as covariate for analysis of SCC as treatment was

administered after samples were collected and due to potential difference in Log 10 SCC between groups.

The effect of treatment on milk production was determined using PROC MIXED with milk production as the dependent variable in a repeated measure analysis. Independent variables included treatment (control; PEG), time (2 and 1 d BDRY, and 5, 10, 14, 30, 60, and 120 d ACAVL) and the interaction of treatment and time, with cow (treatment) as a random effect. Milk yield on day 7 BDRY was included as covariate as treatment was administered after samples were collected and due to potential difference in milk yield between treatment groups.

The percentage difference in udder width for each rear quarter was calculated by dividing the rear udder measurements taken at each period by the measurements at day 7 BDRY. The effect of treatment on change in udder width was determined using PROC MIXED with the percentage change as the dependent variable in a repeated measure analysis. Independent variables included treatment (control, PEG), time (2 and 1 d BDRY, DRY, 1, 2, 4, 7, and 14 d ADRY) and the interaction of treatment and time, with cow (treatment) as a random effect. The Bonferroni test for multiple comparisons was used to separate means for Log 10 SCC, milk production, and udder width.

RESULTS

Characteristics of cows

Lactating cows (n = 20) ending their 1st to 4th lactations were enrolled in this pilot study. The SCC and RHA of enrolled cows at their final monthly DHI test prior to dry-off were 92,750 ± 21,830 cells/mL and 13,078 ± 634 kg, respectively. Cows were dried at 323 ± 8.3 DIM and were dry for 58 ± 1.2 d. No differences in parity, DIM, number of days dry, SCC, or milk yield were found between treatment groups ($P > 0.22$; Table 1).

Incidence of intramammary infection

A total of 640 quarter milk samples were collected across 8 sampling periods. Of milk samples collected from cows in the control group (n = 320), 292 (91.3%) samples were culture negative, 4 (1.3%) were contaminated, and 24 (7.4%) were culture positive. Among culture positive samples, non-aureus staphylococci were most common pathogen, accounting for 75% (18 samples from 6 quarters of 4 cows) of IMI, followed by *Streptococcus s*pp. (4 samples from 4 quarters of 1 cow), *Aerococcus viridians* (1 samples from 1 quarter of 1 cow), and *Trueperella pyogenes* (1 sample from 1 quarter of 1 cow) (Table 2). Non-aureus staphylococci spp. were composed by *Staphylococcus haemolyticus, Staphylococcus chromogenes, and Staphylococcus xylosis.* Of these IMI, only one cow had clinical mastitis (caused by *Streptococcus* spp.) during the dry period and received dry cow therapy after the last pre-calving sample was collected. One quarter from a cow in the control group had an IMI caused by non-aureus staphylococci at day 7 BDRY and remained infected across all samplings. Nine quarters from 3 cows developed a new IMI during the dry period and three quarters from 2 cows developed a new IMI after calving.

Of milk samples collected from cows in the treatment group (n = 320), 297 (92.8%) samples were culture negative, 5 (1.6%) samples were contaminated, and 18 (5.6%) samples were culture positive. Among culture positive samples, non-aureus staphylococci were the most common isolated pathogen, accounting for 77.7% (14 samples from 4 quarters of 3 cows) of IMI, followed by *Staphylococcus aureus* (3 samples from 1 quarter of 1 cow) and *Aerococcus viridians* (1 sample from 1 quarter of 1 cow) (Table 2). Three quarters (3 cows) already had an IMI at day 7 BDRY. Of these IMI, two were caused by non-aureus staphylococci (2 quarter of 2 cow) and one (1 quarter of 1 cow) was caused by *Aerococcus viridians*. Only 1 quarter (1 cow)

developed a new IMI during the dry period and two quarters (2 cows) developed a new IMI caused after calving.

Quarters that had IMI at day 7 BDRY were not included in the analysis of new IMI, and the incidence of new IMI was greater for quarters in the control group (30.7%) than for quarters in the treatment group (8.1%) (X^2= 6.1; P= 0.01; Figure 3). Approximately 23% (9/39 quarters) of the quarters in the control group had a new case of IMI during the dry period (7 d ADRY and 14 d ADRY) as compared to none of the quarters in the PEG group. Across all sampling periods, as compared to mammary gland quarters in the treated group, quarters in the control group had 5 times (95% CI:1.3, 19.7) greater odds of IMI.

Somatic cell count, & palpation score

Least square mean (LSM) Log 10 SCC of milk samples collected across all sampling periods from cows in the control and treatment groups were 4.59 ± 0.05 and 4.68 ± 0.05 cells/mL, respectively and were not affected by treatment ($P = 0.23$). Log10 SCC varied by sampling period ($P < 0.001$) and was least at 14 DIM and greatest at day 7 ADRY (Figure 4). An interaction between treatment and sampling period was found ($P = 0.01$). Somatic cell count from cows treated with PEG were 13% greater on day 2 BDRY and 11% greater at DRY as compared to milk samples from cows in the control group ($P = 0.002$).

