Presented at: World Buiatrics Congress, Santiago Chile. November 14-18, 2010.

The Application of Evidence Based Veterinary Medicine to Mastitis Therapy

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Introduction

While considerable progress has been made in controlling contagious mastitis, mastitis continues to be the most frequent and costly disease of dairy cows. In some countries Staph aureus remains a significant cause of mastitis (Unnerstad, et al., 2009) while in other areas, widespread implementation of effective control measures has significantly reduced its' prevalence (Makovec and Ruegg, 2003; Pitkala et al., 2004). Control of mastitis caused by Streptococcus agalactiae and Staphylococcus aureus has resulted in reductions in bulk tank somatic cell count (SCC) but many herds continue to struggle with treatment of clinical mastitis caused by environmental pathogens (Table 1). Common environmental mastitis pathogens include both Gram negative bacteria (such as E. coli and Klebsiella spp.) and Gram positive bacteria (such as Streptococcus uberis and Streptococcus dysgalactiae). Environmental pathogens tend to be less adapted to survival in the udder and infection often triggers an immune response that results in mild or moderate clinical symptoms. The duration of infection with environmental pathogens is associated with the degree of host adaptation of the pathogen. Some environmental pathogens (such as most E. coli), are truly opportunistic and the immune response successfully eliminates them after a brief period of mild clinical disease. Other environmental pathogens (such as Streptococci spp) have become more host adapted and may present as mild clinical cases that erroneously appear to resolve when the case has actually returned to a subclinical state. Both of these scenarios make it very difficult for the veterinary practitioner to discern success of mastitis treatments.

Most cases of clinical mastitis are mild to moderate in severity (Table 2), and are not examined by veterinarians. On many farms, detection, diagnosis and administration of treatments for mild and moderate cases of clinical mastitis are the responsibility of farm personnel and veterinarians are often consulted only when a case becomes life-threatening. It is vitally important for veterinarians to be involved in developing and evaluating treatment protocols for clinical mastitis but the ability to assess the results of treatment is often limited because of inadequate records (Hoe and Ruegg, 2006). The purpose of this paper is to review principles of evidence-based veterinary medicine and to discuss the application of these principles to treatment of clinical mastitis.

Definition of Evidence Based Veterinary Medicine

Evidence based veterinary medicine (EBVM) is an application of the principles of evidence based medicine used by physicians to clinical decision making for animals receiving veterinary care. One proponent of evidence based medicine has stated "Evidence based medicine is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients. The practice of evidence based medicine means integrating individual clinical expertise with the best available external clinical evidence from systematic research" (Sackett, et al., 1996). When applied to veterinary medicine, one definition of EBVM is "the use of current best evidence in making clinical decisions (Cockcroft and Homes, 2003)." On a practical basis, EBVM is an attempt to use results of research to make better treatment decisions for individual cases. The types of research that are most applicable for EBVM are the results of studies that help to improve diagnoses (such as epidemiological studies that define sensitivity and specificity of diagnostic tests) and the results of clinical trials that help to better define the effects of treatments or prognoses of specific diseases. Practitioners interested in applying concepts of EBVM seek out specific information that will help better define the course of treatment for individual clinical cases (Holmes and Cockcroft, 2004). The use of EBVM is growing and there are now websites (www.ebvm.org) and veterinary organizations devoted to promotion of these concepts.

Bovine practitioners who are interested in applying EBVM to mastitis therapy may pose a clinical question such as "Is the use of intramammary (IMM) treatment plus a non-steroidal anti-inflammatory for this case of clinical mastitis better than the use of IMM treatment alone?" According to Cockcroft and Holmes, (2004), this clinical question would then be separated into 4 elements: 1) Patient. How would you describe a group of similar patients? For example, Holstein cows in mid-lactation with mild cases of mastitis caused by E. coli; 2) Intervention. What are the interventions that are being considered? For example, IMM treatment using a commercially available 3rd generation cephalosporin plus the use of flunixin meglumine; 3) Comparison. What is the alternative that is being considered? For example, use of only IMM treatment with commercially available 3rd generation cephalosporin; and 4) Outcome. What benefits or disadvantages are known? Is there a clinical, economic or welfare advantage to one of the therapies? For example, do animals receiving both therapies return to milk earlier, have fewer side effects (such as pain) or produce more milk? After the questions are defined, the practitioner seeks out appropriate sources of external evidence. The hierarchy of research evidence ranges from the weakest evidence (such as opinions and testimonials) to the strongest (such as systematic review of randomized clinical trials). The use of systematic reviews and meta-analyses that are based on

results of blinded randomized clinical trials (RCT) are considered the gold standard of evidence but there are only a few of these types of studies that address bovine mastitis therapy.

