# HERD-LEVEL VARIABLES ASSOCIATED WITH ANTIMICROBIAL USE IN DAIRY HERDS

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**ABSTRACT**

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The aim of this study was to identify variables associated with antimicrobial drug use for bovine mastitis. Intramammary tubes used for lactating cows, discarded by farm personnel, were collected in dedicated trash bins for 12 months on each of 104 herds (Michigan n = 49, Pennsylvania n = 55). Antimicrobial use was quantified using two metrics: arithmetic (number of discarded tubes) and defined daily dose (DDD) per 1,000 cow-days. Mastitis control practices, attitudes, and behaviors were also collected using a survey of herd owners and managers conducted during herd visits. Additionally, a human resources questionnaire survey about dairy producers/manager's beliefs and practices regarding employee communications, training, and education was also collected for each herd. Across all herds, intramammary tube use based on arithmetic count was 4.43 tubes per1,000 cow-days and 3.52 per 1,000 cow-days based on DDD. Multivariate models had similar results, regardless of the metric used to determine intramammary drug use; there was a positive correlation between drug use and the new subclinical mastitis rate (based on monthly DHI SCC testing) and the producer's willingness to improve the image of the dairy products. Use of natural remedies for mastitis treatment was negatively correlated with intramammary drug use Also, intramammary drug use is highly associated with the dynamics of subclinical mastitis as measured in individual cows, and a herd average level.

This thesis is dedicated to my family, especially my parents, as well as to my friends and advisor Ronald Erskine, who have all supported me unconditionally throughout this journey.

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INTRODUCTION

**Mastitis**

The United States is the second largest milk producer, accounting for 28% of world milk production, with a value of $38.8 billion (USDA, 2018). However, the current dairy industry faces several challenges, such as a sagging global economy, rising cost of production, labor issues, and low milk prices. Economic challenges also arise from mastitis, which despite widespread implementation of disease-control strategies, continues to be the costliest infectious disease in adult dairy cattle (Barkema et al., 2006; Thompson -Crispi et al, 2014).

Mastitis is defined as inflammation of the mammary gland and is caused by a broad spectrum of pathogens, which are predominately bacterial, although mycotic, algal, and viral agents have been identified. Infection causes damage to the epithelial cells, which decreases milk quality and production (Ruegg, 2017; Erskine and Ruegg, 2020). Mastitis is also associated with increased culling and health care expenses, such as treatment, discarded milk, and labor (Rollin et al., 2015). Mastitis may also pose a risk to public health because of potential zoonotic diseases and pathogen-derived toxins (Zadoks and Fitzpatrick, 2009).

Intramammary infections (IMI) are generally classified into two forms: contagious and environmental (Ruegg, 2017). Infected mammary glands are the reservoir of contagious IMI, with transmission occurring from the gland of an infected animal to a noninfected animal. Typical pathogens that are predominately transmitted by this means are S*taphylococcus aureus*, S*treptococcus agalactiae*, M*ycoplasma spp*, and C*orynebacterium bovis* (Barkema et al., 2006). Intramammary infections that are classified as environmental (e.g., the reservoir of infection is bedding, housing, pasture) include *E. coli, Klebsiella spp., Streptococcus uberis,* and numerous other pathogens, especially gram-negative organisms (Hogan, 2017). Currently, the most common bacteria isolated from bovine mastitis are staphylococcal species (Leitner et al., 2011; Erskine and Ruegg, 2020), which include both *S. aureus* and non-aureus staphylococci (Tremblay et al., 2013). However, pathogens that are classified as environmental can exhibit contagious transmission within herds such as *Prototheca bovis* (Klaas and Zadoks, 2017). Thus, mastitis control must include a comprehensive program to reduce microbial exposure to the teat, regardless of the reservoir of pathogens that are isolated from a herd.

Mastitis symptoms are often divided into two stages. The first stage, subclinical mastitis, is more prevalent and is the costliest type of mastitis (Seegers et al., 2003; González and Wilson, 2003). Subclinical mastitis is recognized by the presence of infection, without signs of either local or systemic inflammation. Detection can only be done with measures of milk inflammation, such as leukocytosis (somatic cell count; SCC), electroconductivity, diffusion of serum-based proteins, or from microbiological culture (Abebe et al., 2016).

Clinical mastitis, in contrast, is an inflammatory response to infection that results in visible change in the milk (fibrin clots, changes in color) or changes in the udder (e.g., pain, swelling, redness, edema). More pronounced cases may exhibit systemic signs, such as fever or anorexia (Erskine and Ruegg, 2020). Clinical mastitis can be graded according to a severity score: grade one is a mild case, causing abnormal milk with no other signs; grade two leads to abnormal milk, redness, and swelling of the affected quarter; and a score of three, often termed a severe case, includes abnormal milk, redness, and swelling of the quarter, as well as systemic signs. Severe clinical mastitis represents about 15% of clinical mastitis cases (Pinzón-Sánchez and Ruegg, 2011).

Typical signs of severe clinical mastitis are anoxia, fever, decreased milk production, tachycardia, and shock (Wenz et al., 2006). Severe clinical mastitis is most often caused by coliform bacteria (*E. coli* or *Klebsiella* sp.) and is responsible for a higher incidence of agalactia, culling, or death than other pathogens (Wenz et al., 2006).

Chronic mastitis is defined as a persistent IMI and generally presents as subclinical mastitis, although repeat episodes of clinical mastitis may occur (Erskine and Ruegg, 2020). With increased duration of infection, atrophy of the parenchyma of the udder may occur, with fibrosis and micro-abscess formation. This invariably leads to loss of gland function. (Radostits et al., 2007). Milk losses increase with increasing duration of infection, nearly doubling in the amount of lost income ($1.20/day) from the first month of infection to the tenth month of infection ($2.10/day; Hadrich et al., 2018).

**Mastitis Economics**

Mastitis is one of the costliest diseases in the dairy industry, for both producers and processors (Nielsen et al., 2010; Hogeveen et al., 2011). The calculation of the economic cost varies from region to region and country to country due to elements such as milk regulation standards between countries and market status (Halasa et al., 2007). However, several researchers have attempted to determine a standard strategy to deduce possible losses (Hertl et al., 2014). The fundamental consequences of this disease include production losses, culling, labor, cost of veterinary services, decreased milk quality, reduced cow longevity, and an increased risk factor for other diseases (Halasa et al., 2007; Janzen, 1970; Guimarães et al., 2017; Aghamohammadi et al., 2018).

According to Rollin et al. (2015), the estimated cost of each clinical mastitis case in early lactation is $444. Costs were divided into direct costs ($128/case)—which includes diagnosis, therapy, veterinary services, labor, non-salable milk, and death losses—and indirect costs ($316/case), which include future milk production losses, premature culling, replacement losses, and future reproductive losses (Rollin et al., 2015). A Canadian study similarly revealed that the median cost of clinical mastitis per case in Canada is CAD $662 (Aghamohammadi et al., 2018). In Sweden, the average cost of clinical mastitis is €278, or €60 (Nielsen et al., 2010) per cow in the herd. Hogeveen reported that the cost of clinical mastitis in the Netherlands ranged from around €61 to €97 per cow per farm (Hogeveen et al., 2011).

The impact of clinical mastitis on milk yield depends on the stage of lactation and parity, with higher losses early in lactation and after the second parity (Riekerink et al., 2007). The average loss for the first lactation ranged from 31 kg to 749 kg per case and the losses during later lactations ranged between 117 kg and 860 kg (Heikkilä et al., 2012).

The cost of subclinical mastitis has also been estimated. In a study of 38,150 test day observations from several states, lost milk yield production was about 0.04 kg/day, with each increase of about 1% of the natural log of the SCC (Holland et al., 2015). This model revealed that future lost milk production would be 28% of total economic losses.

Discarded milk from abnormal milk and treatment also affects farm productivity. This is especially the case for withheld milk to avoid drug residues (Nielsen et al., 2010).

Labor cost is a vital element that should also be calculated when considering the economic impact of mastitis on dairy farms. This cost is difficult to calculate or interpret because it differs from farm to farm, especially if the farm uses external labor sources (Halasa et al., 2007; Blosser, 1979). However, labor costs for the additional hours spent for the treatment mastitis, changing the units, discarding milk, segregation of affected cows, and extra washing of equipment are substantial. Rollin et al. (2015) estimated the labor cost to be about $21 per clinical case. In addition, the average cost for extra labor in Canadian herds due to clinical mastitis was estimated at CAD $657 per 100 cows per year (Aghamohammadi et al., 2018).

Culling is a high-cost impact of mastitis in the dairy farm industry (Petrovski et al., 2006; Aghamohammadi et al., 2018). Culling is a last-resort decision that can be made by the producer if sufficient replacements are available.

A New York study suggested that mastitis was the most frequent cause of culling, accounting for 14.5% of culled cows (Gröhn et al., 2003). Rollin et al. (2015) reported the cost of culling per case of clinical mastitis in the first 30 days of lactation was $32, and premature culling and replacement loss amounted to $182. Canadian research has suggested the cost of culling a first lactation cow from mastitis was CAD $1,350 (Aghamohammadi et al., 2018). In the case of subclinical mastitis, the average cost of culling from mastitis was CAD $6,743 and CAD $8,571 per 100 cows/year, for first lactation and older cows, respectively (Aghamohammadi et al., 2018).