Changes in udder width were not affected by treatment ($P = 0.23$) but were affected by period ($P < 0.001$; Figure 5). In comparison to udder width at 7 BDRY, beginning at 2 d ADRY, udder width of control cows increased until d 7 while udder width of treated cows decreased. As compared to udder width at 7 BDRY, mammary glands width increased approximately 12% at day 1 ADRY and subsequently reduced approximately 16% at day 14 ADRY (Figure 5).

Milk production

Milk yield was associated with sampling period ($P < 0.001$) and increased during the early lactation observation period. Across all sampling periods, no difference in milk yield was observed based on treatment group (Control= 41.5 kg, PEG= 43.6 kg; $P = 0.20$), but there was a significant interaction between treatment and sampling period ($P = 0.001$; Figure 6).

DISCUSSION

It is well established that cows are most susceptible to new IMI during early dry and peripartum periods (Oliver and Sordillo, 1989), due to increased risk of milk leakage, impairment of neutrophils (Drackley, 2004), and persistence of IMI acquired during the previous lactation (Oliver and Sordillo, 1989; Pantoja et al., 2009; De Prado-Taranilla et al., 2020). Cows are more resistant to new IMI when the mammary gland reaches a steady involution phase (Oliver and Sordillo, 1989). Thus, acceleration of mammary gland involution could help reduce risks of IMI during the dry and subsequent peripartum periods. While this study did not directly measure mammary gland involution, effect of PEG administration on clinical measures of mammary gland health were evaluated. Administration of PEG is known to stimulate production and mobilization of neutrophils and may be a mechanism to speed mammary gland involution, therefore reducing the risk of IMI during this critical period. If effective, the novel approach of altering the administration schedule for PEG could reduce the need for administration of antimicrobials as well as providing a dosing schedule that coordinates with normal handling periods.

We evaluated mammary gland health based on the incidence of IMI during the dry period and after calving, as well as monitoring SCC during the same periods. Incidence of IMI was calculated at the quarter-level even though treatment was applied at cow-level, as quarters

become independently infected. Quarters infected at day 7 BDRY were removed from this analysis and only new cases were included in our analysis. Although the prevalence of existing IMI before treatment (day 7 BDRY) was greater for quarters in cows that received PEG, the incidence of new IMI was greater in control quarters as compared to quarters of cows that received PEG. Of IMI cases in quarters of cows in the control group, approximately 75% occurred during the dry period as compared to no new IMI during the dry period of cows that received PEG. In both groups, the greatest proportion of IMI were caused by non-aureus staphylococci, which are commonly identified during this period (Green et al., 2005; Pantoja et al., 2009). Neutrophils and macrophages are the primary cellular defense against intramammary infection, and the administration of PEG before dry-off might help prevent development of new IMI during the dry period and reduce the incidence of clinical mastitis cases post-calving. Some infections persisted into the subsequent lactation, which agrees with previous researchers who reported that the occurrence of subclinical or clinical mastitis in future lactations is influenced by IMI that persist from the previous lactation and by development of new IMI during the dry period (Pantoja et al., 2009). Even with promising results, due to the pilot nature of this study we enrolled only 10 cows per treatment, but our results provide preliminary indication of efficacy that should be confirmed using more animals and in multiple herds.

While the commercially available PEG product is labeled for administration prior to calving, the impact of PEG on mammary gland health when administered during lactation has been evaluated. Powell et al.,(2018), treated mid-lactation cows (n = 5; 141.3 ± 20.6 DIM) 7 d before challenging a single quarter with *Escherichia coli*. They reported that the concentration of neutrophils was almost three times greater than reported in studies performed during the peripartum period. In addition, they found that milk samples from cows treated with PEG had

reduced numbers of bacteria post-challenge and were protected from IMI. While we did not assess the number of neutrophils, similar to (Powell et al., 2018), the administration of PEG at dry-off may have resulted in greater concentration of neutrophils, thus providing additional resistance against IMI and explaining the difference in incidence of IMI between the treatment groups in this study.

The transition from lactation to involution is marked by changes in udder size, structure, function, and composition of mammary gland secretion. Among methods used to evaluate mammary gland involution, udder measurement is an indirect, but practical method that has been previously used to assess this period (Larsen et al., 2021). Larsen et al. (2021) measured changes in mammary gland dimensions to assess associations of reductions in feed intake, milking frequency, and cabergoline injection with dry-off. They reported that change in udder engorgement was effectively assessed when interventions were used to modify the speed of dry-off. While we did not observe an overall impact of treatment on udder width, we were able to detect differences in udder width among periods, indicating that the method of measurement was sufficient to detect physiological changes occurring during the early dry period.

