

## **ANTIMICROBIAL RESIDUES AND RESISTANCE: UNDERSTANDING AND MANAGING DRUG USAGE ON DAIRY FARMS**

Pamela Ruegg, DVM, MPVM, University of Wisconsin, Madison

### **Introduction**

In modern dairy cattle operations, antimicrobials are administered for both therapeutic and prophylactic purposes. Most antimicrobials are used therapeutically but some antimicrobials are used to prevent disease in healthy animals during periods of increased susceptibility. Mastitis is one of the most frequent infectious diseases in dairy cattle and accounts for most of the doses of antibiotics given to dairy cows (Pol and Ruegg, 2006a). Lactating cows may be treated for clinical mastitis or to pursue bacteriological cure of a subclinical case. Antimicrobials are also used to treat other infectious diseases of dairy cows, including respiratory and uterine infections and infectious foot disease.

The use of antimicrobials to treat food animals has the potential to affect human health through 2 mechanisms: 1) increasing the risk of antimicrobial residues, and 2) influencing the generation or selection of antimicrobial resistant foodborne pathogens (Yan and Gilbert, 2004). The risk of antimicrobial residues is well known and has been addressed through the use of appropriate regulatory mechanisms but there is increasing concern about the impact of antimicrobial usage in food animals on the development of antimicrobial resistance. The objective of this paper is to review data about the relationship between antimicrobial resistance and the use of antimicrobials in dairy cattle with emphasis on antimicrobials used for treatment of mastitis.

### **Antimicrobial Concepts and Definitions**

The terms antimicrobial and antibiotic are often used interchangeably but are not synonymous. In technical terms, "antibiotics" refer only to substances of microbial origin (such as penicillin) that are active against other microbes while "antimicrobial" refers to any substance (including synthetic compounds) which destroys microbes (Guardabasse and Courvalin, 2006). Antimicrobial agents interfere with specific bacterial processes needed for growth or division of cells. Compounds that inhibit bacterial growth are termed bacteriostatic while those that kill the bacteria are termed bactericidal. Antibacterial agents can be bacteriostatic when they reach the minimum inhibitory concentration (MIC) but become bactericidal when they reach a higher concentration, (the minimum bactericidal concentration (MBC)). If the MIC and the MBC are distinctly separated, the agent is considered bacteriostatic. If the MBC is close to the MIC, the compound is considered to be bactericidal (Prescott, 2000a).

Bacterial resistance can be intrinsic or acquired. An example of intrinsic resistance is a Gram-negative bacterium that has an outer membrane that is impermeable to the antibiotic. Acquired resistance occurs when a previously susceptible bacterium becomes resistant through mutation (vertical evolution) or acquisition of new DNA (horizontal evolution). Mutation is the result of a random event that occurs spontaneously. The presence of antibiotic will subsequently select for the resistant mutants (Prescott, 2000b).

Methods of horizontal evolution include conjugation, uptake of free DNA, and transduction. Transduction occurs when bacteriophages transfer genetic material from one bacterium to another. This process may involve genetic material that confers antibiotic resistance, like the transference of the  $\beta$ -lactamase gene from resistant to susceptible staphylococci (Prescott, 2000b). Mobile DNA segments (transposons) can transfer resistance among plasmids or to the genome. A specific transposon (integron) can accumulate resistance genes and may be a mechanism of development of multiple-antimicrobial resistance (Hall and Collis, 1995).

Several mechanisms of resistance have been described. Some bacteria contain enzymes that inactivate antibiotics. The most well known example is  $\beta$ -lactamase. These enzymes inactivate  $\beta$ -lactam antibiotics by cleaving the  $\beta$ -lactam rings. Some bacteria develop resistance by preventing the antibiotic from entering the bacterial cell or by increasing the removal of the drug out of the cell (faster than it enters the cell). Reduced ability to enter the cell occurs with resistance to fluoroquinolones, aminoglycosides and

penicillins and can be overcome by increasing the drug concentration (Neu and Gootz, 2005, Levy, 2002). Increased removal of tetracycline is encoded by genes such as tet(A) and results in resistance of many enterobacteriaceae. Similar mechanisms have been described for resistance to erythromycin, chloramphenicol and ciprofloxacin (Levy, 2002). Structural changes in some bacteria can block activity of some antibiotics that successfully enter bacterial cells. For example, proteins responsible for cell wall synthesis of enterococci have low affinity for cephalosporins making these bacteria inherently resistant (Neu and Gootz, 2005). Elimination of the binding site by resistance genes carried on plasmids can result in resistance to macrolide, lincosamide, and streptogramin B (MLS resistance). Likewise, the tet(M) gene, produces a protein that can inhibit binding of tetracycline (Levy, 2002). Bacteria can also produce alternative binding sites that are resistant to inhibition. This mechanism is observed in methicillin resistant *Staphylococcus aureus* (MRSA), which produce the penicillin binding protein PBP2 encoded by *mecA* (Neu and Gootz, 2005).

#### Antimicrobial Susceptibility Tests

Antimicrobial susceptibility tests measure the ability of an antimicrobial agent to inhibit bacterial growth in vitro and are performed using methods that are based on either dilution or diffusion (Walker, 2000). The agar disc diffusion (ADD) is one of the most common methods and is referred to as the "Kirby-Bauer method." A standardized suspension of bacteria is streaked over a Muller-Hinton agar plate and antimicrobial impregnated discs are applied. During an overnight incubation, a gradient of antimicrobial concentration is established around the discs. The highest concentration is closest to the disc and progressively lower concentrations occur as distance from the disc increases. If the bacteria are susceptible to the antimicrobial tested, a distinct inhibition zone will be observed. If the bacteria are resistant to the antimicrobial, bacterial growth will be observed close to the antimicrobial disc. The diameter of each inhibition zone is recorded and the outcome is interpreted for each antimicrobial using standards based on the size of the zone of inhibition (Walker, 2000; Clinical and Laboratory Standards Institute, 2002). The ADD test is the most widely used method in veterinary medicine because it is inexpensive, does not require specialized equipment and is flexible enough to test different drugs. The major disadvantage is that results are qualitative, therefore the antimicrobial dose cannot be adjusted to maximize clinical outcome (Walker, 2000).