Loss of milk quality is also associated with mastitis. Depending on markets, this lost quality can lead to decreased premiums or penalties related to bulk tank SCC (Seegers et al., 2003; Aghamohammadi et al., 2018). Decreased milk quality also negatively affects cheese yield and consumer satisfaction with dairy products (Seegers et al., 2003).

Mastitis may also affect fertility. Several studies have associated subclinical mastitis with a decreased frequency of estrus cycles, increased risk of embryonic loss, and reduced conception rates (Schrick et al., 2001 Chebel et al., 2004; Santos et al., 2004; Hudson et al., 2012).

Taken together, mastitis is a costly disease for the dairy industry and provides incentives for herd management that focus on prevention to increase herd productivity. Mastitis control requires a multifactorial approach that includes milking machine function and milking processes, housing and hygiene management, immunization, dry-cow therapy, genetic selection, nutrition, human resource management, monitoring of infections, and therapeutic decisions (De Vliegher et al., 2012; Ruegg, 2017).

**Mastitis Management**

Hygiene and housing management are critical factors in controlling IMI, especially with pathogens that typically have an environmental reservoir. Key to control of environmental mastitis is the quality and hygiene of the bedding (Green et al., 2007; McDougall et al., 2009). Inorganic bedding, such as sand, is especially effective at reducing exposure to coliform organisms, and thus the IMI caused by these organisms. Hogan and Smith (2012) concluded that control should focus during wet and hot periods of the season and periparturient cattle. Recommended practices include avoiding overstocking of cows; taking precautions to eliminate stagnant water around cows; maintaining clean, dry bedding; and reducing moisture and organic contamination.

Proper operation and maintenance of milking equipment, in addition to milking protocols that effectively reduce the exposure of teats to pathogens, are also critical for mastitis control (McDougall et al., 2009; Hovinen and Pyörälä, 2011). Essential practices for milking include cleaning udders of bedding and applying effective germicides both pre- and post-milking to prevent the transmission of new infections (Parkey et al., 1984; McDougall et al., 2009; De Vliegher et al., 2012). Separate towels for drying teats and wearing gloves for milking staff have also been advocated for mastitis control (Ruegg, 2017). Additional measures include having a latency period between 60 and 120 seconds between first touch of the udder and attaching the milking clusters, regular evaluation of milking equipment to ensure stable teat-end vacuum, proper pulsation, and routine inspection of liners and rubber air hoses (McDougall et al., 2009; Ruegg, 2017).

Management of mastitis requires disposable materials such as disinfectants, which have short-term economic costs, and non-disposable materials, such as milk parlors, which lead to long-term economic consequences. The costs of both these expenses can be estimated (Halasa et al., 2007). In Canadian herds, preventive costs, from such practices as teat dipping, represented 15% of the total cost of mastitis (Aghamohammadi et al., 2018). Thus, the cost of mastitis control offers a return on investment by reducing the losses caused by IMI such as lost production, culling, and so on. For example, a recent Dutch study showed the total cost of mastitis was 240 euro per cow/year, and preventive practices cost 120 euro per cow/year. Labor was the biggest contributor to preventive practices (at 82 euro), followed by consumables and investments (€34 and €4 per cow/year, respectively. However, a minimum amount of loss due to disease and cost of prevention of mastitis will always exist (Soset et al., 2016).

**Mastitis Treatment**

The essential aim of treatment for bovine mastitis is to improve milk quality and production, decrease the probability of recurrent cases, and eliminate pathogens (Waller et al., 2016). However, several factors should be considered before initiating treatment, as well determining the effect of therapy.

Cow factors such as SCC before therapy, parity, duration of infection, the number of infected quarters, and the type of pathogen can all affect therapeutic efficacy (McDougall et al., 2007a, 2007b). Wagner and Erskine (2013) highlighted the key elements of treatment decisions. First, it is important to determine whether a case of clinical mastitis is new or recurring. In particular, therapy for repeat cases is frequently unsuccessful, although some researchers have advocated extended therapy (Mestorino and Oscar, 2012; Waller et al., 2016). Pinzón-Sánchez and Ruegg (2011) found that factors such as bacteriological cure and parity were related to recurrence probability, and SCC of >200,000 cells/mL before the onset of clinical mastitis cases decreased the probability of successful therapy. The lactation stage and presence of other health issues also affect therapeutic outcome (Sanchez and Ruegg, 2011).

Besides the factors relating to individual cows, it is also important to consider the causative pathogen to determine the approach and possible efficacy of treatment. Some pathogens such as *Str. agalactiae* and *Str. dysgalactiae* are considered to be highly responsive to many antimicrobials, whereas other pathogens may not benefit from drug intervention (Wagner and Erskine, 2013). These include *S. aureus*, *Mycoplasma s*p., *Prototheca*, mycotic organisms, and gram-negative bacteria, including mild cases caused by coliform organisms (Hektoen et al., 2004). In addition, Lago et al. (2011) reported there were no differences in days to clinical cure, bacteriological cure risk, or new intramammary infection risk, following the decision to apply culture-based therapy to mild clinical mastitis compared to cows that were treated empirically. In this model, cows with mild clinical mastitis were first cultured for bacteria and treatment withheld until results were obtained 24 hours later. Cases that yielded no organisms or gram-negative organisms were not treated, as opposed to treatment of cases with gram-positive cocci (Lago et al., 2011). Fuenzalida and Ruegg (2018a, 2018b) further confirmed that treatment of mild clinical mastitis cases that were culture negative or caused by *E. coli* did not improve outcomes such as relapse rate, culling, or SCC. However, clinical cases caused by *Klebsiella* sp. may be more difficult (Fuenzalida and Ruegg, 2018b).

The duration of therapy can affect the efficacy of antimicrobial therapy of bacterial infections. However, IMI provides unique challenges to antimicrobial efficacy relative to other tissues, and extended therapy may unnecessarily increase discarded milk costs out of proportion to benefits. Therefore, therapy should be of short duration. Preliminary studies have suggested that extended use of antimicrobial treatment improves the cure rate for some pathogens, including *S. aureus* and some environmental streptococci (Oliver et al., 2004). However, for *E. coli* and coagulase-negative *staphylococci*, there is no evidence that an extended period of therapy improved the cure rate of pathogens or long-term outcomes (Pinzón-Sánchez et al., 2010).

Typically, the udder has the ability to clear some microbial infections without treatment; for example, the self-cure rates can reach levels as high as 90% in microbes like *E. coli* (Sandholm, 1995; Ruegg, 2018). Moreover, 30 to 40% of mild cases end in no microorganisms being isolated from bacteriologic cultures of milk samples (Ruegg, 2018).

In reality, mastitis therapy applied on individual herds is often based on personal experience and herd data (Pyörälä, 2009). Ideally, mild clinical mastitis cases should be treated only after bacteriologic results are obtained (Lago et al., 2011). However, bacteriologic milk cultures prior to treatment is not commonly done on many dairy herds. In the United States, for example, < 15% of herds reported that bacteriologic cultures were used to help determine mastitis treatment decisions (Hovi and Roderick, 1996; Kayitsinga et al., 2017). In some herds, such as organic herds, total avoidance of antimicrobial therapy for clinical mastitis was practiced.

The case severity is also a key determinant of therapy. Cases that include systemic illness, such as from coliform pathogens, require antimicrobials and supportive therapy. Parenteral therapy, in addition to intramammary infusions, may be indicated to help ameliorate the deleterious effects of bacteremia (Wagner and Erskine, 2013). A similar study confirmed the vital role of systematic antimicrobials for the treatment of severe coliform mastitis (Poutrel et al., 2008).

Organic dairy farms in the United States use a variety of herbal and homeopathic remedies as an alternative to antimicrobial therapy for mastitis (Pol and Ruegg, 2007). Several non-antimicrobial therapies, such as clay, acupuncture, oxygen therapy, and products that include colostrum-derived antibodies and botanical and herbal remedies have all been proposed as being efficacious. Ramey (2007) stated that, “With few exceptions, controlled studies on the clinical effects of herbal or botanical preparations in veterinary medicine appear to be essentially nonexistent" (Ramey, 2007). A small randomized clinical trial, investigating the treatment of subclinical mastitis with a variety of nonantimicrobial compounds, revealed there was no clear or significant impact from those treatments on either bacteriological cure or SCC (Tikofsky and Zadoks, 2005).

**Measuring Antimicrobial Drug Use**

Zwald and Ruegg (2004) studied 131 dairy herds in Minnesota, Michigan, New York, and Wisconsin to compare reported antimicrobial drug use and management factors between conventional and organic dairy herds in the United States. The data collection relied on questionnaires administered on-site for each participating herd and recall of drug use during the previous 60 days. Overall, 79.8% of conventional herds used antimicrobials for mastitis treatment, compared to none of the organic herds. However, although more conventional herds used antimicrobials in calves and heifers (74.7%), a substantial proportion of organic herds also used antimicrobials for younger animals (21.9%).