After dry-off, increased SCC is expected as part of the normal physiological process of involution (Sordillo et al., 1987; Silanikove et al., 2013). Polymorphonuclear cells and macrophages are the primary leukocytes in the mammary gland. After cows are dried off, these cells play an important role in resorption of milk components and in removal of degenerated epithelial cells (Sordillo et al., 1987; Zhao et al., 2019). Thus, increased numbers of neutrophils stimulated by administration of PEG at dry-off may effectively speed mammary gland involution by facilitating removal of milk components and degenerate cells. Animals treated with PEG have been reported to maintain an increased number of neutrophils in blood relative to control animals

for more than 7 d after treatment (Canning et al., 2017; Zinicola et al., 2018). Based on physiological changed after cessation of milking, an increase in SCC is expected based on migration of neutrophils into the mammary gland. When assessing all sampling periods, it is likely that no effect of PEG administration was found for SCC, due to the expected duration of effect of treatment using PEG. When the same model was used to test if SCC was affected by treatment only on day 2 BDRY and at DRY (days more likely to have an effect of treatment based on mode of action of this product), an effect of treatment on SCC was found, and PEG cows had a greater SCC than control cows (data not shown).

Surprisingly, as compared to the control cows, cows treated with PEG experienced a gradual increase in milk yield in subsequent lactation that increased until 30 DIM and then persisted until 120 DIM. The significant interaction between treatment and sampling period was unexpected. After Bonferroni correction, we were unable to demonstrate differences in milk yield at individual periods, but as a pilot study, the data is intriguing and indicates that larger studies are potentially justified. Faster involution of milk secretory cells that result in enhanced productive capacity is a potential mechanism that warrants further investigation (Oliver and Sordillo, 1989; Capuco et al., 2003). Results from this pilot study indicate that use of an altered dosing schedule for PEG may warrant larger studies. Further studies that include more cows are necessary to understand the role of PEG on mammary gland cells renewal during dry period.

CONCLUSION

The labeled administration schedule for PEG is based on prevention of IMI by enhancing the innate immune system during the peripartum period. Preliminary data from this pilot study indicates potential for use of PEG at dry-off to prevent new IMI and potentially speed mammary gland involution. As compared to the label schedule, administration of PEG during this period

aligns with normal management schedules. In this pilot study, we observed that cows treated with PEG at dry-off had reduced incidence of IMI during dry period and after calving while maintaining low SCC and high milk yield in the subsequent lactation.

ACKNOWLEDGEMENTS

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APPENDICES

Table 5.1. Description of the study population (n=20) enrolled in a randomized clinical trail from November 2019 to January 2020.

	Control				Pegbovigrastim				
Variable	Mean	SE	Minimum	Maximum	Mean	SE	Minimum	Maximum	*P*-value
Parity	1.7	0.3	1.0	4.0	1.9	0.3	1.0	4.0	0.64
DIM	322.7	10.6	302.0	408.0	324.6	13.3	302.0	439.0	0.91
Days dry	58.9	1.8	45.0	65.0	56.0	1.4	49.0	63.0	0.22
Log 10 SCC[1]	4.7	0.1	4.1	5.3	4.9	0.2	4.1	5.6	0.33
Milk yield (kg)	12,910	866	9,459	16,968	13,246	971	9,364	19,682	0.80

Table 5.2. Quarter-level incidence of IMI for cows receiving pegbovigrastim[1] (n= 40 quarters) or saline (n= 40 quarters) group during follow up periods.

	Control									Pegbovigrastim								
Pathogen	Before dry-off		At dry-off	Dry period		After calving			Total	Before dry-off		At dry-off	Dry period		After calving			Total
	7	2	0	7	14	5	10	14		7	2	0	7	14	5	10	14	
No growth	39	38	39	33	31	36	38	38	292	37	38	38	36	34	39	38	37	297
Staphylococcus aureus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	3
Non- aureus staphylococci	1	1	1	4	4	3	2	2	18	2	2	2	2	3	–	1	2	14
Environmental streptococci	–	–	–	–	4	–	–	–	4	–	–	–	–	–	–	–	–	–
Aerococcus viridans	–	–	–	–	1	–	–	–	1	1	–	–	–	–	–	–	–	1
Trueperella pyogenes	–	–	–	–	–	1	–	–	1	–	–	–	–	–	–	–	–	–
Total IMI[2]	1	1	1	4	9	4	2	2	24	3	2	2	2	3	1	2	4	18
Contaminated[3]	–	1	–	3	–	–	–	–	4	–	–	–	2	3	–	–	–	5
Number of new cases (incidence[4])	–	–	–	3 (7.7%)	6 (15.4%)	3 (7.7%)	–	–	12 (30.8%)	–	–	1 (2.7%)	–	–	1 (2.7%)	–	1 (2.7%)	3 (8.1%)

[1]Imrestor©, Elanco Animal Health, Greenfield, IN.
[2]IMI: intramammary infection.
[3]Contaminated: presence of three or more colony types in the same sample.
[4]Incidence: number of new intramammary infections divided by the number of non-infected quarters. Intramammary infection identified at day 7 before dry-off was not included in the analysis.

