# FACTORS AFFECTING THE SUCCESS OF ANTIBIOTIC TREATMENT AT DRY OFF

Ynte Schukken[1], Linda Tikovsky[1], David Wilson[1], and Olav Østerås[2]
[1]Cornell University, Ithaca, New York
[2]TINE Dairies Association, Ås, Norway

## Introduction

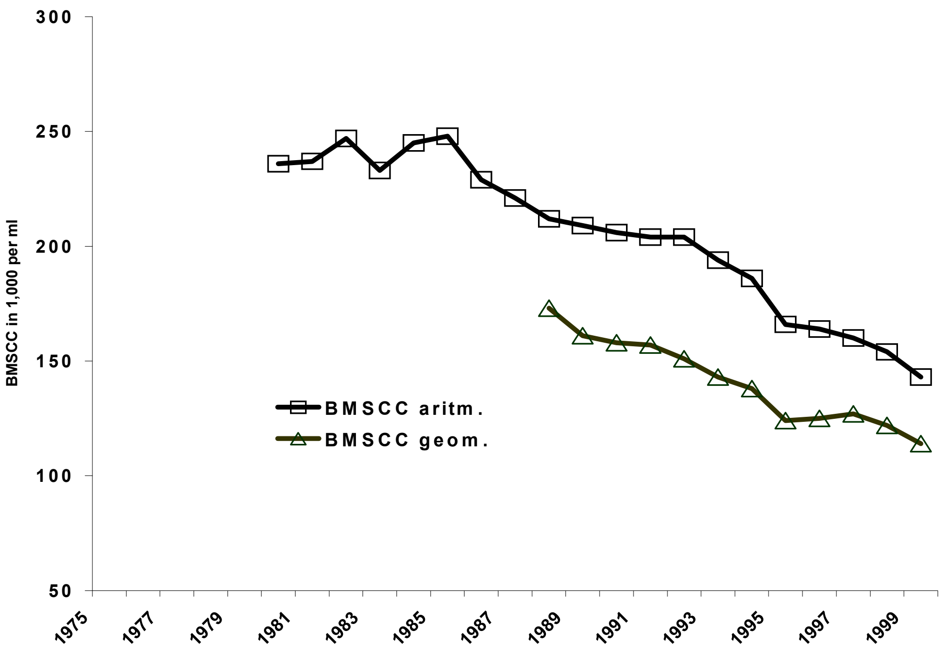
Dry cow therapy is widely used at the start of the dry period. Currently the National Mastitis Council advises to treat all quarters of all cows with antibiotics at the time of dry off. The goal of dry cow therapy is to cure existing intramammary infections (IMI) at the time of dry off, and to prevent new infections in the early weeks of the dry period. As with all treatments, there is no guarantee of success. It is currently believed that of those cows that are infected at drying off with major pathogens, the proportion that cure infection due to dry cow treatment is approximately 40% to 60% (Browning et al. 1990, Erskine et al. 1994, *Østerås* et al. 1999, Sol et al. 1994). This means that anywhere between 40% to 60% of cows infected with major pathogens at drying off do not cure during the dry period.

Similarly, dry cow treatment with antibiotics prevents new infections in the dry period with major pathogens in approximately 5% to 10% of cows (Hassan et al. 1999, Schukken et al. 1993). Hence, anywhere between 90% and 95% of non-infected cows will not develop a new infection whether treated with antibiotics at dry-off or not. In herds with a very low somatic cell count, or in countries with very low somatic cell counts (such as Norway), it is reasonable to assume that very few cows are infected with major pathogens at the time of dry-off. The National mean Bulk Milk Somatic Cell Count in Norway is shown in Figure 1. In herds with very low bulk milk somatic cell counts treatment at the time of dry off would mostly be geared towards prevention of new infections because these herds have relatively few existing IMI's (Berry et al. 1997).It would be of great value if cows that were more likely to become infected with mastitis pathogens in the early dry period could be identified. Some factors affecting new intramammary infection risk have been identified, but more work needs to be done in this area (Schukken et al. 1993).