In a subsequent study, antimicrobial drug use was monitored in 20 organic and 20 conventional herds (Pol and Ruegg, 2007). The criteria for herd enrollment included an average herd SCC of ≥ 250,000 cells/ml for at least six months, use of blanket dry cow therapy in the conventional herds for at least five years, and at least three years of certification for the organic farms. Quantification of antimicrobial use was conducted by an on-site survey of treatment practices and herd records of udder health, clinical mastitis treatment, respiratory diseases, metritis, and lameness for a period of two months to one year (Pol and Ruegg, 2007).

Moreover, to measure drug use for each farm, this study relied on the defined daily dose (DDD) metric, the highest dose that an animal can receive with standard bodyweight (680 kg) if it is treated according to the FDA-approved label dose. It is calculated with the formula DDDa = MG $_{DDDa}$ * U $_{DDDa}$ *F $_{DDDa}$. MG is the mg/mL of the compound, as available in the product; U is the number of millimeters administered for each dose; and F is the number of doses administered per day (Pol and Ruegg, 2007). The total mean antimicrobial use for conventional farms was 5.4 DDD per cow per year; intramammary treatments represented about two-thirds of the total (3.58 DDD per cow per year), which was divided further into clinical mastitis treatment (2.02 DDD), and dry cow therapy (1.56 DDD). Furthermore, as in the Zwald and Ruegg study (2004), use of ceftiofur was higher than that of other compounds (Pol and Ruegg, 2007).

Carson et al. (2008) developed a novel method to determine systemic antimicrobial use in 24 herds of beef cattle and compared two metrics to measure drug use across one year. Producers were asked to dispose of empty drug containers in garbage cans; to keep any receipts from drug purchases; to complete a survey about herd housing, management, and routine use of antimicrobials; and to document drug use in a treatment log. Drug use was expressed in terms of mg of active compounds administered, in addition to the animals' defined daily dose (ADD), and the mean rate of use for both units was determined for 1,000 days/animal. ADD was calculated as the dose of active compound (mg) divided by labeled daily dose (mg)* body weight (kg). The results indicated that, compared to drug use recorded by evaluating discarded drug containers, producers recorded about 60% of injectable antimicrobial use in treatment logs, and only 22% of treated animals had a complete record of all doses (Carson et al., 2008). This suggests that relying on herd records to quantify antimicrobial drug use in beef cattle may not be accurate.

Relative ranking of drug use of different classes of antimicrobials differed, depending on whether actual drug consumption (mg of active compound) or ADD calculations were employed.

Additionally, the study revealed that penicillin products were frequently used in larger doses than was labelled (Carson et al., 2008).

Although this study included a small sample size of herds, over- and underestimation of treatment logs were noted. Thus, disposal bins were suggested because they are more accurate than treatment logs. Similar results were found in a study of 34 Canadian swine farms—a collection of discarded containers was more accurate than the farm treatment records, and the latter tended to underestimate antimicrobial use (Dunlop et al., 1998).

In another study, 20 dairy farmers in Peru were asked to dispose of empty drug containers in receptacles for six months. At the end of the study period, the farmers were interviewed and asked to recall antimicrobial use during the six-month period (Reding et al., 2014). The agreement between the discarded containers and farmer recall was weak with respect to the quantity and type of antimicrobials used. In contrast, self-reported drug use was more reliable regarding intermammary infusion use and in short-term data collection. However, these results might have been due to over-reporting and selection bias by the farmers (Redding et al., 2014).

Saini et al. (2012) conducted a comprehensive study of 89 Canadian dairy farms to quantify and describe antimicrobial use. Average herd size was 88 cows, with a range of bulk tank SCC from 91,000 to 500,000 cells/ml. Herd selection criteria included having at least 80% of the herd as Holsteins, participation in DHI, and a milking schedule of three times daily. This study also determined antimicrobial drug use by counting the number of discarded drug containers and infusion tubes. The unit of drug use was DDD, using an adjusted average weight of adult dairy cows of 600 kg. The rate of antimicrobial drug use was defined as a herd-level and time-sensitive parameter. The antimicrobial drug use rate (ADUR) was defined as the active compound used during the study period (kg) divided by the number of days in the study period * DDD. The number of dairy cattle in each herd was calculated as the average number of cows in the herd during the study period—as collected by DHI test dates (Saini et al., 2012). The mean ADUR for all herds was 4.35 DDD/1,000 cow-days, and the most common antimicrobials used were cephalosporins and penicillin. The rated parenteral administration of antimicrobial drugs was higher than intramammary infusions, but this study included drug use of both adult cows and replacement animals.

Furthermore, intramammary drug use was more frequent for clinical mastitis treatment than dry cow therapy, and penicillin combinations were the most commonly used antimicrobial drug for clinical mastitis (Saini et al., 2012). The milk production level was positively associated with ADUR, the use of pirlimycin for clinical mastitis treatment, cephalosporin use in dry cow therapy, and ceftiofur use for systematic administration. Herd size was positively correlated with ADUR and the systemic administration of ceftiofur. However, there was no significant association between ADUR and herd SCC or type of housing (Saini et al., 2012).

In a follow-up study of 51 Canadian dairy herds, estimated antimicrobial drug use recorded from discarded drug container inventory and farm treatment records was 31,840 ADD and 14,487 100 cow-years ADD, respectively (Nobrega et al., 2017). This suggests that, for every entry in the treatment record, 2.2 times more treatments were accounted for by disposal inventory. The mean ADD estimated from disposal inventory was consistently higher than that of the treatment records for all classes of antimicrobials.

The authors suggested that the limitations of the inventory method might include overestimation because of off-label therapies, overdosing, and the discarding of partially used drug containers (Nobrega et al., 2017). Mastitis, reproductive diseases, and dry cow therapy were the most frequent causes for the use of antimicrobials, and penicillin was the most frequently used antimicrobial drug (Nobrega et al., 2017).

In a Pennsylvania study (Redding et al., 2019), 235 dairy producers completed a survey during a six-month period. Drug use was quantified by three metrics. First, defined daily dose (DDD), which in the case of intramammary therapy for lactating cows, one tube represented one DDD, and four tubes was one DDD for dry cow treatment. Parenteral therapy DDD was then calculated, as described earlier. Animal-defined daily doses (ADD) was calculated as the average labeled dose (defined daily dose the DDD (mg/kg) multiplied by the animal standard bodyweight (50 kg for calves, 200 kg for heifers, and 600 kg for adult cows.) Finally, the treatment incidence (TI) per 1,000 animal-days was calculated as the ADD divided by the number of animals at risk × days at risk corrected for 1,000. Days of antimicrobials in therapy (DOT) was calculated as the number of treated cases multiplied by the duration of treatment. The final formal was TI(DOT) = DOT / number of animals at risk × days at risk × 1,000 (Redding et al., 2019).

The overall rate of antimicrobial use was 4.2 ADD/1,000 animal-days and 3.3 DOT/1,000 animal-days. The most commonly used class of antimicrobial was first generation cephalosporins, and the most common indication for antimicrobial use was mastitis. The rates of antimicrobial use were positively associated with herd size. The rate of DOT was relatively lower than ADD. However, the two metrics were correlated. Additionally, 40% of producers did not keep written treatment protocols (Redding et al., 2019).

Numerous other studies and research efforts have attempted to obtain accurate methods for data collection and quantified antimicrobial use in both humans and animals with different tactics and approaches. Examples include records of drug sales (Grave et al., 1999; Chauvin et al., 2005; Kools et al., 2008; Bondt et al., 2013), national-level or state surveillance systems (Dewey et al., 1999; Ruegg and Tabone, 2000; Merle et al., 2012; Bos et al., 2013), in-person interviews with producers (Luna-Tortos et al., 2006; Timmerman et al., 2006; Hill et al., 2009; Callens et al., 2012; Persoons et al., 2012), drug concentrations in tissues (Jones and Seymour, 1988), pharmaceutical data records (Stewart and Lynch, 2012), patient diaries (Parker et al., 2007), and on-farm treatment records (Meek et al., 1986).

All methods of data collection have limitations. However, the literature to date suggests the inventory method or collection of discarded drug packaging is considered one of the most suitable and easiest ways to determine the use of antimicrobial drugs in dairy herds.

In addition to the determination of techniques for acquiring or collecting antimicrobial drug use in dairy farms, there is a clear lack of a gold-standard metric to quantify ADUR. The total mg of an active component was one of the first metrics used because it was easy to calculate and to understand, and it is suitable for comparison in dairy farms with different numbers of cattle using the same dose of a particular drug per animal (Mills et al., 2018). However, the total mg metric suffers from differences in doses across different classes of antimicrobial drugs and potential variations in administered doses between individual veterinarians and herds. For example, the use of lincomycin and tylosin is sometimes higher than advisable in footbaths, which could reflect the actual rate of use (Mills et al., 2018).

The total mg/kg of body weight for an active compound was also used because it was easy to calculate and understand, and it is suitable for comparison in dairy farms with different numbers of cattle using the same dose of a particular drug per animal (Mills et al., 2018). However, this metric also suffers from differences in doses across different classes of antimicrobial drugs and potential variations in administered doses between individual veterinarians and herds. Moreover, using estimated or inaccurate body weights might lead to over- or underestimation of actual antimicrobial drug use (Mills et al., 2018).