Figure 5.1. Representation of the protocol used to manually measure the distance from rear mammary glands. Mammary glands were marked with a permanent paint and the dots were used as the reference points for all measurements across the study. The distance from the right rear quarter to the left rear quarter was measured using a ruler following the curve of the udder.

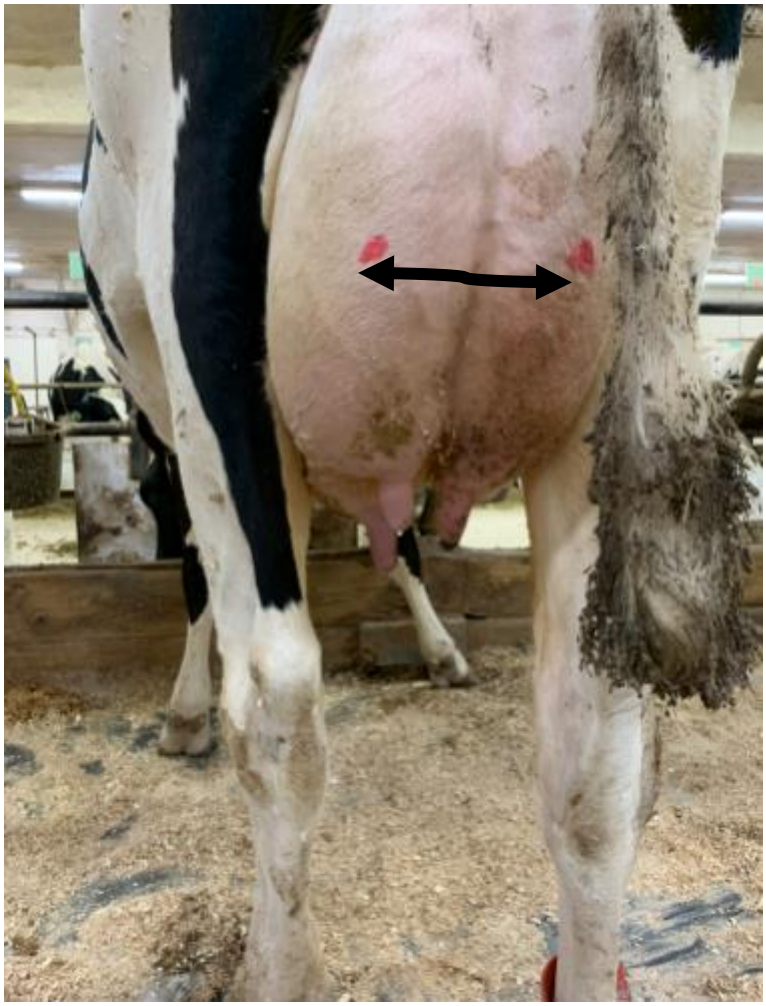


Figure 5.2. Sampling schedule for the outcomes of interest.