In cows infected with major mastitis pathogens the outcome of treatment is either cure or non-cure, and it would be of value to understand why cows cure or don't cure. Cure following treatment is likely not a random event. It would be of great value to be able to predict which cows are more or less likely to be cured of IMI. In recent years a number of studies have been published that have specifically looked at factors affecting the outcome of dry treatment in cows infected with major pathogens (Sol et al. 1994, *Østerås* et al. 1999, *Østerås* and Edge 2000).

Because antibiotic treatment in farm animals is currently under greater scrutiny then ever before, and essentially dry cow treatment is the only blanket use of antibiotics in the dairy industry, it is of value to consider the opportunities for a somewhat selective and more rational use of dry cow antibiotic treatment. It is important to emphasize that most US dairies with average milk quality would still benefit from dry cow treating all or almost all of their cows. In this presentation we will attempt to summarize the factors associated with the success of dry cow therapy, and evaluate whether these factors could be used as predictors of the likelihood of cure.

Figure 1. Mean Bulk Milk Somatic Cell Count in Norway from 1980 to 2000




Factors associated with the success of treatment will be divided into cow, bacterial and treatment factors.

Cow Factors

Important cow factors that are associated with bacterial cure of major pathogens (in most herds either S. aureus or environmental streptococci) are especially the age of the cow, the mean somatic cell count before dry off, and the number of quarters infected (Sol et al. 1994, *Østerås* et al. 1999, *Østerås* and Edge 2000). Older cows, cows with higher cell counts at dry off and cows with more then one quarter infected were shown to be less likely to cure. The relationship between cure of S.aureus and age and cell count before dry off is depicted in Figure 2. It can be seen that these factors appear to have a strong impact on the likelihood of cure (Sol et al. 1994). As an example, a cow in $4^{th}$ parity with a cell count at drying off of 8,000,000 and two quarters infected has a likelihood of cure of 8%, where as a heifer with one quarter infected and a cell count of 2,000,000 has a likelihood of cure of 83%.

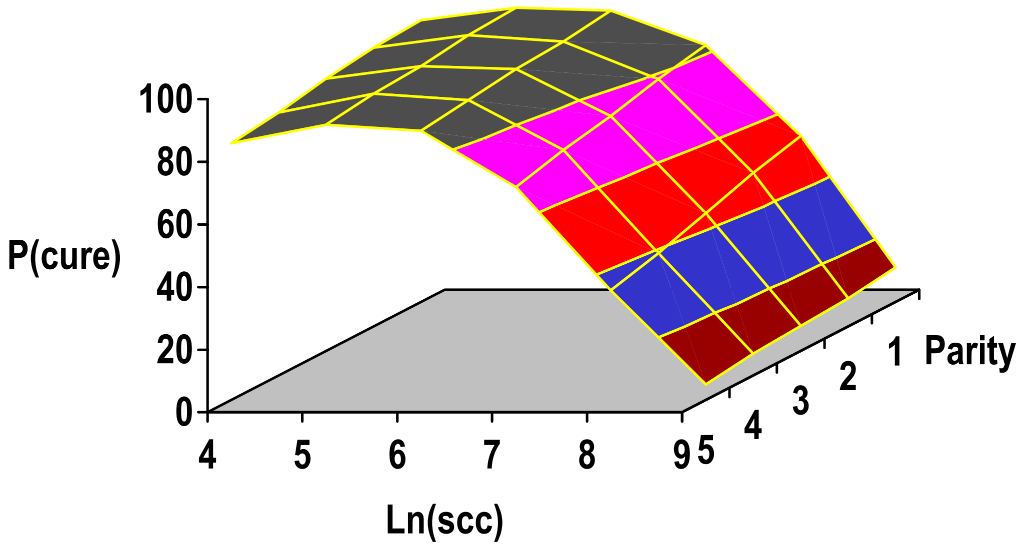



Figure 2. Relationship between cure and the natural logarithm of somatic cell count before dry off and the parity of the cow at dry off.