Finding Evidence for Clinical Decision Making For Treatment of Bovine Mastitis

Finding accessible and appropriate evidence upon which to base treatment decisions is the foundation of evidence based medicine. As of June, 2010, the U.S. online registry of human clinical trials (www.clincialtrial.gov) listed 91,083 current clinical trials located in 173 countries. For almost any human disease, hundreds of published clinical trial results can be found in the online U.S. Library of Medicine database (www.pubmed.org). In contrast, the limited scope of research to address many veterinary diseases is a weakness of EBVM and this is especially evident for bovine mastitis. Performing an English language literature search for the term "bovine mastitis clinical trials" using PubMed or Google Scholar results in <300 peer reviewed prospective studies published since 1990, of which <35 actually compare specific treatments for clinical mastitis occurring during lactation in a manner that can be used to guide therapeutic decisions (Table 3&4). The pool of evidence is further reduced because availability of antimicrobials varies among countries and registration and labeling requirements have not been standardized. An informal survey of veterinarians located in several countries indicated that the most popular IMM treatments vary among countries without any supporting evidence that treatments used in one region are superior to treatments used in other regions (Table 5). The U.S., Food and Drug Administration (FDA) requires combination products to demonstrate synergistic activity between active ingredients and perhaps as a consequence, none have been approved for use as mastitis treatments. In other countries, combination products are commonly used for treatment of mastitis. As a result of these regional differences, practitioners seeking evidence to improve mastitis therapy may prefer research that was performed in their country but that requirement further reduces available evidence. In the mastitis treatment literature search (Tables 3 & 4), the distribution of regions of studies retrieved was 34% (USA), 44% Europe, 10% Asia and 12% other countries (mostly New Zealand). Of studies conducted in the U.S., none had been published for at least 5 years.

The use of scientific evidence for improving treatment decisions is further complicated because of complex issues related to designing and performing clinical trials for evaluation of mastitis treatment. Due to the active nature of dairy farms, animals are often lost from studies due to failure to follow protocols or by exclusion of bacteriologically negative cases from statistical analysis. For example, both Serieys et al., (2005) and McDougall et al., (2007b) enrolled sufficiently large numbers of animals in their studies but ended up using only about 60% of the cases in statistical analyses because farmers changed therapy or failed to collect the appropriate data. Interpretation of

results is further complicated because treatments are usually administered before etiology is determined and some antimicrobials will not be effective against some pathogens. For example, Serieys et al., (2005) conducted a study using antimicrobials that were expected to be active only against Gram positive organisms but about 25% of the pathogens recovered from enrolled cases were Gram negative. Similarly, Hoe and Ruegg (2005) evaluated outcomes of mastitis cases which received IMM pirlimycin after farm personnel detected the cases but before microbiological analysis was performed. Of 133 enrolled cases, only 75 (56%) cases were caused by Gram positive pathogens that would be expected to respond to therapy using pirlimycin. In both instances the authors appropriately interpreted the positive outcomes from those cases as apparent spontaneous cures rather than results of treatment.

Finding appropriate evidence is also limited because of the difficulty in identifying trials that have been appropriately designed to meet statistical assumptions. Most mastitis trials are performed using natural exposure to pathogens occurring on commercial dairy herds. Dairy farmers may not be willing to withhold treatment from cows so few randomized clinical trials for bovine clinical mastitis include non treated animals (a negative control group). Of 32 prospective trials found in the literature search (Table 3 & 4), only 9 included a negative control group and only six included more than 200 animals. Studies that lack a negative control group often conclude with statements that the "new treatment" is equivalent to an existing treatment. However the ability to demonstrate equivalence is dependent on inclusion of sufficient numbers of animals in the comparison groups and a priori determination of the maximum difference permitted for determination of equivalence (Schukken and Deluyker, 1995). Of reviewed studies, only 2 (McDougall, et al., 2007a; McDougall et al., 2007b) included a statement that the study was designed to detect equivalence and a description of the statistical reasoning behind the sample size calculation. Most statistical analyses are designed to minimize the probability that the experiment will find a difference when none actually exists (Type I error – usually set at 5% in statistical analysis). The determination that no difference exists when in reality there is a difference (Type II error) is usually considered less egregious but that outcome can be quite misleading. For example, if the expected bacteriological cure rate of an existing mastitis treatment was 60% then at least 238 cows would have to complete the study (119 cows per treatment group) to determine if the "new treatment" was equivalent to the "existing treatment" (Figure 1). The failure to include enough animals would result in a finding of no difference between the old and new treatment, even if the cure rate of the new treatment was less than the old treatment. In general, when a negative control group is not used, at least 120 to 300 cases are required to meet statistical requirements to determine equivalence (Figure 1).

Determining Outcomes of Mastitis Therapy

The use of EBVM for improving mastitis therapy is also difficult because metrics used to determine success of mastitis therapy are not standardized. For most farmers, the practical goal of treatment is to rapidly produce a reduction in clinical symptoms, eventually reduce SCC, prevent recurrence of additional clinical cases and maintain expected milk yield. Interpretation of treatment outcomes can be confusing because most cases of mastitis caused by environmental pathogens present with mild or moderate clinical signs (Table 2). Clinical signs will normally abate for the majority of cows within about 4-6 days with or without treatment, but disappearance of clinical signs does not always indicate that the quarter has been successfully cured. While the milk appears normal, many of these cases may have simply regressed to a subclinical state. This occurrence is especially true for Gram positive pathogens.