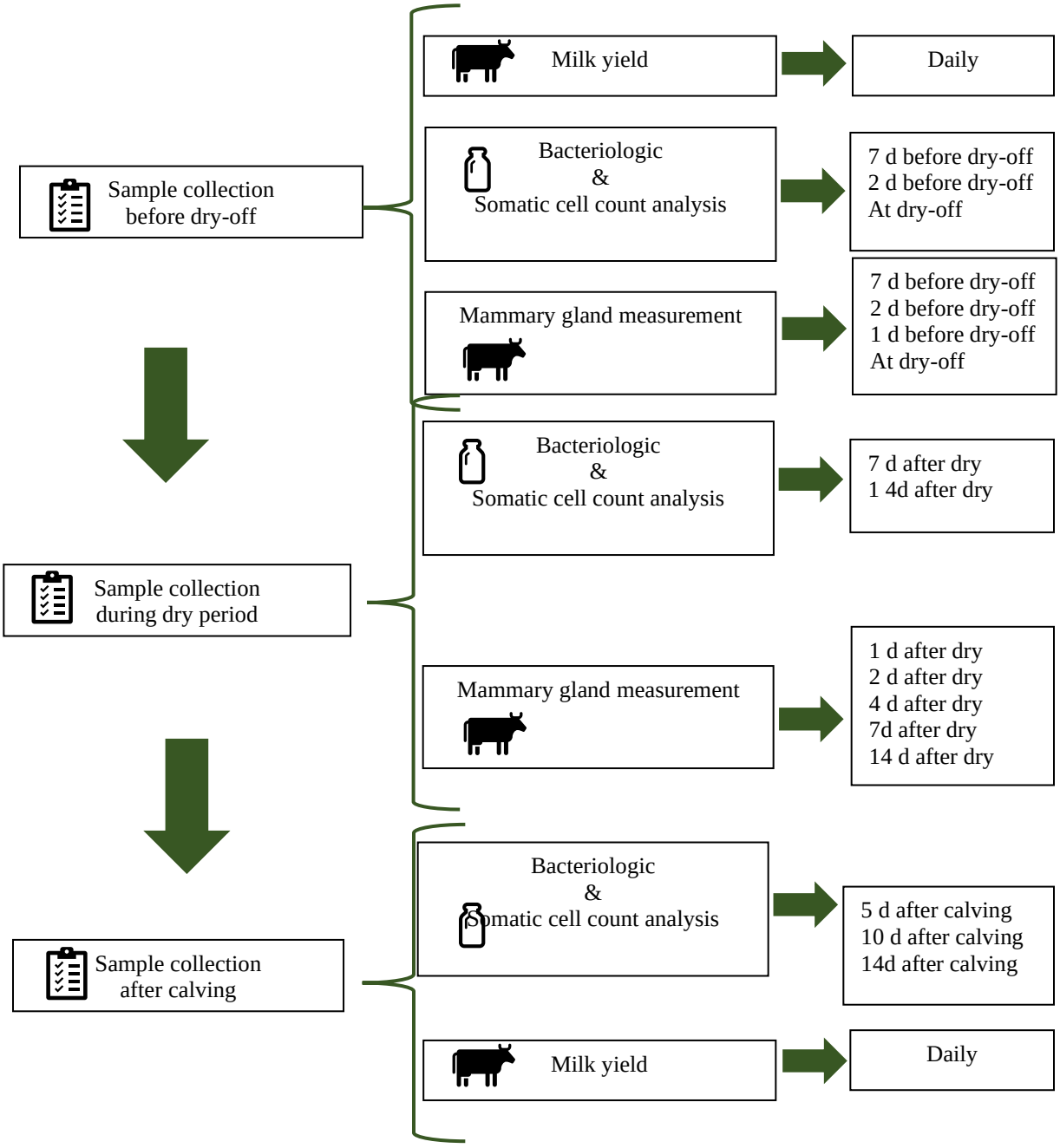
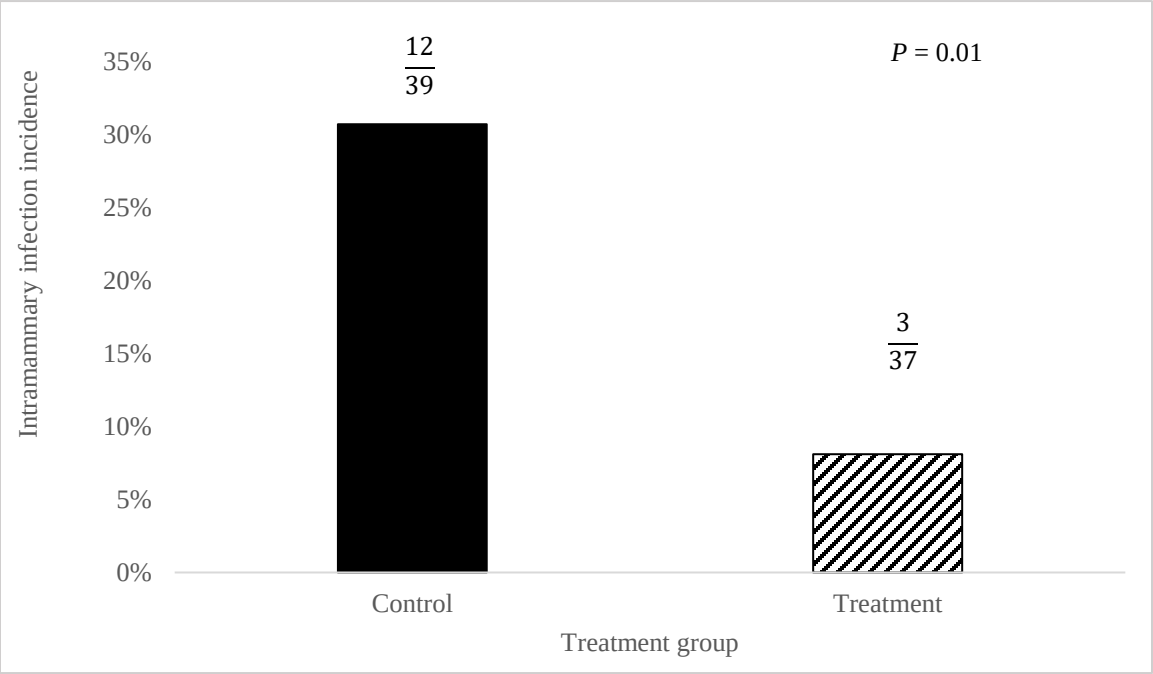