In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates (agar dilution) or in broth (broth dilution) containing dilutions of the antimicrobial agent. These methods generate both quantitative and qualitative outcomes. Agar dilution is considered the "gold standard" but is costly and cumbersome (Walker, 2000). The use of broth microdilution is becoming more common in veterinary laboratories due to the development of automated systems. Serial dilutions of antimicrobials are inoculated with a standardized inoculum of bacteria and are incubated at 35° C for 16 to 20 hours. The MIC is considered to be the lowest concentration of antimicrobial agent that completely inhibits bacterial growth (Walker, 2000; Clinical and Laboratory Standards Institute, 2002). Like diffusion tests, outcomes of microdilution may be expressed as categorical outcomes (susceptible, intermediate, or resistant) based on breakpoints determined by the Clinical and Laboratory Standards Institute (CLSI) standards (Walker, 2000). The CLSI definition of susceptible implies "that there is a high likelihood of a favorable clinical outcome when the drug is administered at label dosage, because of adequate pharmacodynamic parameters relative to the MIC of the causative organism." (Clinical and Laboratory Standards Institute, 2002). The intermediate category is applicable to isolates that are "moderately susceptible" to an antibiotic that can be used for treatment at a higher dosage because of its low toxicity or because the antibiotic is concentrated at the focus of infection (e.g. urine). The resistant category implies that there will not be a favorable clinical outcome, because the achievable systemic concentrations of the agent will be lower than the MIC of the causative organism with normal dosage schedules and/or fall in the range or where specific microbial resistance mechanisms are likely (e.g.  $\beta$ -lactamase), and clinical efficacy has not been reliable in treatment studies." (Clinical and Laboratory Standards Institute, 2002).

Interpretive criteria (breakpoints) for MIC or zone diameter values used to indicate whether the isolate is susceptible or resistant are determined by a national panel of experts (CLSI, formerly called the National Committee on Clinical Laboratory Standards or NCCLS) (Clinical and Laboratory Standards Institute,

2002). Only a few antimicrobials (penicillin/novobiocin, pirlimycin and ceftiofur) have a breakpoint for bovine mastitis. Other antimicrobials (ceftiofur, enrofloxacin, florfenicol, spectinomycin, and tilmicosin) have a veterinary breakpoint for bovine respiratory disease. Extrapolation of susceptibility criteria to other veterinary diseases (such as mastitis) may lead to incorrect prediction of clinical outcome. For example, in vitro susceptibility tests do not always predict clinical efficacy, especially for mastitis (Walker, 2000; Constable and Morin, 2003, Hoe and Ruegg, 2005). Breakpoints for pirlimycin are veterinary validated for mastitis but also appear to be poor predictors of treatment outcomes of mild and moderate clinical cases of mastitis (Hoe and Ruegg, 2005). Various factors may explain this issue. Antimicrobial susceptibility is tested in vitro, and laboratory conditions are different than the mammary gland environment (Walker, 2000). Proteins in milk may bind to antimicrobials therefore decreasing their activity (Fang and Pyorala, 1996). Finally, incomplete knowledge of the pharmacokinetics of intramammary compounds may lead to uncertain concentration of the antimicrobial at the site of infection (Sandholm et al., 1990).

#### Usage of Antimicrobials in Agriculture

The most comprehensive data about antimicrobial usage in the U.S. comes from the National Animal Health Monitoring System (NAHMS) (USDA, 2005). NAHMS estimated that more than half (55%) of the operations utilized medicated milk replacers (usually tetracyclines (+/- neomycin) or decoquinatone). Chlorotetracycline and sulfamethazine were the most commonly used antimicrobials in heifer rations (USDA, 2005). About 10% of calves were treated for pneumonia or diarrhea and one third of treated cases received florfenicol. Macrolides (16% of the treatments),  $\beta$ -lactams (not cephalosporins) (14%), and cephalosporins (14%) were also used. Calves with diarrhea received sulfonamides (24% of the treatments), tetracyclines (22%),  $\beta$ -lactams (not cephalosporins) (14%), aminoglycosides (11%) and cephalosporins (11%) (USDA, 2005). Weaned heifers were treated less frequently. Overall, 5% and 0.5% of all weaned heifers were treated for pneumonia and diarrhea, respectively. Tetracyclines were used for about one third of pneumonia treatments, florfenicol (26%) and macrolides (17%). Cephalosporins were used to treat more than half of the diarrhea cases, while  $\beta$ -lactams (not cephalosporins) (13%), tetracyclines (12%), and sulfonamides (11%) were used less frequently (USDA, 2005).

Foot infections in adult cows occurred in 52% of the dairy farms, while 50% and 42% of the producers reported the occurrence of pneumonia or metritis in adult cows, respectively (USDA, 2005). Most cases of pneumonia received antimicrobial treatment but only two thirds of the animals with metritis and foot infections received antimicrobials. The relatively low incidence of many diseases, resulted in a small proportion of the adult cows receiving antimicrobial treatments (< 14% of animals were treated for pneumonia, metritis or foot infections). Almost all (99%) of producers used antimicrobials to treat pneumonia (99%) and metritis or foot infections (90% each). Cephalosporins (67%),  $\beta$ -lactams (13%), and tetracyclines (12%) were used most frequently for treatments of pneumonia. Tetracyclines (41%),  $\beta$ -lactams (31%), and cephalosporins (23%) were used most frequently for treatments of metritis. Tetracyclines (42%), cephalosporins (30%), and  $\beta$ -lactams (17%) were used most frequently for treatments of foot infections.