Nevertheless, an attempt in the United Kingdom to improve the accuracy of the estimated bodyweight of different breeds across England examined 2,774 dairy cattle of different breeds from 20 farms using Lely Automatic Milking Systems, which provided weigh scales in the floor. The results revealed that the average body weight of adult lactating cattle was 617 kg, and the average bodyweight varied across different breeds, with a range from 636 kg for Holsteins to 466 kg for Jerseys (Schubert et al., 2018). Another tactic could be used as an alternative to total mg/kg: the use of production information instead of bodyweight, such as mg/1,000 L of milk production (Mills et al., 2018).

Several European countries (EVAS) have attempted to create a unified DDD bovine metric that uses a fixed daily dose and a standard weight of 425 kg. Furthermore, the DDD is defined for specific active compounds and route of administration (Mills et al., 2018). However, this approach does not consider different drug labels and use between countries, as well as variability in access to drugs for the herd (Mills et al., 2018). Moreover, intramammary infusions have a relatively low drug dose compared to systemic therapy, which skews mg/kg of body weight considerations. Therefore, it may cause an underestimation of drug use on dairy farms (Mills et al., 2018). Moreover, dry cow therapy can confound the DDD metric. The DDD can be improved by individualizing for specific country-labeled indications, relevant fixed daily doses, and standardized animal bodyweights. The daily dose system in different countries' national AMU monitoring systems has been named the animal defined daily dose (ADD), defined animal daily dose (DADD), and defined daily dose animal (DDDA; Mills et al., 2018).

**Herd-Level Factors Associated with Antimicrobial Use**

Several studies have tried to discover the variables that explain the differences in antimicrobial use. Kayitsinga et al. (2017) surveyed over 600 herds from Michigan, Florida, and Pennsylvania and determined that a majority of herds do not utilize management practices that are considered essential to help limit the unwarranted use of antimicrobial drugs in the treatment of mastitis. Only 56% of farmers kept treatment records, and 49% of farmers reviewed records before administering treatment. Furthermore, the percentage of farmers who cultured milk samples for bacteriology, at least occasionally, with elevated SCC was only 17%, 18% for cows with clinical mastitis, and 13% for bulk tank milk. The multivariable analysis revealed that the use of treatment records was associated with increased use of both intramammary infusion and systemic treatment of clinical mastitis. Decreasing intramammary use for clinical mastitis was also related to membership in an Amish community, the use of organic therapies, the use of bacterins for controlling *Staphylococcus aureus*, and a lower level of education (Kayitsinga et al., 2017).

Conversely, use of internal teat sealants at dry-off, financial incentives for employees, application of conductivity to measure subclinical mastitis in herds, and sole proprietorship were associated with the increasing systemic use of antimicrobial drugs for clinical mastitis. Moreover, sand bedding was linked to a decrease in the systemic use of antimicrobial drugs, which may reflect a lower incidence of mastitis, typical for herds that house with this material. However, limitations from this study include possible inaccurate answers from farmers and recall bias (Kayitsinga et al., 2017).

McDougall et al. (2016) attempted to discover the factors related to the selection of antimicrobials by veterinarians and the attitudes of both veterinarians and producers toward antimicrobial use and resistance in New Zealand dairy farms. A focus group of 22 farmers and dairy managers, as well as 206 veterinarians that responded through an anonymous online survey, were used in this study. Veterinarians based their prescription decisions on perceived diagnosis (82%), response to previous medication (65%) withholding period (25%), and their client–farmer's preferences (22%). In the focus groups, 85% of the farmers stated they tended to choose antimicrobial therapies based on veterinary consultations, although a majority (68%) also based therapy on personal experience. Antimicrobial culture and susceptibility testing were not widely used (McDougall et al., 2016).

Swinkels et al. (2015) conducted interviews with 38 farmers (17 from the Netherlands and 21 from Germany) from herds that had to be a nonorganic, with ≥ 50 cows, and they were committed to staying in business for at least five more years. In fact, 30 of the 38 farm owners routinely extended clinical mastitis treatment beyond prescribed regimens, and on an additional seven farms, the dairy managers occasionally extended clinical mastitis treatment. The participants expressed sensitivity toward the social norm of other farmers to be a good herdsman by extending treatment. Moreover, feeling insecure about the efficacy of treatment and being described as a poor herdsman led farmers to follow the practices of other farmers. Veterinarians also extended treatment regardless of the cost if a farm was losing milk. Furthermore, using antimicrobial drugs led to the emotional reward of being "a good stockman" and thus making this behavior acceptable. Additionally, societal concerns had little effect on farmer decisions due to the gap between them and the public (Swinkels et al., 2015).

Mastitis is a multifactorial disease and has a substantial impact on the productivity of dairy cattle. Therefore, progress has been made in milk quality over the past quarter century, as understanding and application of critical management procedures, including enhanced housing, defined milking protocols, consistent ration formulation, and knowledge of the pathogens have helped mitigate the impact of this disease. However, mastitis is still the primary reason for using antimicrobials in dairy herds.

As a result, we conducted research to gain an understanding of the most accurate method for quantifying antimicrobial use in dairy farms and gain knowledge of the variables that could affect antimicrobial use. The available published research demonstrates there are diverse strategies to quantify AMU in dairy herds. Despite growing evidence that the inventory method of collecting empty drug containers may be a more accurate measure of AMU, DDD remains the most popular metric. However, DDD suffers from limitations such as differences in prescriptions and dose labels between countries. This can be especially problematic with intramammary therapy, both for lactating and dry cows.

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# CHAPTER 2:

# HERD-LEVEL VARIABLES ASSOCIATED WITH ANTIMICROBIAL DRUG USE IN DAIRY HERDS

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ABSTRACT

The aim of this study was to identify the variables associated with antimicrobial drug use for bovine mastitis. Intramammary tubes used for lactating cows, discarded by farm personnel, were collected in dedicated trash bins for 12 months from 104 herds (Michigan *n* = 49, Pennsylvania *n* = 55). We quantified antimicrobial use through two metrics: arithmetic (number of discarded tubes) and defined daily dose (DDD) per 1,000 cow-days. We also collected mastitis control practices, attitudes, and behaviors using a survey of herd owners and managers conducted during herd visits. Additionally, we compiled a human resources questionnaire survey about dairy producers/manager's beliefs and practices regarding employee communications, training, and education for each herd. Across all herds, intramammary tube use based on arithmetic count was 4.43 tubes per 1,000 cow-days and 3.52 per 1,000 cow-days based on DDD. A higher trend of antimicrobial use (5.22 tubes per 1,000 cow-days and DDD of 4.1 per 1,000 cow-days) was found in Pennsylvania than in Michigan (3.61 tubes per 1,000 cow-days and the DDD 2.91 per 1,000 cow-days). The ranking of antimicrobial use showed that ceftiofur was the most frequently used antimicrobial in the study, followed by cephapirin, pirlimycin, amoxicillin, hetacillin, and cloxacillin. A majority of producers believed they had sufficient knowledge of mastitis treatment and control, but only 23% of them had a written treatment protocol, and less than half of them (44%) reviewed animal health history before treating infected cows. Moreover, 80% disagreed that culture of milk samples was needed to make mastitis treatment or management decisions. Multivariate models had similar results, regardless of the metric used to determine intramammary drug use; there was a positive correlation between drug use and the new subclinical mastitis rate (based on monthly DHI SCC testing) and the producer's willingness to improve the image of the dairy products. Use of natural remedies for mastitis treatment was negatively correlated with

intramammary drug use. Our results indicate there continues to be a gap between producer practices and beliefs and best practices to reduce antimicrobial use on dairy farms. Additionally, intramammary drug use is highly associated with the dynamics of subclinical mastitis, as measured in individual cows and a herd average level.

# INTRODUCTION

Research has established an association between antimicrobial use in animal agriculture and antimicrobial resistance. Mastitis is considered to be the most common reason for using antimicrobials in dairy farms (Pol and Ruegg, 2007; Saini et al., 2012) and is also one of the costliest diseases in the dairy industry (Nielsen et al., 2010; Hogeveen et al., 2011). Estimated costs of clinical mastitis cases include direct costs associated with diagnosis, therapy, veterinary services, labor, and non-salable milk (Rollin et al., 2015; Aghamohammadi et al., 2018). Thus, efficient treatment strategies for mastitis are needed to decrease the probability of recurrence and to eliminate pathogens (Waller et al., 2016), as well as foster the prudent use of antimicrobial therapy.

Several factors should be considered before initiating mastitis therapy: cow factors (e.g., SCC, parity, duration of infection, number of infected quarters), type of pathogen, and duration of therapy (Oliver et al., 2004: Wagner and Erskine, 2013; Fuenzalida and Ruegg, 2018b). Essential for determining therapeutic efficacy is the ability to describe antimicrobial use in dairy farms. Zwald and Ruegg (2004) compared the reported rate of antimicrobial use between conventional and organic dairy herds in the United States using an on-site survey based on producer recall and a review of animal health records of antimicrobial use in the previous 60 days; 79.8% of conventional herds used antimicrobials for mastitis treatment, compared to none of the organic herds. Subsequently, Pol and Ruegg (2007) monitored antimicrobial use in 20 organic and 20 conventional herds, using the defined daily dose (**DDD)** metric. Mean antimicrobial use for the conventional farms was 5.4 DDD per cow per year and intramammary treatments represented about two-thirds of the total.