In Figure 2 the probability of cure is depicted for cows where the logarithm of the last SCC before dry treatment varied from 4 to 9, and parity varied from 1 to 5. This graph is created using the prediction equation:

$$P(\text{cure}) := \frac{1}{1 + e^{-\left[\left(\left(-5.57 + 3.55 \cdot \text{lnscc} - .34 \cdot \text{lnscc}^2 - .19\ \text{par} - .56 \cdot \text{nquart}\right)\right)\right]}}$$

Additionally, the presence of clinical mastitis in the current lactation was also found to be associated with a lower probability of cure (*Østerås* and Edge 2000). This is currently not included in the prediction equation above, but would need to be incorporated.

Bacterial Factors

Studies that evaluated bacterial factors associated with cure after antibiotic therapy, or with the chronicity of infection have focused mostly on *S.aureus* (Fitzgerald et al. 2000, Mullarky et al. 2001, Owens et al. 1997, Pyörälä and Pyörälä 1998, Pyörälä et al. 2000, Sol et al. 1997, Sol et al. 2000, Tollersrud et al. 2000). The components of pathogenicity that have been studied in greatest detail are antibiotic susceptibility (Pyörälä and Pyörälä 1998, Pyörälä et al. 2000, Sol et al. 1997), capsule and slime formation (Sutra and Poutrel 1994) and production of enterotoxins (Fitzgerald et al. 2000, Mullarky et al. 2001, Tollersrud et al. 2000).

Antibiotic susceptibility has been reported to be associated with cure after treatment (Sol et al. 1997, Pyörälä et al. 2000, Owens et al. 1997). Most of these studies used results from treatment of clinical or subclinical cases, not necessarily associated with dry cow treatment. Presence of penicillin resistance, or ß-lactamase production by the S.aureus isolate was predictive for substantially lower cure rates. Pyörälä et al. (2000) reported a cure rate of 69% in penicillin sensitive strains versus 30% in strains that were penicillin resistant. All strains that were penicillin sensitive were treated with procaine penicillin G, whereas penicillin resistant strains were treated with a product containing amoxicillin – clavulanic acid. Similar results were reported by Sol et al. 1997, where penicillin resistance was also significantly associated with lower cure rates. In this study all treatments were done with antibiotic products that are not broken down by ß-lactamase. Penicillin sensitive strains had a cure rate of 58.7% while penicillin resistant strains showed a cure rate of 40.3 % ($p<.05$). Despite the use of appropriate antibiotics, the penicillin resistant strains were less likely to cure. The mechanism responsible for this phenomenon is not understood. In recent publications the presence of pathogenicity islands in the genome of S.aureus have been identified. These pathogenicity islands carry several genes that code for pathogenic characteristics (Fitzgerald et al. 2001). Hence, clusters of antibiotic resistance genes might be present in the genome of S.aureus. However, it has not been shown yet that multiple antibiotic resistance genes are coded on these genomic islands.

Production of enterotoxins by S.aureus has been studied for a number of years. A recent Norwegian study (Tollersrud et al. 2000) did not find an association between production of enterotoxins (A, B, C, D and TSST) and clinical mastitis outcome. A study from Ireland also observed no statistically significant relationship between enterotoxin (enterotoxin A and B, TSST and C) production and clinical mastitis outcome (Fitzgerald et al. 2000), although there appeared to be a weak relationship between enterotoxin C and a more severe clinical mastitis outcome. A US study reported a relationship between the production of enterotoxin A and neutrophil killing ability (Mullarky et al. 2001). Enterotoxins have also been associated with modulating the immune response of susceptible lymphocytes (Ferens et al. 1998). These recent studies show varying results with regard to a possible correlation between toxin production and clinical outcome of an intramammary infection. However, ongoing research may show that these enterotoxins are indeed involved in the clinical pathogenicity of S.aureus.