Pol and Ruegg (2006) recently developed a method to quantify antimicrobial usage and treatment practices. Data on disease prevalence and treatment practices of farms (n = 20) with bulk tank SCC >250,000 cells/ml were obtained during a farm visit and a standardized estimate of antimicrobial usage was developed using a Defined Daily Dose (DDD). Density of antimicrobial usage was expressed as the number of DDD per adult cow per year. Farmers reported that penicillin was the compound most commonly used for dry cow therapy and cephalosporins were most commonly used for treatment of clinical mastitis (Table 1). Additional antimicrobial exposures occurred for treatment of other diseases (foot infections, respiratory disease and metritis) and consisted of ceftiofur (0.59 DDD/cow/year; used on 17 farms), tetracycline (0.17 DDD/cow/year; 12 farms), penicillin (0.52 DDD/cow/year, used on 7 farms), ampicillin (0.07 DDD/cow/year, used on 8 farms) and sulphonamides (0.57 DDD/cow/year, used on 4 farms).

Table 1. Use of antimicrobials for treatment and prevention of mastitis on selected WI dairy farms with bulk tank SCC >250,000 cells/ml (n = 20) (from Pol and Ruegg, 2006)

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Dry Cow Therapy	Treatment of Clinical Mastitis
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Product	Herds Using (%)	DDD <sup>a</sup>	Herds Using (%)	DDD <sup>a</sup>
Intramammary usage				
Penicillin	18 (90%)	0.62	0	
Streptomycin	18 (90%)	0.62	0	
Cephapirin	15 (75%)	0.55	18 (90%)	1.31
Novobiocin	3 (15%)	0.22	0	
Pirlimycin	0		15 (90%)	0.70
Amoxicillin	0		8 (40%)	0.66
Cloxacillin	0		2 (10%)	0.32
Erythromycin	0		2 (10%)	0.08
Parenteral usage				
Penicillin	5 (25%)	0.43	7 (35%)	0.68
Tetracycline	4 (20%)	0.37	7 (35%)	0.12
Tylosin	4 (20%)	0.13	0	0
Ceftiofur	0		6 (30%)	0.47
Ampicillin	0		6 (30%)	0.04
Sulphonamides	0		4 (20%)	2.51

<sup>a</sup>defined daily doses per cow per year

The estimated overall exposure to antimicrobials was 5.43 DDD/cow/year composed of 3.58 and 1.85 DDD of intramammary and parenteral antimicrobials, respectively (Table 2). Intramammary antimicrobials used for treatment of mastitis accounted for 56% of the intramammary usage and for 38% of the total usage. Parenteral antimicrobials used for treatment of mastitis accounted for about half of the parenteral usage and 17% of the total usage. About 80% of all antimicrobials were used for treatment or prevention of mastitis (DCT, 28%; intramammary compounds for clinical mastitis, 38%; parenteral compound for clinical mastitis, 17%).

Table 2. Descriptive statistics of estimated Defined Daily Doses (DDD) per cow per year

	No Herds <sup>1</sup>	%	DDD per cow per year <sup>2</sup>				Route <sup>3</sup> (%)	Total <sup>4</sup> (%)
			Mean	SE	Min	Max		
Intramammary								
Clinical Mastitis	20	100.0	2.02	0.70	0.08	11.82	56.0	38.0
DCT <sup>5</sup>	20	100.0	1.56	0.08	1.00	2.00	43.0	28.0
Total IMM	20	100.0	3.58	0.74	1.21	13.77	100.0	66.0
Parenteral								
DCT <sup>5</sup>	7	35.0	0.52	0.26	0.02	1.71	9.8	3.3
Clinical Mastitis	13	65.0	1.43	0.67	<0.01	7.83	50.3	17.1
Foot	16	80.0	0.39	0.14	0.01	2.42	16.8	5.7
Respiratory	19	95.0	0.14	0.03	<0.01	0.61	7.4	2.5
Metritis	18	90.0	0.32	0.05	0.02	0.83	15.6	5.3
Total parenteral	20	100.0	1.85	0.51	0.05	8.59	100.0	34.0
Total	20	100.0	5.43	1.06	1.43	19.93	100.0	100.0

<sup>1</sup> Farms could report the use of multiple products; <sup>2</sup> For farms reporting usage; <sup>3</sup> Percentage of each route by reason to treat; <sup>4</sup> Percentage of the total use of each reason to treat or route

Eleven producers (of 20) reported extra-label use of antimicrobials via an intramammary route. Ampicillin was used for intramammary treatments on 6 farms, ceftiofur and gentamycin were used for intramammary treatments on 3 farms each. Two farmers reported the extra-label use of penicillin, 1 producer reported the extra-label use of miconazole, and 1 producer used extra-label oxytetracycline for IMM treatments.

Compounds prepared by veterinarians with unknown ingredients were used in 2 farms. Two producers reported use of a prohibited compound for IMM treatments (sulfamethoxazole/trimethoprim).

Considerable among farm and region variability has been reported for mastitis therapeutics (Zwald et al., 2004; Sato et al., 2005; Kirk et al., 2005). Almost 80% of the producers (n = 99) surveyed by Zwald et al. (2004) reported the parenteral use of antimicrobials to treat some cases of clinical mastitis but in a smaller study (n = 30) Sato et al. (2005) found that < one third of WI producers utilized systemic antimicrobials for mastitis treatments. About 40% and 60% of the California producers reported the usage of injectable antimicrobials to treat mild and severe cases of mastitis, respectively (Kirk et al., 2005). Sato et al. (2005) reported that only 60% of the conventional farms regularly used intramammary tubes to treat clinical mastitis but his estimates contrasts with the 93% reported in NAHMS survey of dairy cattle (USDA, 1996).