Saini et al. (2012) applied a novel approach to quantify antimicrobial use on Canadian dairy farms. The investigators counted the number of empty drug containers and infusion tubes in trash bins and compared the count for each drug to treatment records. Intramammary antimicrobial use was more frequent for clinical mastitis treatment than for dry cow therapy (Saini et al., 2012). In a follow-up study involving 51 Canadian dairy herds, Nobrega et al. (2017) estimated antimicrobial use from discarded containers and farm treatment records was 31,840 and 14,487 animal defined daily doses (ADD), respectively. The authors suggested that disposal inventory was more accurate than treatment records for estimating antimicrobial use.

Researchers have also sought to discover the explanatory variables for differences in the rate of antimicrobial use between herds. Kayitsinga et al. (2017) surveyed over 600 herds from Michigan, Florida, and Pennsylvania and found the use of treatment records was associated with increased use of both intramammary infusion and systemic treatment of clinical mastitis. Decreasing intramammary use for clinical mastitis was related to the use of organic therapies, internal teat sealants at dry-off, and financial incentives for employees; sole proprietorship was associated with increasing systemic use of antimicrobials for clinical mastitis. Moreover, the use of sand bedding was linked to a decrease in the systemic use of antimicrobials, which may reflect a lower incidence of mastitis, typical for herds that are housed with such bedding. Rates of antimicrobial use have also been positively associated with herd size (Saini et al., 2012; Redding et al., 2019). Other studies have revealed that farmer and veterinarian perceptions are associated with antimicrobial use in dairy farms ; Swinkels et al., 2015). However, the explanatory variables related to the farm labor structure are poorly understood.

The objectives of this study were to compare antimicrobial drug use rate (ADUR) in dairy herds, quantified by two methods, for intramammary infusion tubes (IMT) in lactating cows.

Additionally, we sought to better elucidate the potential explanatory variables for the rate of IMT use across herds, including both social variables regarding labor structure and herd management practices.

## MATERIALS AND METHODS

### Dairy Farm Selection

This study was part of a larger project in which 124 dairy herds in Florida, Michigan, and Pennsylvania participated in a 15-month trial to develop an evaluation of mastitis and herd management practices. The 104 herds (Michigan $n$ = 49 and Pennsylvania $n$ = 55) that were part of the larger project and included in this study were visited by the investigators twice between January 2016 and May, 2017 to complete a herd profile and management survey and also perform an evaluation of milking behaviors, performance, and cow hygiene. Enrolled herds participated in the Dairy Herd Improvement (DHI) individual cow somatic cell count (SCC) option and had a targeted herd size ≥ 60 cows. Because the overall objectives of the umbrella project included employee-related factors on milk quality and antimicrobial use on dairy farms, we excluded organic dairies and herds that milked with automated milking systems. All survey information was collected following approval and performed within the guidelines set by the Institutional Review Board of Michigan State University.

### Herd Profile and Management Culture

During the initial herd visit, we explained the study design and conducted a herd profile to record milking times and groups, type of milking facility, housing, employee structure, and other general information. Within 30 to 60 days, we returned to conduct a milk quality evaluation that included (1) milking behaviors and proficiency, (2) milking systems, (3) cow environment, (4) monitoring and therapy of infected cows, and (5) farm labor structure. To capture information relative to management practices and beliefs, we interviewed dairy producers and/or managers with an 84-question survey relative to their mastitis control practices, attitudes, and behaviors (Schewe et al., 2015). Additionally, we administered a separate 16-question human resources survey to describe producer/manager beliefs and practices regarding employee communication, training, and education. Approximately 90 minutes was needed to conduct the surveys and review the project with each producer.

For this study, we selected a subset of questions from the larger sample of 84 original questions that we deemed to be possibly associated with antimicrobial drug use and decisions related to mastitis therapy. For example, questions related to use of SCC or milk bacteriology for selecting cows for therapy were retained in our study, but questions such as those pertaining to frequency of changing liners or the number of cows milked per employee per hour were excluded. Thus, for our study, we initially selected 34 questions from the herd surveys to be included for our analysis.

**Assessing and Collecting Intramammary Infusion Tubes**

Both the compliance and existence of records for the treatment of mastitis varied widely between herds. To quantify intramammary antimicrobial drug use for clinical mastitis treatment, we placed plastic bins in locations that farm personnel selected (in some herds, more than one location). Herd personnel who were responsible for the treatment of mastitis in milking cows were requested to discard any used IMT, including partially used tubes, in the bins, rather than the trash. To help acclimate herd personnel with IMT disposal in the study bins, we conducted a pre-trial period for three months, during which we visited each herd at least twice to ensure compliance with the protocol. Following this period, we commenced the trial and visited each herd on a regular basis for 12 months. The frequency of herd visits varied, dependent on herd size and perceived drug use, to ensure that the bins did not reach full capacity between visits. In general, larger herds tended to be visited more often to accommodate their larger quantity of drug use.

Nearly all herds (96%) used dry cow therapy, and 90% of the herds used blanket dry cow therapy. Thus, after the pre-trial period, we decided not to quantify dry cow tube use because >80% of discarded IMT were labelled for dry cows across all herds. This decision (1) reduced the burden of tube disposal for herd personnel, which facilitated compliance of the study protocol for our primary interest (i.e., treatment of clinical mastitis in milking cows), and (2) reduced the frequency of herd visits by the investigators, which better utilized travel resources and ensured that disposal bins remained below capacity between visits.

During our visits, we logged and counted discarded IMT while on the farm and classified according to the type of products (amoxicillin, hetacillin, penicillin, pirlimycin, ceftiofur, cephapirin, and cloxacillin). Herd management was always notified of impending visits and given a record of discarded drug results. We did not offer any advice on treatments, and questions about therapy protocols were referred to the herd veterinarian.

**Statistical Analysis**

***Dependent Variables.*** The dependent variable was the rate of use for discarded IMT labelled for the treatment of clinical mastitis in lactating cows. This included partially dispensed IMT, but not IMT that were judged by the investigators to be full—the occurrence of the latter was rare. We entered the quantity of discarded IMT for each herd into an Excel sheet for data management and quantified the rate of IMT use by two metrics: arithmetic count of IMT and DDD (as described below). To account for differences in the duration of the trial period and herd size between participating herds, we estimated the rate in terms of 1,000/cow-days by using the following formula:

1,000 cow-days = (days herd in trial* mean cows milked (DHI)/1,000)

The following formula were used to describe the arithmetic IMT rate in each herd:

IMT in bins/1,000 cow-days = (total number of tubes/1,000 cow-days)

We calculated DDD for each IMT product based on the labeled frequency of dosing. Thus, for products labeled for one dose per 24 hours, one IMT = one DDD. Likewise, for products labeled for consecutive doses administered during successive milking, two IMT = one DDD. The corrected DDD rate of IMT use for each drug was then applied per 1,000 cow-days, as with the total IMT use, as described above (DDD/1,000 cow-days).

***Independent Variables.*** The independent variables originated from the surveys described above and DHI SCC for each herd. Additionally, each herd was evaluated based on udder hygiene scores (UHS), as described by Schreiner and Ruegg (2003). We analyzed SCC data based on four metrics: (1) the 12-month mean of monthly herd average SCC; (2) the 12-month mean of the proportion of cows with a linear SCC score (LSCC) of ≥ 4 (200,000 cells/mL), essentially the monthly prevalence of subclinical mastitis; (3) incidence of new subclinical mastitis, defined in our study as the number of cows with a LSCC ≥ 4 during the current test date that had a LSCC < 4 during the previous test date, divided by the total cows that had a LSCC < 4 during the previous test date; and (4) prevalence of chronic subclinical mastitis, defined as the proportion of the tested cows with a LSCC ≥ 4 during both the current and previous test date. For UHS, we determined the combined prevalence of lactating and dry cows with a UHS ≥ 3. We evaluated numbers of cows for UHS in each herd based on sample size calculations to attain a 95% CI dependent on herd size.

We classified the survey variables according to producers' knowledge about mastitis control (e.g., how to treat mastitis cows), use of individual SCC to identify problems, and attitudes, (e.g., perceptions of culture of milk samples) to make treatment decisions and employee communication and compliance with protocols. The analysis of explanatory herd-level variables for IMT use was similar for both simple arithmetic and DDD metric and performed in two steps: bivariate followed by multivariate analysis.

***Bivariate Analysis.*** We conducted bivariate analysis to discover the correlation between IMT use (dependent variable) and explanatory variables (independent variables) through negative binomial regression (Schukken et al., 1991) to account for the skewed distribution of the dependent variable. Furthermore, we classified the independent variables into (1) producers' attitudes and knowledge about mastitis controls, (2) mastitis bacteriology, (3) farm protocols, (4) therapy of infected cows, (5) communications and employee compliance, and (6) DHIA SCC data. We transformed continuous independent variables with skewed distribution by natural log to attain normality. We chose a cutoff point of $P < 0.25$ (2-tails) to determine the eligibility of independent variables for inclusion in the multivariable analysis.

***Scale Factor Analysis.*** We used scale factor analysis to test for several independent variables that were considered to be highly correlated. Nevertheless, none of the factors or variables satisfied the criteria, which included a Cronbach's $\alpha > 0.7$ and Eigenvalue > 1.