## Antibiotic Treatment Factors

A proper choice of antibiotics has been shown to be somewhat associated with the expected cure of IMI at the time of dry off or during lactation (Browning et al. 1990, Erskine et al. 1994, Owens et al. 1988, Sandholm et al. 1990, Soback et al. 1990, Wilson et al. 1999). There is some value in matching the in vitro antibiotic susceptibility to the choice of antibiotics for in vivo treatment (Owens et al. 1988). This is especially the case for S.aureus isolates that produce ß-lactamase. Combination therapy at dry off, whereby intramammary treatment is combined with parenteral treatment was shown to increase the probability of cure (Sol et al. 1997), although a carefully controlled trial by Erskine et al. (1994) did not show a significant beneficial effect of added parenteral therapy with oxytetracycline.

<u>Other Factors</u>

Additional factors that have been associated with observed cure included herd level factors such as hygiene (Sol et al. 1994), bulk milk somatic cell count level (*Østerås* and Edge 2000), number of new infections as judged by increase in somatic cell count (*Østerås* and Edge 2000) and observed prevalence of S.aureus in the herd. One of the reasons for this might be the risk of re-infection in herds with high prevalence of infection. Hence, treatment might result in cure, but due to a high risk of new infection the post-partum samples still show the presence of pathogenic bacteria. This might then be a situation of misclassification due to inappropriate diagnostics. One way of trying to understand this better would be the use of genetic techniques to try and distinguish different isolates before and after treatment from one another. This more precise diagnostic tool would allow a distinction between a combination of cure of strain A and a new infection with strain B, something that would be called a treatment failure when using conventional diagnostics.

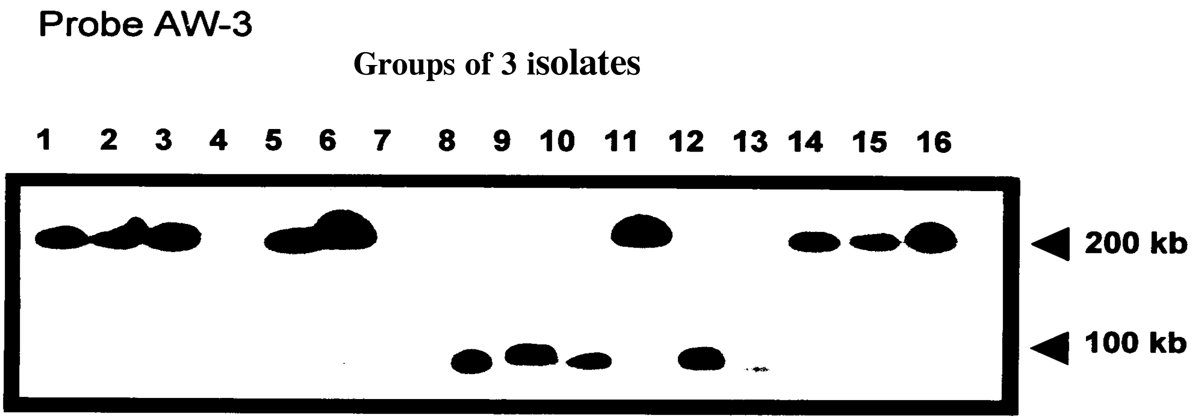



Figure 3. Binary typing of S.aureus isolates from a single herd. The numbers above the box indicate the isolate numbers (from Zadoks et al. 2000).

For example, using the coding of figure 3, isolates 1,2,3 would be from the same quarter of the same cow before and after treatment, this would be correctly classified as a treatment failure (the same is true for 5,6,7 and 8,9,10 and 14,15,16. In the case of isolates 11 (before treatment) and 12 and 13 (after treatment) this would be a situation of a cure and a new infection combined. There would be a real cure, but this would be classified as a treatment failure using conventional bacteriological diagnostics.