#### Usage of Antimicrobials and Susceptibility

Methodological and reporting differences among studies complicate the ability to determine if resistance to antimicrobials is emerging in mastitis pathogens. However, similar retrospective studies have reported comparable findings (Erskine et al., 2002; Makovec and Ruegg, 2003). Erskine et al. (2002) reported the trends in antimicrobial susceptibility of about 2,800 mastitis isolates obtained from samples submitted by veterinarians to a central laboratory during 7 years. Antimicrobial susceptibility was tested using ADD. The proportion of susceptible isolates did not change during the 7-yr period for most of bacteria-antimicrobial combinations. Makovec and Ruegg (2003) reported similar conclusions in a larger study (8,900 samples), where a reduced resistance to  $\beta$ -lactams antimicrobials was observed in *S. aureus*, CNS, and *Strep. spp.*

Several studies have described differences in susceptibility of isolates obtained from farms with different histories of potential exposure to antimicrobials (Tikofsky et al., 2003; Sato et al., 2004; Rajala-Schultz et al., 2004; Berghash et al., 1983). Tikofsky et al., (2003) used ADD to study antimicrobial susceptibility of *S. aureus* isolated from cows on organic (n = 144) and conventional herds (n = 117). Significantly fewer isolates obtained from conventional herds were susceptible to ampicillin, penicillin or tetracycline. However, antimicrobial usage was not quantified. Sato et al. (2004) studied *S. aureus* isolated from bulk milk obtained from organic (Wisconsin n = 179; Denmark n = 75) and conventional (Wisconsin n = 152; Denmark n = 7) herds in Wisconsin and Denmark. Isolates with MIC higher than MIC<sub>90</sub> (MIC needed to inhibit 90% of isolates) were classified as isolates with reduced susceptibility. Isolates with a MIC lower than MIC<sub>90</sub> were termed as isolates with high susceptibility. The quantity of antimicrobial used by conventional farmers was unknown. The probability of having a resistant isolates in conventional herds was higher only for ciprofloxacin in Wisconsin and for avilamycin in Denmark. Significant differences were found in the susceptibility pattern among countries. Wisconsin isolates had a higher probability of reduced susceptibility (defined as MIC > MIC<sub>90</sub>) for bacitracin, gentamycin, kanamycin, penicillin, sulphamethoxazole, tetracycline and trimetoprim.

Rajala-Schultz et al. (2004) studied the antimicrobial susceptibility of CNS isolated from primiparous and multiparous cows using a microbroth dilution system. A total of 139 isolates were studied but the number of isolates by parity was not reported. The antimicrobial usage pattern in the single herd studied was not evaluated quantitatively. Penicillin, ceftiofur, cephapirin and dihydrostreptomycin were the drugs used more frequently. The authors hypothesized that first lactation cows might be exposed to a lower level of antimicrobial exposure due to differences in antimicrobial therapies among the two groups. The study failed to detect significant differences among primiparous and multiparous cows. However, a trend was observed in the differences of the proportion of CNS isolates resistant to penicillin.

One early study attempted to compare herds with exposures to different amounts of antimicrobials (Berghash et al., 1983). In that study, antimicrobial susceptibility of *Strep. agalactiae*, *Strep. spp.* and *S. aureus* was studied using agar dilution. Herds were classified as either high antibiotic-use or low antibiotic-use. The "high antibiotic-use" group used comprehensive DCT with cephapirin. The "low antibiotic-use" group used IMM antimicrobials only for treatment of clinical cases of mastitis. Isolates of *Strep. agalactiae* obtained "high antibiotic-use" (n = 53) presented high levels of resistance to  $\beta$ -lactams,

tetracyclines, aminoglycosides and novobiocin as compared to isolates obtained “low antibiotic use” herds (n = 49). No differences were found in the antimicrobial susceptibility of *S. aureus* isolates or *Strep* spp. among groups.

Pol and Ruegg (2006b) recently analyzed relationships between usage of antimicrobials on dairy farms and results of antimicrobial susceptibility testing of mastitis pathogens. Exposure to selected antimicrobials (n = 10) was standardized using DDD. Farms (n = 40) were categorized based on levels of antimicrobial exposure: Organic (no usage); Conventional - low usage, Conventional - high usage. The MIC of selected antimicrobials was determined using a microbroth dilution system for isolates of *Staphylococcus aureus* (n = 137), coagulase negative *Staphylococci* (n = 294) and *Strep. spp.* (n = 95) obtained from subclinical mastitis infections. Most isolates were inhibited at the lowest dilution tested of most antimicrobials. For *Staph. aureus* and CNS, the MIC was associated with level of exposure to penicillin and pirlimycin (Fig 1). For CNS, farm type was also associated with the MIC of ampicillin, and tetracycline. For *Strep spp.*, farm type was associated with MIC of pirlimycin and tetracycline. For all mastitis pathogens studied, the MIC of pirlimycin increased with increasing exposure to defined daily doses of pirlimycin. The level of exposure to most other antimicrobials was not associated with minimum inhibitory concentration of mastitis pathogens. Usage of two compounds commonly administered for treatment of intramammary infections (penicillin and pirlimycin) was associated with resistance of mastitis pathogens but usage of many other commonly used compounds was not. A dose-response effect between pirlimycin usage groups and pirlimycin MIC was observed for all isolates studied. The usage of penicillin was associated with reduced susceptibility of *Staph. aureus* (data not shown) and CNS isolates (Fig 1). However, the usage of cephapirin (a widely used antimicrobial for intramammary infections treatments) was not associated with reduced susceptibility of any of the studied pathogens.