***Multivariate Analysis.*** We compared several regression model approaches to determine the final and most applicable model for this study due to the highly skewed dependent variable, including Poisson regression, overexpressed regression, and negative binomial regression. We determined that negative binomial regression had an excellent, and best fit for the data through comparing measurements of goodness of model fit (Akaike information criterion (AIC), Bayesian information criterion (BIC), and degree of freedom value) for each approach. Thus, we applied negative binomial regression models created for both arithmetic and DDD IMT in our final model. In addition, we added location by state (MI or PA) to improve the model. We added herd size to the final model in an attempt to discover if it affected the results. However, it did not have any significant effect on the final model and was removed. After the analysis, only biological fit variables that were significant at $P < 0.05$ and with significant exponentiation of the $\beta$ coefficient were retained in the final model using Wald Chi-Square values, *P*-values, and 95% confidence intervals for the coefficients.

# RESULTS

Of the original 120 herds from PA and MI that participated in the broader mastitis evaluation study, 16 were excluded from our study due to noncompliance with the study protocols or leaving the study before the trial period was finished. The overall mean herd size in the remaining 104 herds was 401 cows (ranging from 64 to 2,778 cows). Mean herd size for the 49 herds from Michigan was 469 cows (ranging from 64 to 2,778 cows) and for the 55 herds from Pennsylvania, 314 cows (ranging from 66 to 1,611 cows; Table 1). The rate of IMT use across all study herds was 4.43 tubes/1,000 cow-days arithmetically and 3.52 DDD/1,000 cow-days (Table 1). Furthermore, the mean IMT use in Michigan was 3.61 tubes/1,000 cow-days and 2.91 DDD/1,000 cow-days, whereas mean IMT use in Pennsylvania was 5.22 tubes/1,000 cow-days and 4.1 DDD/1,000 cow-days. Figure 1 presents the distribution by herd of arithmetic IMT use and DDD.

Ceftiofur IMT were used in 86.5% of the herds, which ranked higher than any other class of drug (Table 2). Conversely, only one herd used a procaine penicillin product. Ceftiofur was also the most frequently used IMT across herds. The mean rate of use was 2.37 per 1,000 cow-days for both IMT count and DDD. Cephapirin was the second most frequently used drug (64.4% of herds), with a mean arithmetric rate of 2.18 tubes/1,000 cow-days and 1.09 DDD/1,000 cow-days. Rates for pirlimycin (0.95 tubes and DDD/1,000 cow-days, 51% of herds), amoxicillin (1.84 tubes/1,000 cow-days and 0.92 DDD/1,000 cow-days, 22% of herds), hetacillin (0.36 tubes and DDD/1,000 cow-days, 16% of herds), and cloxacillin (0.74 tubes and DDD/1,000 cow-days, 6.8% of herds) followed in ranking. As expected, based on the formula for DDD, there was a two-fold discrepancy between arithmetic and DDD IMT use for products that were labeled for twice/day dose frequency.

Improving the public image of dairy products was important for 91% of the dairy herds that participated in the study (Table 3), with 66% ranking this concern as very important. Moreover, 83% of dairy producers ranked veterinarian advice as important or very important for mastitis treatment practices, compared to 27% considering the advice of drug company representatives as important or very important for mastitis treatment practices (Table 3).

As described above, 93% of dairy farms used blanket dry cow therapy (3% selective), and 73% of herds used internal sealants at dry off. In addition, only 35% of herds segregated treated cows from untreated cows by use of separate milking groups or facilities, and 11% of herds operated separate milking parlors for treated and hospital cows. Moreover, only 24% consistently reviewed treatment records before administering mastitis therapy, and 59% recorded all mastitis treatments (Table 4). Although 79% of dairy herd managers had a treatment protocol, only 23% had a written treatment protocol. Additionally, 83% of herds reported that they strictly follow their veterinary-prescribed protocols for mastitis treatment, always or frequently (Table 4).

Although we did not quantify systemic use of drugs in this study, 29% and 44% of herds reported that they used systemic antimicrobial/anti-inflammatory therapy, or oxytocin, at least sometimes for mild mastitis therapy (Table 4). Treatment of mastitis in some herds was not restricted to clinical mastitis only because 31% reported that they frequently or always treat cows based solely on subclinical mastitis (determined by SCC, see Table 4). Overall, 84% of herds agreed they had good knowledge of mastitis therapy (Table 5).

Regarding milk bacteriology, 38% of herds reported that they always or frequently employ culture-based therapy (wait for milk culture results) before treating mastitis cases (Table 4), even though 62% disagreed with the statement that “culture of milk samples doesn’t provide useful results” (Table 5). Likewise, 72% of herds agreed the milk culture should be used more often in their mastitis treatment decisions, but, conversely, 32% did not agree that culture results were useful, 61% agreed that it took too long to attain culture results, 21% agreed it took too much time to collect milk samples, and 15% thought milk culture was too expensive (Table 5). Regarding use of SCC, 58% of the herds reported they did not use individual somatic cell count to track new infection rates in milking cows. However, 83% of dairy herds used individual SCCs to track chronic/high SCC cows (data not shown).

Because of herds that lacked employees or by management decision, 39% of the herds responded that employees did not treat cows with antimicrobials. However, of the remaining herds, 9% reported that primary training for mastitis therapy was self-taught or provided by other employees, rather than from herd managers or veterinarians (data not shown). Employee training for the treatment of mastitis, or, by comparison, for milking protocols, was either nonexistent or only given when first hired in 27% and 38% of the herds, respectively.

The bivariable analysis revealed 12 variables that met the $P < 0.25$ criterion for inclusion in the multivariable analysis for both metrics of quantifying IMT use: (1) improving the image of dairy products, (2) producer intent on reducing the use of antimicrobials, (3) intent to reduce labor costs, (4) the use of oxytocin for milk letdown, (5) are teats disinfected after milking (post-dip), (6) the number of cows dried per towel during milking preparation, (7) the percentage of cows with an udder hygiene score ≥ 3, (8) are hospital pen/treated cows separate from all other cows, (9) the use of natural remedies for mastitis treatment, (10) the mean new subclinical infection rate, (11) the mean test date herd average SCC, and (12) the mean prevalence of subclinical mastitis (see Table 6).

Nevertheless, for a better fit and to enhance the multivariate models, we only included variables with substantial biologically relevant lower *P* values and excluded variables that were closely correlated. For example, the four DHI SCC parameters were highly correlated, and we thus chose a new subclinical mastitis rate because of the higher correlation value. Nevertheless, we tried to explore the effect of DHI SCC on IMT use in dairy herds by replacing it with a new mean subclinical infection rate in the final model. However, the correlation coefficient and *p*-value for the new mean subclinical infection rate was more significant than DHI SCC. Therefore, it was not included in the final model.

The final variables included in the multivariable analysis were (1) the use of natural remedies for mastitis treatment, (2) improving the image of dairy products, (3) the number of cows dried per towel during milking preparation, (4) are teats disinfected after milking post-dip, (5) are hospital pen/treated cows separate from all other cows, and (6) the mean new subclinical infection rate.

Table 7 illustrates the final multivariable models for both metrics of IMT use. The new subclinical mastitis rate and improving the image of dairy products on the part of the producer were positively associated with IMT use ($P < 0.01$). We found a negative correlation between IMT use and the use of natural remedies for mastitis treatment ($P < 0.05$). The two models for both IMT use metrics had similar outcomes.

DISCUSSION

To our knowledge, our study was the first to consider human resource information and individual cow SCC within the explanatory variables. Although participating herds discarded a wide variety of antimicrobial products, labeled for both parenteral and intramammary administration, we limited our estimation of antimicrobial use to IMT. We did this because (1) the lack of consistent record keeping of drug therapy across all herds, (2) most discarded multi-dose vials were likely used for reasons other than treatment of mastitis, and (3) wanting to focus on mastitis therapy, the most likely cause of antimicrobial use in most dairy herds (Wagner and Erskine, 2013; Ruegg, 2018).

Our study found that ceftiofur was the most frequently used IMT, followed by cephapirin, pirlimycin, and amoxicillin. In contrast, penicillin was rarely used for treating mastitis. This is similar to the results found by Zwald and Ruegg (2004). In contrast, Redding (2019) found that the most used class of antimicrobials in dairy herds for several diseases, including mastitis, were first-generation cephalosporins, followed by penicillin and third-generation cephalosporins. Pol and Rugg (2007) found that cephapirin was the most used antimicrobial for treating clinical mastitis, and penicillin-based compounds were the most used for dry cow therapy. In Canadian studies, penicillin combinations were the most frequently used antimicrobials for the treatment of mastitis (Saini et al., 2012; Nobrega et al., 2017).

As expected, the rate of IMT use varied widely between herds, depending on the metric used and labeled frequency of dosing. IMT use, as measured by DDD, was lower compared to the arithmetic count. Our results for DDD (3.54/1000 cow-days) were slightly lower than previous studies, 4.35 DDD and 4.2 DDD/1,000 cow-days, in Canada and Pennsylvania, respectively (Saini et al., 2012; Redding at al., 2019). The lower antimicrobial drug rate in our study may be partly explained in that the previous studies calculated ADUR for treatments other than mastitis.