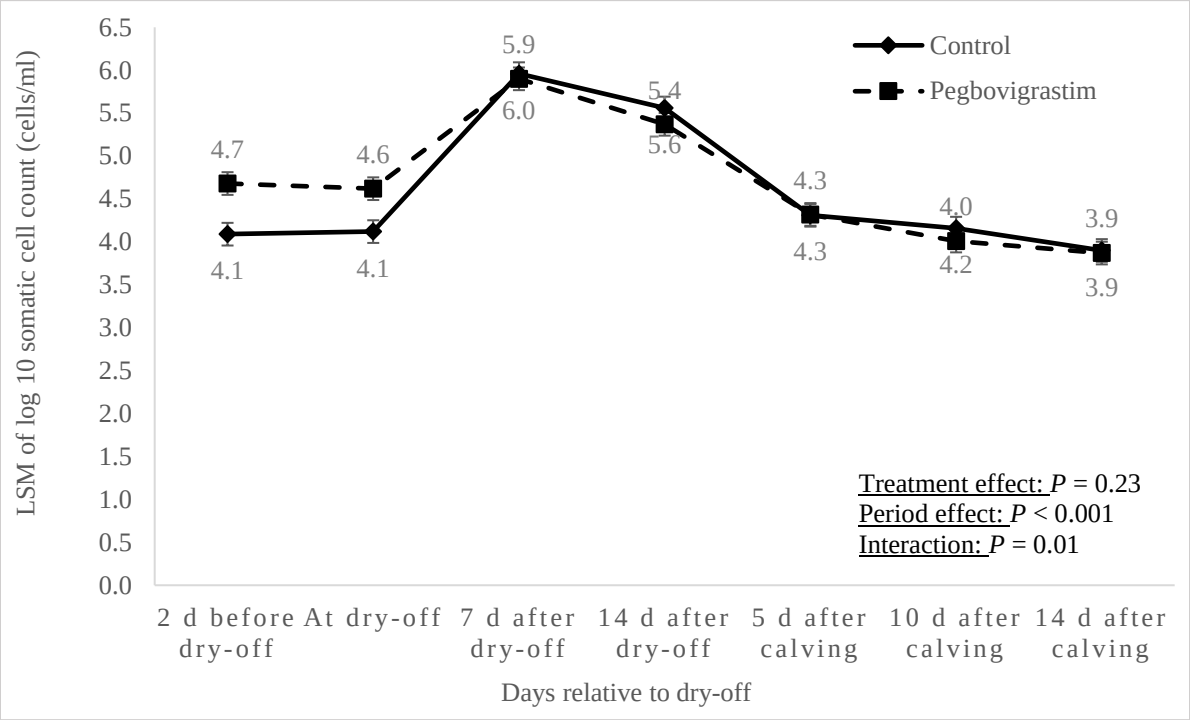