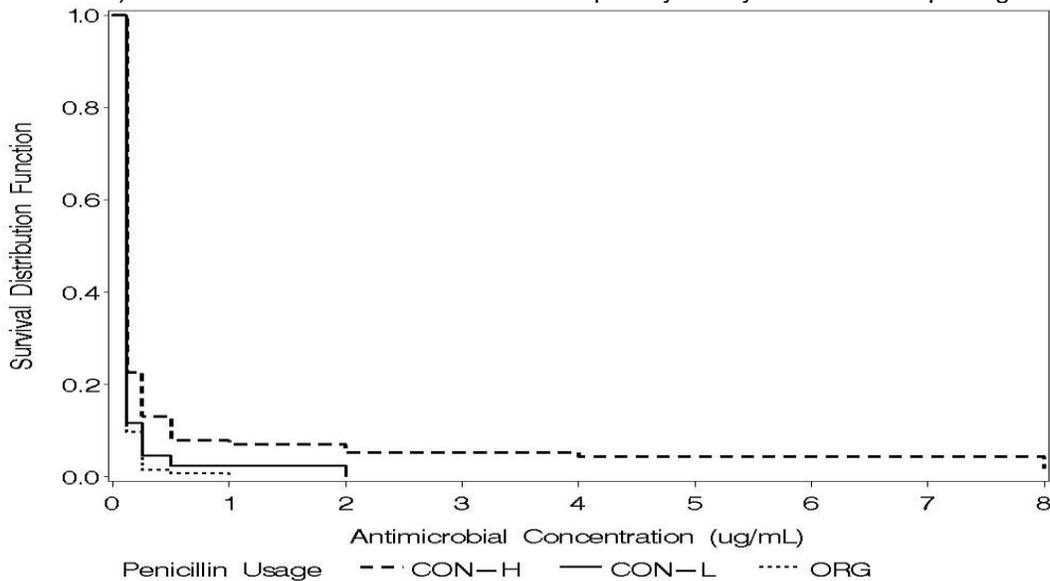


Figure 1. Kaplan-Meier plot showing survival proportion of CNS on organic farms , no use of penicillin or use  $\leq$  than the first quartile of usage (CON-L) or use  $>$  than the first quartile of usage (CON-H).

### Conclusions

Society will continue to be concerned about the development and transfer of antimicrobial resistance from animal agriculture and veterinarians will need to be responsive to their concerns. Antimicrobial usage is difficult to assess on dairy farms but recent studies indicate that many dairy cows are exposed to  $>5$  DDD/cow/year. The majority of antimicrobial usage on dairy farms is related to treatment and prevention of mastitis. The amount of exposure to some antimicrobials has been linked to increased resistance but exposure to other commonly used antimicrobials has not. While exposure to antimicrobial has been linked to resistance, there is no current evidence that resistance is increasing. Further studies should be designed to evaluate the temporal relationship of exposure and resistance and to determine optimal usage patterns of antimicrobials on dairy farms.

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Zwald AG, Ruegg PL, Kaneene JB, Warnick LD, Wells SJ, Fossler C, Halbert LW. 2004. Management practices and reported antimicrobial usage on conventional and organic dairy farms. *J Dairy Sci.* 87(1):191-201.

Most dairy farms occasionally require the use of antibiotics for treatment of sick animals (McEwen, et al., 1991). In a recent survey, 95% of conventional dairy herds (n = 99) reported administering at least 1 dose of antibiotics during the 2 months that preceded the interview (Zwald et al., 2004). Of the surveyed herds, 5% used no antibiotics, 85% administered antibiotics to <10% of their animals, 9% administered antibiotics to 11-25% of their animals and <1% administered antibiotics to >25% of their animals (Zwald et al., 2004). In an earlier survey, McEwen et al. (1991) estimated usage of selected antibiotics using a mail survey of Canadian dairy herds (<50 cows/herd). In that survey, 1.3-1.9 and 1.3-1.6 cows received intramammary and systemic antibiotics each month, respectfully. Approximately 20% of the herds reported the use of medicated feeds (McEwen et al., 1991). In another study, Danish dairy herds (n = 111) reported a median of 63 drug administrations per 100 calvings (Enevoldsen et al., 1996).

Antibiotics are used to treat a variety of diseases and 80% of conventional dairy herds reported the use of antibiotics for treatment of mastitis (Zwald et al., 2004). Mastitis was the first disease of dairy cattle to be treated with antibiotics and remains their most common disease; consequently, the most common reason for administering antibiotics to dairy cows is for treatment of mastitis (Mitchell, et al., 1998). A study of dairy herds in the Netherlands (n = 201) reported the use of 1.9 antibiotic treatments per case of clinical mastitis (Barkema et al., 1998).

Antibiotic residues in milk and milk products are a rare consequence of antibiotic usage on dairy farms. There have been a number of studies looking at reasons for antibiotic residues in milk (Booth and Harding, 1986, McEwen et al., 1991; Oliver et al., 1990; Wilson et al., 1998). The use of intramammary antibiotics and mistakes regarding withholding periods of milk are the most frequently cited reasons for antibiotic residues (McEwen et al. 1991, Wilson et al., 1998). In the U. S., public health is protected by regulations that prohibit the presence of antibiotics in milk intended for human consumption (Anonymous, 2001). The purpose of this paper is to review the relationship between the occurrence of antibiotic residues in milk and bulk tank somatic cell count level.

#### Antibiotic Residues in Milk and Human Health

The occurrence of antibiotic residues in milk intended for human consumption is undesirable for a number of reasons (Allison, 1985). As recently as 30 years ago, the presence of antibiotic residues in milk was considered primarily a manufacturing problem related to inhibition of cheese and yogurt starters (Cogan, 1972). More recently, the presence of antibiotics in milk has been prohibited because of concerns about public health. Initially, public health officials desired to protect hypersensitive individuals from exposure to specific antibiotics. More recently, attention has shifted to the potential for antibiotic residues in milk to contribute to the development and/or transmission of antibiotic resistant bacteria (Allison, 1985, Mitchell, et al., 1995, Mitchell et al., 1988)

Hypersensitivity to antibiotic residues in milk. Allergic reactions to antibiotics are well recognized and hypersensitivity to  $\beta$ -lactam compounds is especially prevalent (de Weck, 1983). The literature regarding allergic responses of humans after exposure to drug residues found in milk is sparse and focused primarily on risks associated with exposure to  $\beta$ -lactams (Boonk and van Ketel, 1981; Boonk and van Ketel, 1982; Borrie and Barrett, 1961; Dewdney and Edwards, 1984; Dewdney et al., 1991; Ormerod, et al., 1987; Vickers, et al., 1958; and Wicher et al., 1969). The immunological characteristics of most other drug classes (including macrolides, tetracyclines and aminoglycosides) makes the development of allergic responses to minute residues unlikely, although it is considered theoretically possible that exposure could result in clinically relevant immunological events (Dewdney and Edwards, 1984).