Clearly, an important factor that affects the observed cure of IMI in known infected cows is the quality and precision of diagnostics. On one hand is the option for underestimating treatment failure when only a single sample is evaluated post treatment (Sol et al. 1994), on the other hand is the option for overestimation of treatment failure as illustrated above.

## Discussion

Cure after dry cow therapy is not a random event ('coin toss'). There are a number of known factors that affect the probability of being cured after antibiotic treatment. Among the most important factors are the age of the cow, cell count level before dry-off, the number of quarters infected, the occurrence of clinical mastitis in the same lactation, being infected with a penicillin resistant strain of S.aureus, and being treated in a herd with a known high prevalence or incidence of infection. These factors can be used to provide an algorithm that will predict the probability of cure for every individual cow. Using such an algorithm would be able to distinguish between cows that could be treated and have a high probability of cure, and cows where treatment would most likely not result in cure. The cows in this latter group that are infected with contagious mastitis bacteria would need to be culled or segregated from the herd to prevent further spread of infection.

Antibiotic treatment at dry off is an important tool to manage the mastitis situation in herds. It is an excellent tool in many herds where it is used to treat existing infection and prevent new infections in the early dry period. The current advice is to treat all quarters of all cows with antibiotics. In this paper we consider that using a number of cow, bacteria and herd characteristics it may be possible to more precisely allocate treatment to cows that are expected to cure. This would be especially useful when blanket use of dry cow antibiotics is coming under increasing scrutiny or when producers want to fine tune the use of antibiotics in their herd.

## References

Baselga R., I. Albizu, and B. Amorena. 1994. Staphylococcus aureus capsule and slime as virulence factors in ruminant mastitis A review Vet. Microbiol. 39:195.

Berry S.L., J. Maas, J.H. Kirk, J.P. Reynolds, I.A. Gardner, and A. Ahmadi. 1997. Effects of antimicrobial treatment at the end of lactation on milk yield, somatic cell count, and incidence of clinical mastitis during the subsequent lactation in a dairy herd with a low prevalence of contagious mastitis. J Am Vet Med Assoc. 211:207.

Browning J.W., G.A. Mein, M. Barton, T.J. Nicholls, and P. Brightling. 1990. Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. Aust Vet J. Dec. 67:440.

Erskine R.J., P.C. Bartlett, P.C. Crawshaw, and D.M. Gombas. 1994. Efficacy of intramuscular oxytetracycline as a dry cow treatment for Staphylococcus aureus mastitis. J Dairy Sci. 77:3347.

Ferens W.A., W.C. Davis, M.J. Hamilton, Y.H. Park, C.F. Deobald, L. Fox, and G. Bohach. 1998. Activation of bovine lymphocyte subpopulations by staphylococcal enterotoxin C. Infect Immun. 66:573.

Fitzgerald J.Rr, S.R Monday, T.J. Foster, G.A. Bohach, P.J. Hartigan, W.J. Meaney, and C.J. Smyth. 2001. Characterization of a putative pathogenicity island from bovine staphylococcus aureus encoding multiple superantigens. J Bacteriol. 183:63.

Hassan Z., R.C. Daniel, D. O'Boyle, and A.J. Frost. 1999. Effects of dry cow intramammary therapy on quarter infections in the dry period. Vet Rec. 145:635.

Mullarky I.K., C. Su, N. Frieze, Y.H. Park, and L.M. Sordillo. 2001. Staphylococcus aureus agr Genotypes with Enterotoxin Production Capabilities Can Resist Neutrophil Bactericidal Activity. Infect Immun. 69:45.

*Østerås* O, and V.L. Edge. 2000. Factors prior to dry period associated with high and low levels of cow milk somatic cell counts in next lactation. Acta Vet Scand. 41:63-77.

*Østerås* O., and L. Sandvik. 1996. Effects of selective dry-cow therapy on culling rate, clinical mastitis, milk yield and cow somatic cell count. A randomized clinical field study in cows. Zentralbl Veterinarmed [B]. 43:555.