Figure 5.3. Intramammary infection incidence[1] across all periods (before dry-off, at dry-off, after dry-off, and after calving) for pegbovigrastim[2] (n= 37) and control (n= 39) group.[1]



[1]Intramammary infection incidence was calculated as the total number of new cases divided by the total number of quarters without intramammary infection across sampling periods. Intramammary infection identified at day 7 before dry-off was not included in the analysis.
[2]Imrestor©, Elanco Animal Health, Greenfield, IN.

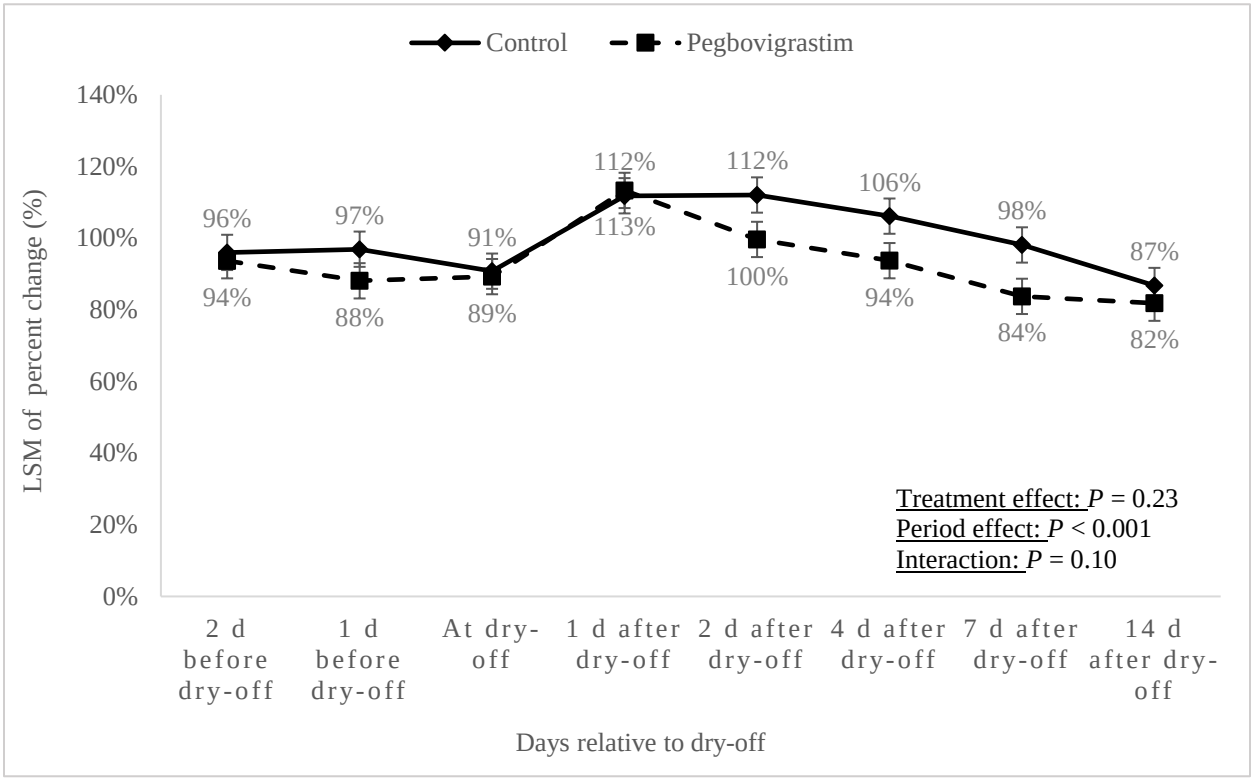
Figure 5.4. Effect of pegbovigrastim[1] administration on somatic cell count before dry-off (2 d before dry-off), at drying-off, during the dry period (7 and 14 d after dried-off), and after calving (5, 10, and 14 DIM) after administration on day 7 before dry-off and at dry-off on treatment (n=10) and control (n=10) group. Repeated measures analysis included the effect of treatment, period, and interaction between treatment and time.[2]



[1]Imrestor©, Elanco Animal Health, Greenfield, IN.

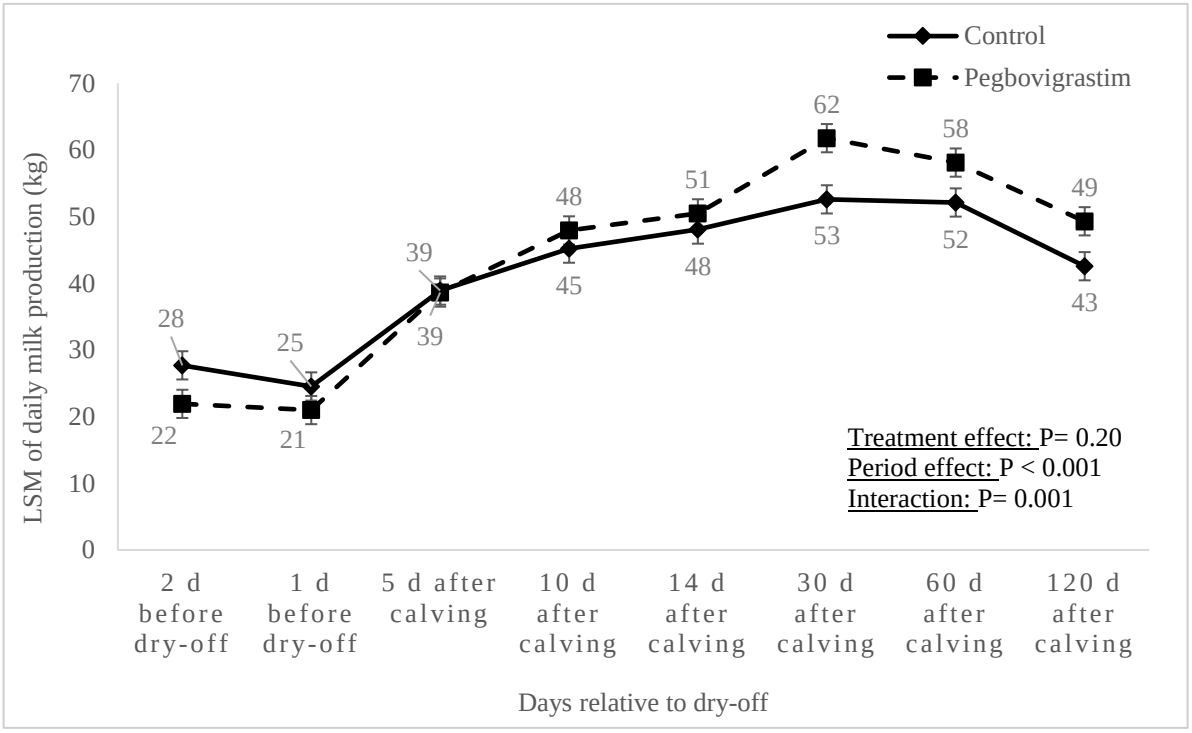
[2]Log 10 SCC from day 7 before dry-off was not included in the analysis as cows had not received treatment prior to sampling.