Allergic reactions to antibiotics develop when an individual is challenged by exposure after a primary sensitization has occurred. Oral administration of antibiotics does not stimulate as rapid or strong of an immunological response as compared to systemic administration and there is no scientific evidence that  $\beta$ -lactam residues present in milk have ever induced primary sensitization in humans (Dewdney and Edwards, 1984). Allergic reactions (dermatitis, pruritis and urticaria) of pre-sensitized individuals caused by  $\beta$ -lactam residues in milk have been documented for a small number of people (Dewdney and Edwards, 1984). Exposure to penicillin residues in milk has been reported as a cause of chronic urticaria (Boonk and van Ketel, 1981, Boonk and van Ketel, 1982, Ormerod, et al., 1987). In one early case report, a highly sensitive individual exhibited a number of generalized symptoms after ingestion of processed milk containing approximately 10 units/ml of penicillin (Wicher et al., 1969). Verified case reports after 1987 are apparently non-existent.

Transfer or development of resistant pathogens. At this point, the relationship between antibiotic residues in milk and the development or transfer of resistant pathogens appears to be hypothetical. There is some indication that mastitis pathogens isolated from dairy cattle with potential exposure to antibiotics may be less susceptible (but not necessarily resistant) than similar pathogens isolated from cattle located on organic dairy farms (Tikofsky et al., 2003). However, mastitis pathogens in general do not appear to be becoming more resistant (Erskine et al., 2002, Erskine, et al., 2004, Makovec and Ruegg, 2003). Direct transfer of resistant organisms to humans through consumption of milk is unlikely because most milk is pasteurized (Teuber and Perreten, 2000). Traditional methods of pasteurization reduce the quantity of bacteria present in milk to negligible levels but will not appreciably reduce the level of antibiotic residues (Moats, 1999). Milk can be contaminated with fecal pathogens that exhibit resistance to antibiotics and raw milk products have been implicated as mechanisms for transferring fecal pathogens from farm environments to humans (Kalman et al., 2000, Villar et al., 1999).

Ingestion of antibiotics present in milk can influence gut flora. Antibiotics present in milk have been shown to increase antibiotic resistance of gut flora in baby calves, but the levels fed were considerably above detection limits of current antibiotic screening tests (Langford et al., 2003). The likelihood of antibiotic residues to create a similar effect on human gut flora is considered extremely remote because of dilution and dairy process mechanization (Allison, 1985).

#### Prevalence of Antibiotic Residues in Bulk Tank Milk

Antibiotic residues occur in milk supplies throughout the world. In some relatively unregulated markets, antibiotic residues may exist in 8-15% of total bulk tank loads (Shitandi, and Sternesjo, 2004; Baynes et al., 1999). In the U. S., the dairy industry bears the primary responsibility for ensuring the safety of milk and milk products (Talley, 1999). The Food and Drug Administration (FDA) is responsible for verifying that the industry is complying with regulations and initiates regulatory action when necessary. The FDA has accepted appendix N of the Grade A Pasteurized Milk Ordinance (PMO) as the official reference regarding testing for drug residues in milk. Appendix N requires that every tanker of milk must be screened for  $\beta$ -lactam residues prior to unloading. Individual bulk milk samples from every farm are tested once monthly 4 times in every 6 month period. Additional random testing for other drug classes is also performed and individual state regulatory agencies or individual milk processors may test more frequently. Results of official drug testing are compiled annually in the National Milk Drug Residue Database (<http://www.cfsan.fda.gov/~ear/milkrp03.html>). The prevalence of positive test results for bulk milk tankers has been steadily declining (Fig 1) and 30% less milk was discarded in fiscal year 2003 as compared to FY 1999 (76,370,000 lbs (34,640,848 kg) versus 107,744,000 lbs (48,871,856 kg)).

In 2003 the value of milk discarded because of positive antibiotic test results exceeded \$7.6 million USD (@ \$10.00/cwt). Additionally, 8 of 54,932 antibiotic tests performed on pasteurized fluid milk and milk products were positive resulting in disposal of 64,000 lbs (29,030 kg) of finished products.

#### Relationship between Antibiotic Residues and SCC

The bulk tank somatic cell count (BTSCC) is used as a key indicator of milk quality and reflects the prevalence of subclinical mastitis in a dairy herd. The BTSCC is an indirect measure of the overall amount of mastitis that a herd is experiencing and herds with high BTSCC have been reported to have higher rates of clinical mastitis and to cull more mastitic cows (Rodrigues et al., 2004). In many regions, the BTSCC is used to define financial incentives paid for high quality milk and herds shipping milk

containing high levels of somatic cells may have a significant financial disadvantage (Rodrigues, et al., 2004). Under regulations contained in the PMO, BTSCC values are monitored for all farms. The current U.S. regulatory limit for SCC is 750,000 cells/ml (anonymous, 2001). Repeated violations of this limit result in significant financial penalties and potential loss of grade A status.

Farms experiencing consistently high BTSCC have considerable motivation to reduce the number of infected quarters. Treatment of infected quarters using antibiotics is one tactic used to control mastitis. The use of antibiotics introduces the risk of having an antibiotic residue. Farmers don't intentionally adulterate milk. Antibiotic residue violations occur primarily because of mistakes regarding withholding periods or identification of treated cows (Booth and Harding, 1986, McEwen et al., 1991). Investigators have consistently identified a relationship between BTSCC and the rate of antibiotic residue violations (Ruegg and Tabone, 2000; Sargeant, et al., 1998; Saville et al., 2000; van Schaik, 2002). In data obtained from both Wisconsin and Ohio, the rate of violative residues per 1000 herd years is clearly associated with BTSCC (Fig. 2).