Bacteriological cure rates are generally used in research studies to assess treatment efficacy but very few farmers or veterinarians evaluate bacterial clearance of pathogen from an affected gland. The ability to achieve a bacteriological cure depends on the pathogen type, case severity, variation in immune response among cows, efficacy of the treatment protocol and the promptness of initiating treatment (Hillerton and Berry, 2003). Laboratory issues can also influence the probability of recovering bacteria from milk samples. Issues such as the frequency of sampling, the volume of milk that is inoculated, the time period after therapy until sampling and time between collection of consecutive samples all contribute to the wide variation in bacteriological cure rates noted in the literature (Ruegg and Reinemann, 2002). Therefore, bacteriological cures should be reviewed critically in both research and clinical settings before therapeutic success can be confirmed.

Useful Evidence for Improving Mastitis Therapy

While research comparing specific IMM treatments is very limited and there is an urgent need for appropriately designed randomized clinical trials of treatments used for bovine mastitis, the existing studies do contain information that practitioners can use to make better mastitis treatment decisions.

Cow Factors Influencing Treatment Outcomes. The relationship between incidence of intramammary infection caused by environmental pathogens and parity (or age) of cattle has been well known for at least 25 years (Smith et al., 1985). Older cattle have a greater risk of both subclinical and clinical mastitis and several studies have indicated that older cattle have poorer responses to treatment as compared to younger cattle. Deluyker et al., (1999) used a rigorous definition of clinical cure (normal milk by 5 d and no relapse within 3 weeks post-treatment) and

reported a reduction in combined "clinical & bacteriological cure rates" from 39% (lactation 1) to 26-30% for older cattle. Sol et al., (2000), McDougall et al, (2007a&b) and Pyorala et al., (1998) all reported that bacteriological cure after mastitis therapy were less for older cows. Age has also been associated with reduced clinical responses to therapy. Hektoen et al., (2004) measured responses to treatment by comparing scores for both acute and chronic symptoms obtained before treatment and at various periods post-treatment. While parity was not associated with differences in acute symptoms of clinical mastitis, the reduction in chronic symptoms (changes in the milk, gland or inflammatory response) were markedly greater in first lactation as compared to older cattle. The effect of parity should be considered by practitioners before initiating mastitis treatments. For example, when IMM compounds are approved for extended duration therapy, veterinarians may want to consider using use longer duration of treatment for cases occurring in older cows. Likewise, older cows (>3 lactation) may not be considered as good candidates for withholding -treatment if that option is used for treating some types of mastitis on particular farms.

Differences Among Pathogens. While it is difficult to incorporate microbiological examination of milk samples in all situations, it is well known that mastitis is caused by a diverse group of bacteria (Table 1) and the probability of cure is highly influenced by the characteristics of the pathogen. While some cases occasionally experience spontaneous cure, therapeutic cure rates for several mastitis pathogens (yeasts, pseudomonas, mycoplasma, prototheca etc.) are essentially zero, regardless of treatment. Combining data from 2 equally efficacious treatments, McDougall et al., (2007) noted the following typical differences among pathogens in bacteriological cure after treatment: Strep uberis (89%, n = 488 cases); Strep dysgalactiae (69%, n = 32 cases), Staph aureus (33%, n = 40 cases), and CNS (85%, n = 71). On farms that have controlled contagious mastitis, approximately 25-40% of clinical cases are microbiologically negative before treatment (Table 1). Clinical and spontaneous cure rates for these "no-growth" samples are often very high with or without treatment (Guterbock et al., 1993, Morin et al., 1998). For example, Hektoen et al., (2004) noted that both acute symptoms and long term responses were significantly improved for mastitis cases which were microbiologically negative as compared to cases from which Staph aureus or other bacteria were isolated. In contrast, mastitis caused by environmental Streptococci typically respond well to IMM antimicrobial therapy but have a low spontaneous cure rate and high rate of recurrence when antimicrobials are not administered (Morin et al., 1998). These differences among pathogen demonstrate that identification of pathogen considerably improves mastitis treatment protocols. With current laboratory methods, it is not feasible for all farms to achieve a microbiological diagnosis before beginning therapy but guiding treatment by use of on-farm culture systems has been shown to be economically beneficial (Lago, et al., 2005, Lago et al., 2008). Even

if a diagnosis is not immediately available, farmers can submit milk samples to laboratories for rapid provisional diagnosis and then readjust therapy when the pathogen is diagnosed 24-48 hours after beginning treatment. In the future, it is likely that rapid methods will become available to guide treatments and consistent and accurate identification of pathogens before initiating therapy should result in improved therapeutic responses.