Figure 5.5. Effect of pegbovigrastim[1] administration on mammary gland width before dry-off (1 and 2 d before dry-off), at drying-off, and during the dry period (1, 2, 4, 7 and 14 d after dried-off) after administration on day 7 before dry-off and at dry-off on treatment (n=10) and control (n=10) group. The width of the rear quarters on day 7 before dry-off was used as a standard point to compare the width of the rear quarters on different periods relative to dry-off. Repeated measures analysis included the effect of treatment, period, and interaction between treatment and time.[2]



[1]Imrestor©, Elanco Animal Health, Indiana- US.
[2]Milk yield (kg) from day 7 before dry-off was not included in the analysis as cows had not received treatment prior to sampling.

Figure 5.6. Effect of pegbovigrastim[1] administration in milk production before dry-off (2 and 1 d before dry-off) and after calving (5, 10, 14, 30, 60, and 120 DIM) after administration at day 7 before dry-off and at dry-off on a treatment (n=10) and control (n=10) group. Repeated measures analysis included the effect of treatment, period, and interaction between treatment and time.



[1]Imrestor©, Elanco Animal Health, Indiana- US

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SUMMARY

Quantifying antimicrobial usage on farms is important to understand how antimicrobials are used and will help implement interventions that will reduce the use of antimicrobials. Reducing antimicrobial usage on farms could be achieved by reducing duration of treatment, identifying cows that would benefit from selective dry cow therapy, or boosting the immune system of cows during periods of increase susceptivity to mastitis. The objective of this dissertation was to explore protocols that aimed to reduce intramammary antimicrobials administration while maintaining udder health. To accomplish this aim, four studies were performed to characterize antimicrobial usage for udder health, compare costs associated with different treatment protocols in lactating and dry cows, and finally evaluate a non-antibiotic option at dry-off.

Chapter 2 described antimicrobial usage for adult cows and preweaned calves in 40 large dairy herds using both a dose-based and a mass-based metric. The results of this chapter demonstrate that great variation in antimicrobial usage was observed among herds and the main routes of antimicrobial usage were dependent on the metric used to quantify antimicrobials. When using a dose-based metric, antimicrobials administered as intramammary applications accounted the largest shares of doses, but when antimicrobials were measured using a mass-based metric, antimicrobials administered as injectable route accounted for the largest share of mass of antimicrobials used per kg of body weight.

Chapter 3 described direct costs associated with treatment of clinical mastitis in 37 of the original 40 herds and compared differences in costs if short-duration of treatments were used. Results from this study demonstrate difference in costs based on treatment protocols and that cost considerably increased with higher milk production. In addition, results from this study

demonstrated that considerable reduction of costs could be obtained by reducing duration of treatment.

Chapter 4 described direct costs at dry-off in 37 of the original 40 herds and compared differences in costs with usage of selective dry cow therapy. Results from this study demonstrate that half of the antimicrobial usage on farm could be reduced by implementing selective dry cow therapy. Costs at dry-off were associated with treatment protocol used and intramammary dry cow therapy product.

Chapter 5 evaluated the effect of an alternative dosing schedule for pegbovigrastim in udder health of cows during dry period. Results from this pilot study demonstrate promising outcomes in incidence of intramammary infection during the dry period and production in subsequent lactation. However, further studies with larger sample sizes are necessary to understand the effect of pegbovigrastim administration at dry-off.

Based on results from these studies, considerable usage of antimicrobials could be reduced by reducing duration of treatments and implementation of selective dry cow therapy. While further studies needed to understand the effects of pegbovigrastim at dry-off, promising results indicated that speeding mammary gland involution could be used to dry-off healthy dairy cows without affecting mammary gland health in subsequent lactation. Further studies should focus on exploring differences in antimicrobial usage among herds and on educational programs that show to dairy farmers economic benefits from reducing antimicrobial usage on farms.