In Wisconsin, data were analyzed for the period of Jan. 1995 through November 1998 and consisted of results of tests performed on 805,772 grade A and 176,763 grade B milk samples (Ruegg and Tabone, 2000). Herd-year SCC averages were used to classify herds (<250,000; 251,000 to 400,000, 401,000 to 550,000, 551,000 to 700,000, >700,000) and the relative risk of antibiotic residue by SCC class was determined. The arithmetic mean SCC values were 334,634 and 480,029 for grade A and grade B milk respectively. SCC values were significantly higher for samples with positive antibiotic residue tests for Grade A milk during all 4 years tested. SCC values were significantly higher for samples with positive antibiotic residue tests for Grade B milk for 3 of 4 years. The rate of antibiotic residue violation per 1000 herd-years increased with SCC class for both grade A and grade B milk. The relative risk of antibiotic residue violation by SCC class was 1.0, 1.43, 2.38, 2.78 and 7.10 for Grade A milk and 1.0, 1.11, 2.67, 4.33 and 5.43 for Grade B milk.

In Ohio, information was analyzed for 1994 through 1997 (Saville et al., 2000). Two separate data sets were assessed: 1) 16,831 herd-years of data obtained from a large milk marketing cooperative and 2) 12,042 herd-years of data obtained from the Ohio Dept. of Agriculture. In the milk marketing dataset, violative antibiotic residues occurred in 153 of 8441 (1.8%) farms included in the study. In the Ohio Dept. of Agriculture dataset, violative antibiotic residues occurred in 482 of 4,022 (12.0%) farms. The large difference in the violative rate was attributed to the regulatory function of the Ohio Dept. of Agriculture and perhaps to intervention by field personnel of the milk marketing cooperative. In both datasets, herds were classified into strata based on BTSCC (<400,000; 400,000 to 750,000; >750,000). The rate of antibiotic residue violation per 1000 herd-years increased for both datasets. The relative risk of antibiotic residue violation by SCC class was 1.0, 2.3 and 5.1 for milk cooperative data and 1.0, 1.3 and 2.2 for data from the Ohio Dept. of Agriculture.

Other researchers have reported similar results. Data obtained from all herds in Ontario was analyzed for the period between March 1985 and July 1994 (Sargeant et al., 1998). The rate of antibiotic residue violations by SCC category was: 1.6% (<150,000); 1.6% (150,000-299,000); 3.4% (300,000-499,000); 3.7% (450,000-599,000) and 5.7% (>600,000). In data analyzed from five large milk plants operating in New York State, farms with SCC levels >750,000 had a much greater rate of antibiotic residue violations as compared to herd producing higher quality milk (van Schaik, et al., 2002).

#### Conclusion

Adulteration of milk supplies with antibiotics is clearly undesirable and the regulation of milk supplies to prohibit antibiotic residues is useful to protect public health. Researchers have identified a consistent relationship between BTSCC and the occurrence of violative antibiotic residues. Interventions that reduce the prevalence of subclinical mastitis and therefore reduce the need for antibiotics may have an added benefit of further reducing the risk of violative residues.

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The first usage of antimicrobials in veterinary medicine was for treatment of mastitis in dairy cows (Mitchell et al., 1998a). Antimicrobial drugs are used on dairy farms as therapeutics and prophylactics. Therapeutic usage is intended to treat bacterial infection associated with disease such as pneumonia, metritis, and mastitis (Schwarz et al., 2001). The use of prophylactic antimicrobials is for treating healthy animals to prevent a disease during periods of increase susceptibility. In specific cases, antimicrobials may be used for therapeutic and prophylactic

purposes. For example, the purpose of antimicrobial dry cow therapy (DCT) is to cure existing intramammary infections and also prevent new infections (Smith et al., 1967). Comprehensive intramammary treatment using long acting antimicrobials is widely used on dairy farms. Antimicrobials used as DCT contain high levels of one or more antimicrobials in a slow release base which will maintain therapeutic levels in the dry udder for a significant length of time (National Mastitis Council, 1999). Of Wisconsin dairy farmers participating in a milk quality improvement program (n = 180), 93% reported treating all quarters of all cows at dry-off with antimicrobials (Rodrigues et al., 2005). In a national study in U.S., 72% of farmers reported use of intramammary antimicrobials at dry-off in all cows (NAHMS, 2007). The most commonly used antimicrobials given to dry cows were cephalosporins (31 % of cows) and penicillin G (procaine)/dihydrostreptomycin (37% of cows) (NAHMS, 2007). Penicillin G (procaine)/dihydrostreptomycin was also the most common antimicrobial reported used by farmers in Wisconsin (Pol and Ruegg, 2007b) and Washington (Mollet et al., 1997).

Clinical illness has been reported as the main reason for initiating antimicrobial treatment on dairy farms (Friedman et al., 2007). The most frequent diseases affecting adult cows reported by farmers are mastitis, lameness, pneumonia, and reproductive tract infections (USDA, 2007b), and because of their infectious nature, these diseases are commonly treated with antimicrobial drugs. The most common antimicrobials used on dairy farms include 6 classes: 1.  $\beta$ -lactams (e.g., penicillin and cephalosporin), 2. Tetracyclines (e.g., oxytetracycline and tetracycline), 3. Macrolides (e.g., erythromycin), 4. Sulfonamides (e.g., sulfadimethoxine), 5. Lincosamides (e.g., pirlimycin), and 6. Amphenicols (e.g., florfenicol).