Treatment of mastitis caused by Staphylococcus aureus. As compared to other mastitis pathogens, there is a much larger body of evidence upon which to base treatment decisions for Staph aureus. Expectations for spontaneous bacteriological cure of subclinical and clinical mastitis caused by Staph aureus are essentially zero (Oliver et al., 2004, Zhen et al, 2009). Most of the evidence agrees that treatment of clinical mastitis caused by chronic infections with Staph aureus is not rewarding and many of these cows will have periodic episodes of mild or moderate clinical mastitis. It is not considered cost-effective to treat clinical mastitis in cows that are chronically infected with Staph aureus because cure rates are typically <35% and in most instances, when the clinical symptoms disappear, the infection has simply returned to a subclinical state. Effective cure of cows infected with Staph aureus have been shown to be strongly related to duration of subclinical infection. In one study, bacteriological cure rates for chronic (> 4-weeks duration) Staph aureus infections were only 35% compared to 70% for newly acquired (< 2-weeks duration) infections (Owens, et al., 1997). Treatment protocols designed for farms where Staph aureus infections are common should not prescribe the use of antimicrobial to treat mild clinical cases occurring in chronically infected cows. In these instances it is more cost effective to simply isolate the cow or affected quarter, discard the milk until it returns to normal and then make a decision about culling or retaining and isolating the cow. An excellent review of factors influencing therapeutic success of mastitis caused by Staphylococcus aureus notes that treatment outcomes can be influenced by cow factors (age, duration of infection, SCC, etc.), pathogen factors (different strains, inherent resistance to penicillin as indicated by presence of β-lactamase) and treatment factors (duration or therapy) (Barkema, et al., 2006). Cure rates for subclinical mastitis caused by Staph aureus have been shown to decrease with age (from 81 % for cows ≤48 months of age to 55% for cows ≥96 months), the number of infected quarters (from 73% for 1 infected quarter to 56% for 4 infected quarters) and increasing SCC (Sol et al., 1997). Similar results have been demonstrated for clinical mastitis and bacteriological cure rates have been shown to be significantly greater if the pathogen is β-lactamase negative as compared to positive. The use of extended duration therapy has been shown to increase cure of clinical mastitis caused by Staph aureus and at least 5 days of therapy is recommended (Pyorala et al., 1998, Sol et al., 2000). Extended duration IMM treatment of clinical cases of Staph aureus may be successful for young cows, in early

lactation with recent single quarter infections but should not be attempted for chronically infected cows.

Duration of Therapy. Discarded milk is the greatest proportion of expense associated with treatment of clinical mastitis. In general, duration of antibiotic treatment is kept as short as possible to minimize the economic losses associated with milk discard. The appropriate duration of antibiotic treatment for clinical mastitis has not been well-defined and varies depending on the causative pathogen. There is considerable evidence that extended administration of antibiotics increases cure rates for pathogens that have the ability to invade secretory tissue (Staph aureus and some environmental Streps). For example, bacteriological cure for subclinical mastitis caused by Staph aureus treated with IMM ceftiofur were 0 % (no treatment), 7% (2 days), 17% (5 days) and 36% (8 days) (Oliver et al., 2004). Cure rates reported for clinical mastitis caused by β-lactamase negative Staph aureus were significantly greater when extended duration therapy was used (50%) versus administration of 3 treatments over 36 hours (38%) (Sol et al. 2000). Likewise, bacteriological cure rates for experimentally induced Strep uberis infections increased from 58% (2-d treatment) to 69-80% for treatments of 5 or 8 days (Oliver et al., 2003). Therefore, for mastitis caused by potentially invasive pathogens, the duration of therapy should be 5 to 8 days. However, research to support the use of extended duration therapy to treat pathogens that infect superficial tissues (for example coagulase negative staphylococci or most E. coli) has not been published and the use of extended duration therapy to treat these pathogens probably increases costs without improving treatment outcomes.

Use of Oxytocin and Frequent Milking. Frequent milking (FM) with or without administration of oxytocin is commonly recommended as an ancillary or primary treatment for clinical mastitis. In recent years, several studies have been conducted to evaluate this practice either alone or in combination with antimicrobial therapy. One researcher experimentally induced E coli mastitis in 8 cows and compared responses to 8 cows enrolled as controls (Leininger et al., 2003). Cows were divided into 4 groups of 4 cows each: 1. induced E coli mastitis, treated with FM & oxytocin, 2. induced E coli mastitis but no treatment, 3. healthy cows with FM & Oxytocin, or 4. healthy cows with no treatment. In cows that developed E coli mastitis (n = 8) the use of FM and oxytocin did not significantly affect SCC response, time to bacteriological cure, time to systemic cure or time required for milk to return to normal appearance. In another small study, Roberson et al., (2004) compared outcomes after dividing cases into 4 groups: 1. use of FM & oxytocin (n = 19 cases), 2. FM and IMM amoxicillin (n = 22), 3. IMM amoxicillin (n = 22) or 4. no treatment (n = 19). Enrolled cases included mastitis caused by environmental streptococci, E coli, Klebsiella and “no