Although the mean rate of IMT use was higher when calculated by arithmetic count than DDD, we did not find a significant difference. However, this is not surprising because ceftiofur, a product that is labelled for one dose per day, was the predominant antimicrobial used across all farms. Moreover, products (ceftiofur, hetacillin, pirlimycin, and cloxacillin) that are labeled for once per day dosing accounted for 58% of the total number of IMT used.

Interestingly, many of our herds used more than one product during the study, and they often used products that were labeled for once per day and twice per day dosing. In part due to labor constraints, most of our study herds administered IMT therapy once per day, regardless of the labeled frequency of dosing. Dosing cows with IMT that are labelled for two consecutive milkings can be especially problematic in herds that milk three times per day. In our study, about 40% of herds were milked three times per day, and all of them stated in their therapy protocols that they administered IMT once per day. However, a third of the 3x milking herds used products that were labelled for dosing at two consecutive milkings. Our observations are aligned with previous investigators' concerns that DDD may not reflect actual IMT use in many dairy herds (Nobrega et al., 2017), especially if using IMT that are labeled for multiple doses per day. Furthermore, from the standpoint of actual antimicrobial drug use (mg over a period of time), the lower rate, as gained from DDD compared to the arithmetic count, is deceptive. For example, if a cow was treated once a day for two consecutive days with ceftiofur (250 mg total antimicrobial administered) this would be two DDD compared to one DDD for a cow dosed with two IMT of cephapirin over the same period (400 mg total antimicrobial administered). Realizing that bioactivity and pharmacokinetics differ between different antimicrobials, this paradox has been described previously (Redding et al., 2019).

As previously mentioned, our concern over inadequate treatment records, which has been reported by other investigators (Redding et al., 2019), was the primary reason we chose to assess IMT use by discarding into containers. This metric may have limitations, such as an overestimation due to discarded IMT that were not completely used, or an underestimation if all used IMT were not placed in bins (Nobrega et al., 2017). However, this approach has been used in several studies and was suggested to better account for IMT use than relying on treatment records (Dunlop et al., 1998; Carson et al., 2008; Redding et al., 2014; Nobrega et al., 2017).

This study revealed that, among herds with employees, employee training for the treatment of mastitis or milking protocols was inconsistent and often given only when first hired. Furthermore, if the training exists, sometimes it was self-taught or provided by other employees, rather than from herd managers or veterinarians. Frequent rapid turnover of employees (Hagevoort et al., 2013) can play a role in inadequate training. In addition, larger dairy farms are often reliant on immigrant labor, which represents about 51% of all US dairy employees (Adcock et al., 2015), and could create extra challenges such as communication in regard to understanding farm goals and disparities in training compared to English-speaking employees (Susanto et al., 2010; Schenker and Gunderson, 2013; Erskine et al., 2015).

Our multivariate models had similar results, regardless of the metric used to determine IMT use. Enhancing the image of dairy products was associated with IMT use, but paradoxically was a positive association. Dairy producers who are concerned about increasing the perception and public demand for and quality of dairy foods would seemingly be more circumspect about IMT use, especially in the decision-making of when to administer therapy. However, Benard and De

Cock-Buning (2013) reported that farmers strongly believe the public lacks knowledge about agricultural practices. Therefore, they believe the public's perceptions of farming operations are irrelevant.

Moreover, perceived IMT use on the part of the herds in this study may be disconnected from therapeutic reality. Reducing antimicrobial use was important for 80% of the dairy herds in our study. Kayitsinga et al. (2017) found a similar result, which indicated that 81% of farmers believe it is essential to reduce antimicrobial use for mastitis. However, only about three-fourths of our study herds had a treatment protocol, and about one-fourth had a written treatment protocol. Furthermore, less than half of the herd managers reported that they reviewed previous treatment records before making therapy decisions for clinical mastitis cases, and, as in a previous Pennsylvania study, only about half of dairy herd managers retained antimicrobial treatment therapy records (Sawant et al., 2005).

This deficit can lead to underestimations on the part of herd managers of actual IMT use over a period of time and a higher probability of ineffective antimicrobial treatment of clinical mastitis (Hess et al., 2003; Barkema et al., 2006; Lago et al., 2011a). Poor therapeutic outcomes and decision-making could have been further exacerbated by the low portion of our study herds (30%) that reported they sometimes or always relied on milk culture results before administering therapy. Lack of knowledge regarding causative pathogens increases the risk of ineffective mastitis therapy (Barkema et al., 2006; Lago et al., 2011a; Oliveira and Ruegg, 2014).

Not surprisingly, our model found a negative correlation between the use of natural remedies for mastitis treatment and IMT use, which was similar to that reported by Kayitsinga et al. (2017).

Perhaps the most novel finding of our multivariate model was the strong positive association between new subclinical mastitis rates and IMT use. On the surface, it would be expected that herds with a higher prevalence of mastitis would also treat more clinical cases and, as in some of our study herds (16%), subclinical mastitis. Furthermore, there was association, though weaker, between herd IMT use and herd average SCC, which would be similar to herd bulk tank SCC. Intriguingly, this suggests that assessing the impact of subclinical mastitis in a dairy herd may be improved if the dynamics of individual cow SCC are included rather than herd-level SCC alone. This may be a consequence of the nonnormal distribution of SCC on the herd level. Thus, even though the incidence of clinical mastitis is an important metric to determine therapy protocols and outcomes for a herd, the impact of subclinical mastitis should also be accounted for.

Saini et al. (2012) found that herd size was not correlated with the overall antimicrobial drug use rate. We found a similar result relative to IMT use in our study; we also explored the effect of the change in herd size during the course of the 12-month study period for each herd, but this was found not to be significant in the bivariate analysis. Furthermore, we attempted to control for herd size by forcing this variable into our multivariable analysis, but this had little effect on the strength of the final model.

The current study has several strengths, including a long follow-up period, excellent compliance from the dairy herd participants in the study procedures, a high level of participation, and a large sample size. Moreover, using self-interview surveys improved screening accuracy, captured verbal and nonverbal cues, and kept the participants focused on questions. The placement of bins for collecting intramammary infusions protected the study from recall bias issues and the problems that come with self-reported surveys, including unverifiable or incomplete treatment records (Saini et al., 2012).

# CONCLUSIONS

Regardless of the metric used to determine the rate of IMT use, there was a strong positive correlation between IMT use and individual cow subclinical mastitis dynamics. Paradoxically, dairy producers who are concerned about increasing the perception and public demand for dairy foods tended to have higher rates of IMT use. The rate of actual antimicrobial drug use, at least when estimating IMT use by arithmetic count compared to DDD, may be misrepresented, especially for products that are labeled for twice per day dosing. Management practices that are related to improving prudent mastitis therapy are infrequently used on many dairy herds, which could lead to inefficiency in mastitis treatment and overuse of antimicrobial drugs.

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# APPENDIX

Table 1 : Descriptive statistics for the use of intramammary tubes (IMT), as described by arithmetic count, defined-daily dose (DDD) per 1,000/cow-days, and herd (12 month) test date averages for cows in herd, somatic cell count, new subclinical mastitis rate (number of cows with a LSCC ≥ 4 during the current test date with a LSCC < 4 during the previous test date, divided by the total cows with a LSCC < 4 during the previous test date), chronic subclinical mastitis (the proportion of cows with a LSCC ≥ 4 during both the current and previous test date), and the proportion of cows with linear SCC score ≥ 4 from 104 dairy herds in Michigan and Pennsylvania.

| IMT Use Metric | Mean | Median | Std. Dev | Minimum | Maximum |
|---|---|---|---|---|---|
| DDD | 3.52 | 1.97 | 4.72 | 0.02 | 27.5 |
| IMT | 4.43 | 2.32 | 5.51 | 0.02 | 28.26 |
| Michigan | | | | | |
| DDD | 2.91 | 1.7 | 3.1 | 0.09 | 13.57 |
| IMT | 3.61 | 2.32 | 3.93 | 0.09 | 16.45 |
| Pennsylvania | | | | | |
| DDD | 4.10 | 2.21 | 5.08 | 0.02 | 27.3 |
| IMT | 5.22 | 2.85 | 6.46 | 0.02 | 28.26 |
| | | | | | |
| Cows in herd | | | | | |
| All herds | 401 | 241 | 463 | 64 | 2,778 |
| Michigan | 469 | 286 | 497 | 64 | 2,778 |
| Pennsylvania | 314 | 232 | 458 | 66 | 1,611 |
| | | | | | |
| TD DHIA SCC (cells/mL) | | | | | |
| All herds | 169 | 158 | 76 | 60 | 560 |
| Michigan | 150 | 133 | 79 | 62 | 560 |
| Pennsylvania | 183 | 177 | 71 | 60 | 321 |

Table 1 (cont'd)

| | | | | | |
|---|---|---|---|---|---|
| All herds | | | | | |
| Michigan | 7.84 | 6.9 | 3.35 | 2.83 | 23.87 |
| Pennsylvania | 7.53 | 6.78 | 3.5 | 2.83 | 23.87 |
| | 8.14 | 7.2 | 3.25 | 3.1 | 21.3 |
| New chronic mastitis rate | | | | | |
| All herds | 73.9 | 85.2 | 17.01 | 22.77 | 91.59 |
| Michigan | 57.34 | 58.81 | 10.93 | 22.77 | 77.65 |
| Pennsylvania | 87.95 | 87.99 | 1.90 | 83.66 | 91.59 |
| % SCS ≥4 | | | | | |
| All herds | 21.87 | 20.5 | 7.88 | 7.5 | 58.3 |
| Michigan | 20.0 | 19.5 | 8.3 | 7.5 | 58.35 |
| Pennsylvania | 23.2 | 51.75 | 7.1 | 8 | 46.3 |