Several surveys have reported antimicrobial usage of dairy herds in different regions

(Zwald et al., 2004; Sawant et al., 2005; Raymond et al., 2006; Pol and Ruegg, 2007b; Hill et al.,

2009). All studies reported use of similar antimicrobial drugs has been reported to treat selected disease and beta-lactams and tetracyclines were the most widely used antimicrobials (Table 1.4). All studies reported mastitis and foot rot as the main reasons for antimicrobial treatment and all conventional farms included in these studies reported treatment of animals for these reasons (Table 1.4). Overall, proportion of lactating cows that were treated for clinical mastitis varied among studies and has been reported as 14% to 38% of lactating cows during an one year period, with exception of one study that collected the data relative to 3 months prior to the farm visit (Raymond et al., 2006) (Table 1.4). In most studies, the most frequently used intramammary antimicrobial to treat clinical mastitis was cephalosporin but these studies were conducted before approval of intramammary ceftiofur (Table 1.4). Between 19% and 30% of

the lactating cows were treated with antimicrobials for foot problems and the most frequently used compound was ceftiofur. Less respiratory disease in adult cows was reported compared to mastitis (about 3% of lactating animals received antimicrobial treatment) and the most frequently used compound was also ceftiofur (Table 1.4). Ceftiofur has an advantage compared to other antimicrobials because there is no milk withholding period. Between 5% and 14% of lactating animals were treated for reproductive disease using antimicrobials. The most frequently used compounds for treatment of reproductive disease differed among studies. About 2% of lactating animals were treated for diarrhea using antimicrobials. In one study in Pennsylvania, cows were treated with spectinomycin for diarrhea which is an extra-label usage as it is only labeled to treat pneumonia in cattle (Sawant et al., 2005). Extra-label usage refers to any use of a drug not specifically listed on the label and it is only legal under the guidance of a veterinarian (FDA, 2011). Among these studies, only Pol and Ruegg (2007b) quantified antimicrobial drugs by calculating herd-level the defined daily doses (DDD). Defined daily dose is a standard measure of antimicrobial

consumption frequently used in health human and developed by World Health Organization (Chauvin et al., 2001). This standard method permits comparison of the exposure to different compounds, regardless of indication route or units (i.e., mg and IU) (Pol and Ruegg, 2007b). The data necessary to calculate the DDD of a specific antimicrobial include dose (based on FDA- approved label dosages), frequency, duration of treatment, and prevalence of treatment; but it is independent of formulation, package size, and market price (Chauvin et al., 2001; Pol and Ruegg, 2007b). This data is difficult to obtain as many dairy farmers do not record all antimicrobial usage in permanent records that may result in underestimation of antimicrobial usage. In one study, the use of treatment records was associated with herd size (Hoe and Ruegg, 2006). In that study, 66% of responders from large herds in Wisconsin (> 200 animals) recorded treatments in computers compared to only 18% of responders from medium herds (< 200 animals). Pol and Ruegg (2007b) used an extensive questionnaire with picture aids and the estimated overall exposure to antimicrobial drugs was 5.43 DDD per cow per year and mastitis treatment accounted for the greatest proportion of DDD compared to other selected diseases. However, only 20 conventional herds were included in that study and the population included only farms with a 6 months average bulk tank SCC equal or greater than 250,000 cells/mL. This criterion may have selected farms that used more intramammary antimicrobials as compared to farms with a lower bulk tank SCC. Extra-label usage and usage of illegal antimicrobials have been reported among studies. Illegal antimicrobial drugs are prohibited from extra-label use in food animals even under a veterinarian prescription. The extra-label uses may increase antimicrobial resistance of pathogens than can cause human illness. A survey was administered in 381 herds in Washington

State, and 23% reported one or more unapproved or prohibited uses of antimicrobial, which the

most common compounds cited were gentamicin, neomycin, and florfenicol (Raymond et al., 2006).

Introduction of antimicrobial therapy has led bacteria to adapt defenses (Levy, 1992). The use of antimicrobials in food producing animals elicits concern from public health authorities about potential development of antimicrobial resistant pathogens. Particular emphasis is being placed on antimicrobial consumption, and potential associations between antimicrobial usage

and the emergence of resistance is necessary to control the spread of such resistance. Use of a standard measure to quantify antimicrobial usage on farms is important to compare the usage among farms and different studies. Quantification of antimicrobial usage at cow level has been not previously reported and the quantity of antimicrobials administered for a cow during her life may be related to antimicrobial resistance of pathogens isolated from this cow.

#### 1.8 ANTIMICROBIAL RESISTANCE

Resistant bacterial infections decrease therapeutic options, increase the cost of health care, and can contribute to increased morbidity and mortality (Levy, 1992). Bacteria may be intrinsically resistant to a specific antimicrobial class due a lack of binding sites or other pharmacological characteristics. Intrinsic resistance is natural to all members of a bacterial group and could be a significant clinical problem in some circumstances but is not a major public health issue (Neu, 1992). Antimicrobial resistance can also be acquired, either by chromosomal mutations or by exchange of genetic material via plasmids, transposons or integrons (Neu, 1992). A bacterium is considered to be resistant to an antimicrobial drug when the concentration at the site of infection is not sufficient to either kill or inhibit replication of the bacteria (Schwarz and Chaslus-Dancla, 2001). Bacteria have developed various mechanisms of resistance, and in a given bacteria several mechanisms may be responsible for resistance to a single antimicrobial

drug: 1) the bacteria may acquire genes that encode enzymes that inactivate the antimicrobial before it can have an effect. The primary mechanism of resistance to beta-lactam, aminoglycosides, and phenicols is via this mechanism (Tenover, 2006); 2) bacteria may acquire efflux pumps that extrude the antimicrobial drug from the cell (tetracycline, macrolides, lincosamide, and phenicols); 3) bacteria may acquire several genes that modify or replace the drug target (penicillin, macrolides, lincosamides, and quinolones); 4) reduced drug intake results in insufficient concentration of the drug at the site of infection (beta-lactams, tetracycline, and cloramphenicol); 5) drug trapping then the antimicrobial drug could not active the target (sulphonamides); 6) drug target protection (tetracycline and quinolone) (Schwarz and Chaslus- Dancla, 2001; Tenover, 2006). While the overall prevalence of resistance to most intramammary compounds is low (Pol and Ruegg, 2007a; Schmitt-van de Leemput and Zadoks, 2007; Sawant et al., 2009; Oliveira et al., 2012), determination of resistance is an important tool for monitoring the potential development of antimicrobial resistance (Pol and Ruegg, 2007b).