growth." Clinical cure was defined as recovery of normal milk without relapse by 36 days after treatment. Clinical cures were 64% (no treatment), 57% (IMM amoxicillin), 25% (FM), and 52% (FM plus IMM amoxicillin). Bacteriological cures were 55% (no treatment), 67% (IMM amoxicillin), 49% (FM), and 53% (FM plus IMM amoxicillin). While the study lacked statistical power, there was no indication that the use of FM improved neither bacteriological cures nor clinical cures. Recently, the addition of two extra daily milkings (4x/day) was compared to twice daily milking for cows that received IMM treatment for mild or moderate cases of clinical mastitis (Kromker et al., 2010). The researchers enrolled 93 cows from a commercial dairy herd. The addition of two extra milkings had no effect on clinical cure or milk yield after treatment. Of enrolled cows (n = 93), approximately 32% of both treatment groups had normal milk, SCC <100,000 cells/ml and bacteriological cures at the end of the observation period. The use of FM seems logical and many veterinarians have been taught to recommend this practice in veterinary school. However, while all 3 studies lack sufficient sample size, all have failed to identify positive outcomes associated with FM and therefore this practice is not supported by available evidence.

Parenteral Treatment of Acute Coliform Mastitis. Use of IMM antibiotics to treat animals experiencing coliform mastitis has been questioned because of the high rate of spontaneous cure and because many antimicrobials have limited activity against Gram-negative organisms (Jones, et al., 1990, Pyörälä, et al. 1994, Roberson et al., 2004). However, the use of parenteral antimicrobial therapy for treatment of acute severe coliform mastitis is often recommended. Erskine et al., (2002) compared survival of cows with acute severe clinical mastitis that received supportive and IMM therapy to survival of cows that received supportive and IMM therapy combined with parenteral treatment using ceftiofur. While a general treatment effect was not noted, cows with mastitis caused by coliform organisms that did not receive systemic ceftiofur were more likely to be culled or die (37%) as compared to cows that received that treatment (14%). A similar study was conducted for cows experiencing mild and moderate cases of coliform mastitis (Wenz et al., 2005). Animals in this study received IMM treatment alone (one of two separate products) or IMM treatment combined with systemic ceftiofur. No significant differences were noted in culling, loss of quarter, bacteriological cure or recurrence of mastitis. More recently, outcomes after administration of systemic danofloxacin were compared to outcomes experienced by a non-treated control group in cows that had acute induced mastitis caused by E coli (Poutrel et al., 2008). The use of systemic danofloxacin improved elimination of E coli and resulted in better clinical outcomes (reduced body temperature, improved appetite etc.). In summary, current research evidence appears to support the use of parenteral antimicrobial treatment to improve survival and

clinical outcomes of cows experiencing severe coliform mastitis but the routine use of systemic therapy is not recommended for mild or moderate cases.

Evidence Regarding the Use of Alternative Treatments for Clinical Mastitis. In the U.S., cows used for production of organic milk may not receive any antimicrobials (Ruegg, 2008) and producers use a variety of herbal and homeopathic remedies for treatment of mastitis (Pol and Ruegg, 2007). Many alternative therapies have some theoretical basis for efficacy but there are almost no peer reviewed studies that demonstrate clinical efficacy. One recent review of veterinary usage of botanical and herbal remedies 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). One small, randomized, controlled clinical trial performed to evaluate treatment of subclinical IMM infections using several alternative therapies reported no significant effects of treatment on either bacteriological cure or SCC (Tikofsky and Zadoks, 2005). While theoretical basis for efficacy may exist no credible evidence has been published that demonstrates effectiveness of herbal compounds currently used as alternatives to antimicrobials.

Homeopathic remedies were first introduced in Germany in the era before microorganisms were identified and a few articles have specifically evaluated veterinary homeopathy. Of 3 published studies investigating the effect of homeopathic nosodes on mastitis outcomes, none have demonstrate efficacy (Egan, 1998; Hektoen et al., 2004; Holmes et al., 2005). Evidence that demonstrates efficacy of veterinary homeopathy is completely lacking and practitioners seeking to apply concepts of EBVM will not be able to support the use of these products.

Conclusion

While it is difficult to acquire solid research evidence, the application of concepts of EBVM to mastitis therapy has the potential to improve treatment protocols and result in better therapeutic outcomes. Research evidence is available to help guide treatment decisions and to better select animals that will benefit from specific treatments. Publication of results from well designed randomized clinical trials evaluating mastitis treatments can help practitioners make more informed treatment decisions.

References

Bar D, YT Grohn, G Bennett, RN Gonzalez, JA Hertl, HF Schulte, LW Tauer, FL Welcome, YH Schukken. 2007. Effect of repeated episodes of generic clinical mastitis on milk yield in dairy cows. J Dairy Sci 90:4643-4653.

Barkema, HW, YH Schukken, RN Zadoks. 2006. The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine Staphylococcus aureus mastitis. J Dairy Sci 89:1877-1895.

Cao LT, JQ Wu, F Xie, SH Hu, Y Mo. 2007. Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. J Dairy Sci 90:3980-3985.

Cockcroft, P.D. and M. A. Holmes. 2004. Evidence-based veterinary medicine. 2. Identifying information needs and finding the evidence? In Pract. 26:96-102.