Table 2: Antimicrobial use of intramammary tubes, by class of drug, for both arithmetical count and DDD (per 1,000/cow-days) from 104 dairy herds in Michigan and Pennsylvania.

| Types of Antimicrobials | Usage of IMT by Herds Number | Metric of Measurement | Mean | Std. Dev | Minimum | Maximum | Total Number of Tubes |
|---|---|---|---|---|---|---|---|
| Hetacillin | 17 (16%) | DDD/IMT | 0.36 | 0.27 | 0.007 | 1.34 | 1,125 |
| Pirlimycin | 53 (51%) | DDD/IMT | 0.95 | 2.29 | 0.004 | 12.29 | 7,581 |
| Ceftiofur | 90 (86.5%) | DDD/IMT | 2.37 | 2.98 | 0.008 | 12.71 | 42,289 |
| Cloxacillin | 7 (6.8%) | DDD/IMT | 0.74 | 0.05 | 0.02 | 0.14 | 338 |
| Cephapirin | 67 (64.4%) | DDD | 1.09 | 1.87 | 0.003 | 8.76 | 22,615 |
| | | IMT | 2.18 | 3.75 | 0.007 | 17.52 | |
| Amoxicillin | 23 (22.2%) | DDD | 0.92 | 2.22 | 0.007 | 10.72 | 14,824 |
| | | IMT | 1.84 | 4.43 | 0.01 | 21.45 | |
| Penicillin | 1 (1%) | DDD | 0.01 | 0 | 0.001 | 0.001 | 1 |
| | | IMT | 0.02 | 0 | 0.002 | 0.002 | |
| | | | | | | Total | 88,773 |

Table 3: Survey responses of owners/managers from 104 dairy herds in Michigan and Pennsylvania regarding perceptions and beliefs about mastitis treatment.

| Response | Very Important | Important | Unimportant | Not at All Important | Legitimate Skip/Not Applicable | Missing |
|---|---|---|---|---|---|---|
| Veterinarian advice for my mastitis treatment practices | 42% | 41% | 9% | 5% | 0 | 4% |
| Drug company representatives' advice for mastitis treatment practices | 2% | 25% | 8% | 49% | 0 | 7% |
| Improving parlor turnover rate (cows/hour) | 22% | 34% | 34% | 5% | 0 | 6% |
| Improving the image of dairy products | 66% | 25% | 5% | 0 | 0 | 5% |
| Reducing use of antibiotics | 40% | 40% | 15% | 1% | 0 | 5% |

Table 4: Bivariate analysis for use of intramammary tubes for both arithmetic count and DDD (per 1,000/cow-days) from 104 dairy herds in Michigan and Pennsylvania.

| | IMT | | | DDD | | |
|---|---|---|---|---|---|---|
| Variable | B | Sig | Exp(B) | B | Sig | Exp(B) |
| Improving the image of dairy products | 1.208 | .001 | .299 | 1.427 | .002 | 4.165 |
| Reducing the use of antimicrobials | -.70 | .007 | .495 | -.70 | .007 | .495 |
| Intent to reduce labor costs | -.992 | .006 | .371 | -.893 | .006 | .451 |
| How often is Oxytocin used for milk letdown? | .743 | .021 | 1.01 | .743 | .007 | 5.725 |
| Are teats disinfected after milking (post-dip)? | -.544 | .066 | 3.054 | -.531 | 0.68 | 3.421 |
| How many cows are dried per towel? | .708 | .008 | .455 | -.787 | .008 | .453 |

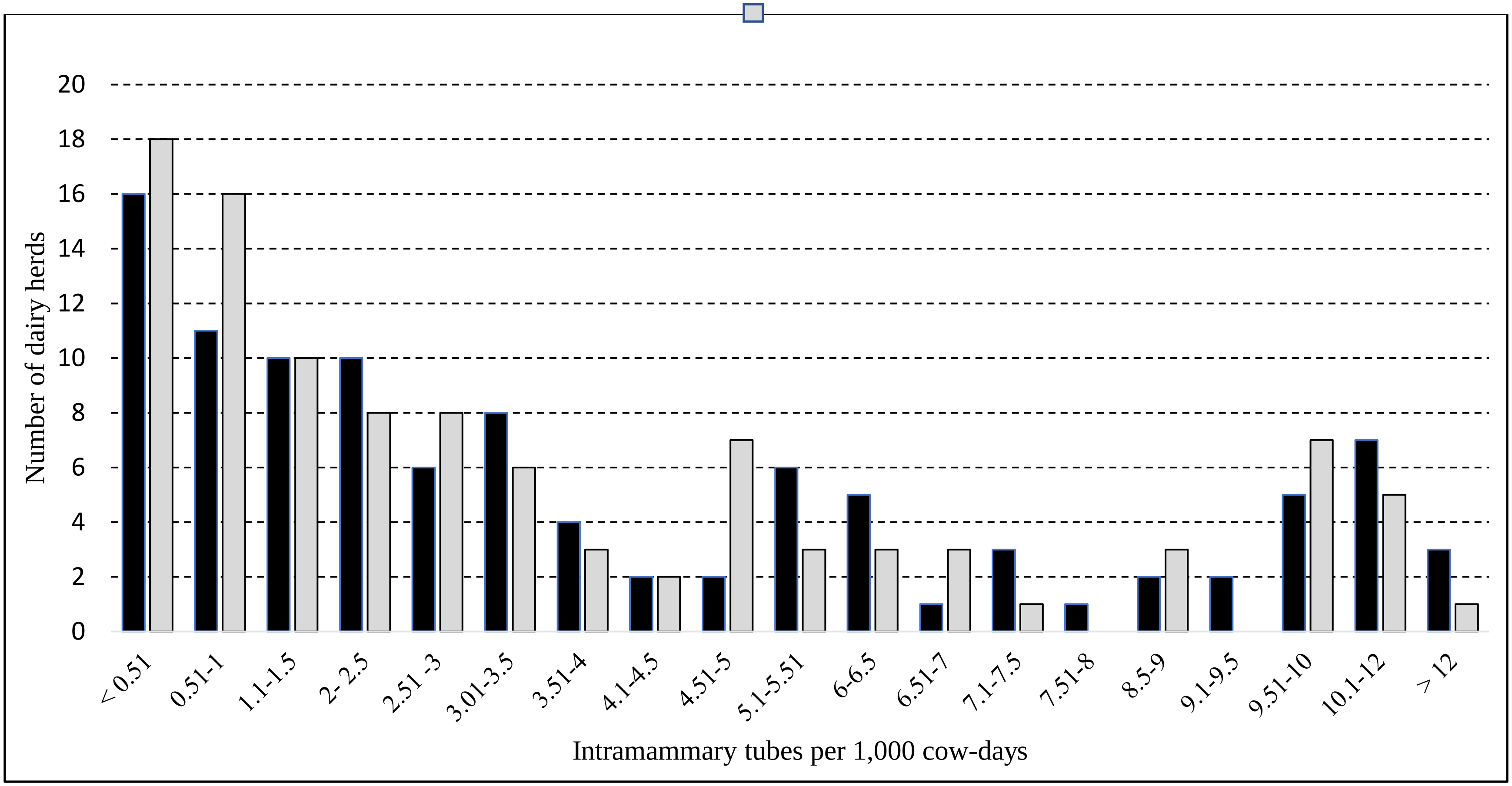
Table 4 (cont'd)

| | | | | | | |
|---|---|---|---|---|---|---|
| The percentage of cows with an udder hygiene score ≥ 3 | .809 | .082 | 2.214 | .726 | .085 | 2.264 |
| Are hospital pen/treated cows separate from all other cows? | .632 | .010 | 1.188 | .741 | .023 | 1.278 |
| Use natural remedies for mastitis treatment | -.937 | .029 | .392 | -.834 | .053 | .434 |
| New subclinical mastitis rate | 1.549 | .018 | 4.707 | 1.652 | .008 | 5.219 |
| The mean test date herd average SCC | .852 | .04 | 2.282 | 0.832 | .05 | 2.482 |
| The mean prevalence of subclinical mastitis | .576 | .04 | .029 | .651 | .005 | .033 |

Table 5: Final multivariable analysis for use of intramammary tubes for both arithmetic and DDD per 1,000 cow-days from 104 herds in Michigan and Pennsylvania.

| | IMT | | | DDD | | |
|---|---|---|---|---|---|---|
| Variable | B | Sig | Exp(B) | B | Sig | Exp(B) |
| Improving the image of dairy products | 1.353 | .004 | 3.870 | 1.550 | .001 | 4.713 |
| New infection rates | 1.745 | .008 | 5.729 | 1.839 | .004 | 6.292 |
| Use natural remedies for mastitis treatment | -1.028 | .017 | .359 | -.949 | .028 | .905 |

Figure 1: Data frequency distribution for intramammary tube use, as described by arithmetic count and DDD per 1000/cow-days from 104 dairy herds in Michigan and Pennsylvania (■ = arithmetic; = DDD).



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