*Østerås* O., S.W. Martin, and V.L. Edge. 1999. Possible risk factors associated with penicillin-resistant strains of Staphylococcus aureus from bovine subclinical mastitis in early lactation. J Dairy Sci. 82:927.

*Østerås* O., V.L. Edge VL, and S.W. Martin. 1999. Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J Dairy Sci. 82:1221.

Owens W.E., C.H. Ray, J.L. Watts, and R.J. Yancey. 1997. Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. J Dairy Sci. 80:313.

Owens W.E., J.L. Watts, R.L. Boddie, and S. C. Nickerson. 1998. Antibiotic Treatment of Mastitis: Comparison of Intramammary and Intramammary Plus Intramuscular Therapies J. Dairy Sci. 71:3143.

Pyörälä S., S. Taponen, A. Jantunen, and E. Pyörälä. 2000. Efficacy of targeted 5-day parenteral and intramammary treatment of clinical *staphylococcus aureus* mastitis caused by penicillin-susceptible or penicillin-resistant bacterial strain. Proc. World Buiatrics Congress, Punta del Esta, p6501-6504.

Pyörälä S.H., and E.O. Pyörälä. 1998. Efficacy of parenteral administration of three antimicrobial agents in treatment of clinical mastitis in lactating cows: 487 cases (1989-1995). J Am Vet Med Assoc. 212:407.

Pyörälä S, and J. Syväjärvi. 1987. Bovine acute mastitis. Part II. Effect of mastitis pathogen, initial infammatory reaction and therapy on the outcome of the disease J. Vet. Med. B 34:629

Sandholm M., L. Kaartinen, S. Pyörälä S. 1990. Bovine Mastitis - Why does antibiotic therapy not work? An overview J. vet. Pharmacol. Therap. 13:248.

Schukken Y.H., J. Vanvliet, D. Vandegeer, and F.J. Grommers. 1993. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. J Dairy Sci 76:2925.

Soback S., G. Ziv, M. Winkler, and A. Saran. 1990. Systemic dry cow therapy--a preliminary report. J Dairy Sci. 73:661.

Sol J., O.C. Sampimon, H.W. Barkema, and Y.H. Schukken YH. 2000. Factors associated with cure after therapy of clinical mastitis caused by Staphylococcus aureus. J Dairy Sci. 83:278.

Sol J., O.C. Sampimon, J.J. Snoep, and Y.H. Schukken. 1997. Factors associated with bacteriological cure during lactation after therapy for subclinical mastitis caused by Staphylococcus aureus. J Dairy Sci. 80:2803.

Sol J., O.C. Sampimon, J.J. Snoep, and Y.H. Schukken YH. 1994. Factors associated with bacteriological cure after dry cow treatment of subclinical staphylococcal mastitis with antibiotics. J Dairy Sci. 77:75.

Sutra L., and B. Poutrel. 1994. Virulence factors involved ion the pathogenesis of bovine intramammary infections due to *Staphylococcus aureus* J. Med. Microbiol. 40: 79.

Tollersrud T., K. Kenny, D.A. Caugant, and A. Lund. 2000. Characterisation of isolates of Staphylococcus aureus from acute, chronic and subclinical mastitis in cows in Norway. APMIS. 108:565.

Zadoks R, W. van Leeuwen, H. Barkema, O. Sampimon, H. Verbrugh, Y.H. Schukken, and A. van Belkum. 2000. Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human Staphylococcus aureus isolates. J Clin Microbiol. 38:1931-9.

Wilson D.J., R.N. Gonzalez, K.L. Case, L.L. Garrison, and Y.T. Grohn. 1999. Comparison of seven antibiotic treatments with no treatment for bacteriological efficacy against bovine mastitis pathogens. J Dairy Sci. 82:1664.

Ziv G., and M. Storper, Intramuscular treatment of subclinical staphylococcal mastitis in lactating cows with penicillin G, methicillin and their esters J. vet. Pharmacol. Therap. 1985, 8: 276-283.