Cockcroft, P. D. and M. A. Holmes. 2009. Handbook of evidence-based veterinary medicine. Oxford, UK; Malden, MA: Blackwell Publishing,

Dascanio JJ, GD Mechor, YT Grohn, DG Kenney, CA Booker, P Thompson, CI Chiffelle, JMM Musser, LD Warnick. Effect of phenylbutazone and flunixen meglumine on acute toxic mastitis in dairy cows. Am J Vet Res 56:1213-1218.

Deluyker HA, ST Chester, SN van Oye. 1999. A multilocation clinical trial in lactating dairy cows affected with clinical mastitis to compare the efficacy of treatment with intramammary infusions of a lincosin/neomycin combination with an ampicillin/cloxacillin combination. J Vet Pharm Ther 22:274-282.

Egan J, 1998. Homeopathic mastitis control: at study on the uptake and efficacy of products in the Republic of Ireland. Page 22-28 in Proc. Brit. Mast. Conf..

Erskine RJ, PC Bartlett, JL VanLente, CR Phipps. 2002. Efficacy of systemic Ceftiofur as a therapy for severe clinical mastitis in dairy cattle. J Dairy Sci 85:2571-2575.

Green MJ, LE Green, PJ Cripps. 1997. Comparison of fluid and flunixen meglumine therapy in combination and individually in the treatment of toxic mastitis. Vet Rec 140:149-152.

Guterbock W M, AL Van Enennaam, R J Anderson, I A Gardner, JS Cullor, CA Holmberg. 1993. Efficacy of intramammary antibiotic therapy for treatment of clinical mastitis caused by environmental pathogens. J Dairy Sci 76: 3437-3444.

Hillerton JE, EA Berry. 2003. The management and treatment of environmental streptococcal mastitis. Veterinary Clinics of N Am. Food Animal Practice 19 157–169

Hoe FGH, PL Ruegg. 2005. Relationship between antimicrobial susceptibility of clinical mastitis pathogens and treatment outcomes. J Am. Vet. Med. Assoc. 227:1461-1468.

Hoe FGH, P L Ruegg. 2006. Opinions and practices of Wisconsin dairy producers about biosecurity and animal well-being. J Dairy Sci 89:2297-2308.

Hoeben D, E Monfardini, C Burvenich, J Hamann. 2000. Treatment of acute Escherichia coli mastitis in cows with enrofloxacin: effect on clinical signs and chemiluminescence of circulating neutrophils. J Dairy Res 67:485-502.

Hohmann KJ, DA Rhoda, PL Ruegg. 2006. Evaluation of clinical mastitis therapy used on commercial dairy farms. J Dairy Sci 89(supp 1): 9.

Holmes MA, PD Cockcroft, CE Booth, MF Heath. 2005. Controlled clinical trial of the effect of a homeopathic nosode on the somatic cell counts in the milk of clinically normal dairy cows. Vet. Rec. 156:565-567.

Hektoen L, SA Odegaard, T Loken, S Larsen. 2004. Evaluation of stratification factors and score-scales in clinical trials of treatment of clinical mastitis in dairy cows. J Vet Med A 51:196-202.

Holmes M, P Cockcroft. 2004. Evidence-based veterinary medicine. 1. Why is it important and what skills are needed? In Pract. 26:28-33.

Jones GF, GE Ward. 1990. Evaluation of systemic administration of gentamicin for treatment of coliform mastitis in cows. J Am Vet Med Assoc 197:731-735.

Klostermann K, F Crispie, J Flynn, RP Ross, C Hill, W Meaney. 2008. Intramammary infusion of a live culture of Lactococcus lactis for treatment of bovine mastitis: comparison with antibiotic treatment in field trials. J Dairy Res 75:365-373.

Kromker V, C Zinke, JH Paduch, D Klocke, A Reimann, G Eller. 2010. Evaluation of increased milking frequency as an additional treatment for cows with clinical mastitis. J Dairy Res 77:90-94.

Kutila T, L Suojala, T Lehtolainen, H Saloniemi, L Kaartinen, M Tahti, K Seppala, S Pyorala. 2004. The efficacy of bovine lactoferrin in the treatment of cows with experimentally induced Escherichia coli mastitis. J Vet Pharmacol Ther 27:197-202.

Lago A, K Leslie, R Dingwell, P Ruegg, L Timms, S. Godden. 2005. Preliminary validation of an on-farm culture system. Proceeding 45th Annual Conference National Mastitis Council, Tampa FL, Jan 22-25, pp 290-291.

Lago A, SM Godden, R Bey, P Ruegg, K Leslie, R Dingwell. 2008. Effect of Using an On-Farm Culture Based Treatment System on Antibiotic Use and Bacteriological Cure for Clinical Mastitis. Proceedings of the 47th Annual Meeting of the National Mastitis Council, New Orleans, LA, Jan 20-23, pp 164-165.

Leininger DJ, JR Roberson, F Elvinger, D Ward, RM Akers. 2003. Evaluation of frequent milkout for treatment of cows with experimentally induced Escherichia coli mastitis. J Am Vet Med Assoc 222:63-66.

Makovec JA, PL Ruegg. 2003. Characteristics of milk samples submitted for microbiological examination in Wisconsin from 1994 to 2001. J Dairy Sci 86:3466-3472.

McDougall S, DG Arthur, MA Bryan, JJ Vermunt, AM Weir. 2007a. Clinical and bacteriological response to treatment of clinical mastitis with one of three intramammary antibiotics. New Zealand Vet J 55:161-170.

McDougall S, KE Agnew, R Cursons, XX Hou, CRW Compton. 2007b. Parenteral treatment of clinical mastitis with tylosin base or penethamate hydriodide in dairy cattle. J Dairy Sci 90:779-789.

McDougall S, MA Bryan, RM Tiddy. 2009. Effect of treatment with the nonsteriodal anti-inflammatory meloxicam on milk production, somatic cell count, probability of re-treatment, and culling of dairy cows with mild clinical mastitis. J Dairy Sci 92:4421-4431.

Morin DE, RD Shanks, GC McCoy. 1998. Comparison of antibiotic administration in conjunction with supportive measures versus supportive measures alone for treatment of dairy cows with clinical mastitis. J AM Vet Med Assoc 213:676-684.

Nash DL, GW Rogers, JB Cooper, GL Hargrove, JF Keown. 2002. Relationship among severity and duration of clinical mastitis and sire transmitting abilities for somatic cell score, udder type traits, productive life, and protein yield. J Dairy Sci 85:1273-1284.

Olde Riekerink RGM, HW Barkema, DF Kelton, DT Scholl. 2008. Incidence rate of clinical mastitis on Canadian dairy farms. J Dairy Sci. 91:1366-1377.

Oliveira L. 2009. Characterization of Staphylococcus aureus isolated from clinical and subclinical cases of mastitis. M.S. Thesis. University of Wisconsin, Madison. Dept. of Dairy Science.

Oliver S, RA Almeida, BE Gillespie, SJ Ivey, H Moorehead, P Lunn, HH Dowlen, DL Johnson, KC Lamar. 2003. Efficacy of extended pirlimycin therapy for treatment of experimentally induced Streptococus uberis intramammary infections in lactating dairy cattle. Vet Ther 4:299-308.

Oliver S, RA Almeida, BE Gillespie, SJ Headrick, HH Dowlen, DL Johnson, KC Lamar, ST Chester, WM Moseley. 2004. Extended Ceftiofur therapy for treatment of experimentally-induced Streptococcus uberis mastitis in lactating dairy cattle. J Dairy Sci 87:3322-3329.

Oliver SP, BE Gillespie, SJ Headrick, H Moorehead, P Lunn, HH Dowlen, DL Johnson, KC Lamar, ST Chester, WM Moseley. 2004. Efficacy of extended Ceftiofur intramammary therapy for treatment of subclinical mastitis in lactating dairy cows. J Dairy Sci 87:2393-2400.

Owens WE, CH Ray, JL Watts, RJ Yancey. 1997. Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility test for bovine mastitis. J Dairy Sci. 80: 313-317.

Pitkala A, M Haveri, S Pyorala, V Myllys, T Honkanen-Buzalski. 2004. Bovine mastitis in Finland 2001—prevalence, distribution of bacteria, and antimicrobial resistance. J Dairy Sci., 87:2433-2441.

Pol M, PL Ruegg. 2007. Treatment practices and quantification of antimicrobial usage in conventional and organic dairy farms in Wisconsin. J Dairy Sci 90:249-261.

Poutrel B., MR Stegemann, O Roy, F Pothier, N Tilt, M Payne-Johnson. 2008. Evaluation of the efficacy of systemic danofloxacin in the treatment of induced acute Escherichia coli bovine mastitis. J Dairy Res 75:310-318.

Pyörälä S, L Kaartinen, H Kack, V Rainio. 1994. Efficacy of two therapy regimens for treatment of experimentally induced Escherichia coli mastitis in cows. J Dairy Sci 77:333-341.

Pyörälä SH, EO 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 Ass 2121:407-412.

Ramey D. 2007. A skeptical view of herbal medicine. Pages 121-135 in Veterinary Herbal Medicine. S. G. Wynn and B. J. Fougere, ed., Mosby, St. Louis, MO.

Roberson JR, LD Warnick, G Moore. 2004. Mild to moderate clinical mastitis: efficacy of intramammary amoxicillin, frequent milk-out, a combined intramammary amoxicillin, and frequent milk-out treatment versus no treatment. J Dairy Sci 87:583-592.

Ruegg PL, DJ Reinemann. Milk Quality and Mastitis Tests. 2002. Bovine Practitioner. 36:41-54.

Ruegg PL, 2009. Management of Mastitis on Organic and Conventional Dairy Farms. J Anim Sci 87:43-55.

Sackett, D. L., W. M. Rosenberg, J. A. Muir Gray, R. B. Haynes, and W. S. Richardson. 1996. Evidence based medicine: what it is and what it isn't. Brit. Med. J. 312:71-72.

Schukken YH., HA Deluyker. 1995. Design of field trials for the evaluation of antibacterial products for therapy of bovine clinical mastitis. J Vet Pharm and Ther. 18:274-283.

Serieys F, Y Raguet, L Goby, H Schmidt, G Friton. 2005. Comparative efficacy of local and systemic antibiotic treatment in lactating cows with clinical mastitis. J dairy Sci 88:93-99.

Shinozuka Y, H Hirata, I Ishibashi, Y Okawa, A kasuga, M Takagi, Y Taura. 2009. Therapeutic efficacy of mammary irrigation regimen in dairy cattle diagnosed with acute coliform mastitis. J Vet Med Sci 71:269-273.

Shipigel, NY, D Levin, M Winkler, A Saran, G Ziv, A Bottner. 1997. Efficacy of cefquinome for treatment of cows with mastitis experimentally induced using Escherichia coli. J Dairy Sci 80:318-323.

Smith KL, DA Todhunter, PS Schoenberger. 1985. Environmental mastitis: cause, prevalence, prevention. J. Dairy Sci. 68:1531-1553.

Sol J, OC Sampimon, HW Barkema, YH Schukken. 2000. Factors associated with cure after therapy of clinical mastitis caused by Staphylococcus aureus. J Dairy Sci 83:278-284.

Sol J, OC Sampimon, JJ Snoep, YH Schukken. 1997. Factors associated with bacteriological cure during lactation after therapy for subclinical mastitis caused by Staphylococcus aureus. J Dairy Sci 80:2803-2808.

Suojala L, H Simojoki K Mustonen, L Kaartinen, S Pyorala. 2010. Efficacy of enrofloxacin in the treatment of naturally occurring acute clinical Escherichia coli mastitis. J Dairy Sci 93:1960-1969.

Taponen S, A Jantunen, E Pyorala, S Pyorala. 2003. Efficacy of targeted 5-day combined parenteral and intramammary treatment of clinical mastitis caused by penicillin-susceptible or penicillin-resistant Staphylococcus aureus. Acta vet Scand 44:53-62.

Taponen S, K Dredge, B Henriksson, AM Pyyhtia, L Suojala, R Junni, K Heinonen, S Pyorala. 2003. Efficacy of intramammary treatment with procaine penicillin G vs. procaine penicillin G plus neomycin in bovine clinical mastitis caused by penicillin-susceptible, gram-positive bacteria – a double blind field study. J Vet Pharm Ther 26:193-198.

Tenhagen BA, G Koster, J Wallmann, W Heuwieser. 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J Dairy Sci 89:2542-2551.

Tikofsky LL, RN Zadoks. 2005. An alternative treatment trial for Staphylococcus aureus mastitis in organically managed dairy cattle. Pages 358-363 in Mastitis in dairy production: current knowledge and future solutions, Proc. 4th Intl. Mastitis Conf. Maastricht, The Netherlands. June 2005. H. Hogeveen ed.

Unnerstad H., A Lindberg, KP Waller, T Ekman, K Artursson, M Nilsson-Ost, B. Bengtsson. 2009. Microbial aetiology of acute clinical mastitis and agent-specific risk factors. Vet Micro. 137:90-97.

Wenz JR, GM Barrington, FB Garry, RP Dinsmore, RJ Callan. 2001. Use of systemic disease signs to assess disease severity in dairy cows with acute coliform mastitis. J Am Vet Med Assoc 218:567-572.

Wenz JR, FB Garry, JE Lombard, R Elia, D Prentice, RP Dinsmore. 2005. Short Communication: Efficacy of parenteral Ceftiofur for treatment of systemically mild clinical mastitis in dairy cattle. J Dairy Sci 88:3496-3499.

Wilson DJ, PM Sears, RN Gonzalez, BS Smith, HF Schulte, GF Bennett, HH Das, CK Johnson. 1996. Efficacy of florfenicol for treatment of clinical and subclinical bovine mastitis. Am J Vet Res 57:526-528, 1996.

Wilson DJ, YT Groh, GJ Bennett, RN Gonzalez, YH Schukken, J Spatz. 2007. Comparison of J5 vaccinates and controls for incidence, etiologic agent, clinical severity and survival in the herd following naturally occurring cases of clinical mastitis. J Dairy Sci 90:4282-4288.

Zhen YH, LJ Jin XY Li, J Guo, Z Li, BJ Zhang, R Fang, YP Xu. 2009. Efficacy of specific egg yolk immunoglobulin (IgY) to bovine mastitis caused by Staphylococcus aureus. Vet Micro 133:317-322.

Figure 1. Sample sizes *per treatment group* required to determine that a "new treatment" results in equivalent proportion of "cure" (bacteriological cure, clinical cure or other dichotomous outcome) as compared an existing treatment that results in 60% "cure" by size of difference in the outcome variable. The assumption is that the study is designed with 95% significance (5% probability of Type I error) and 80% power (20% probability of Type II error).

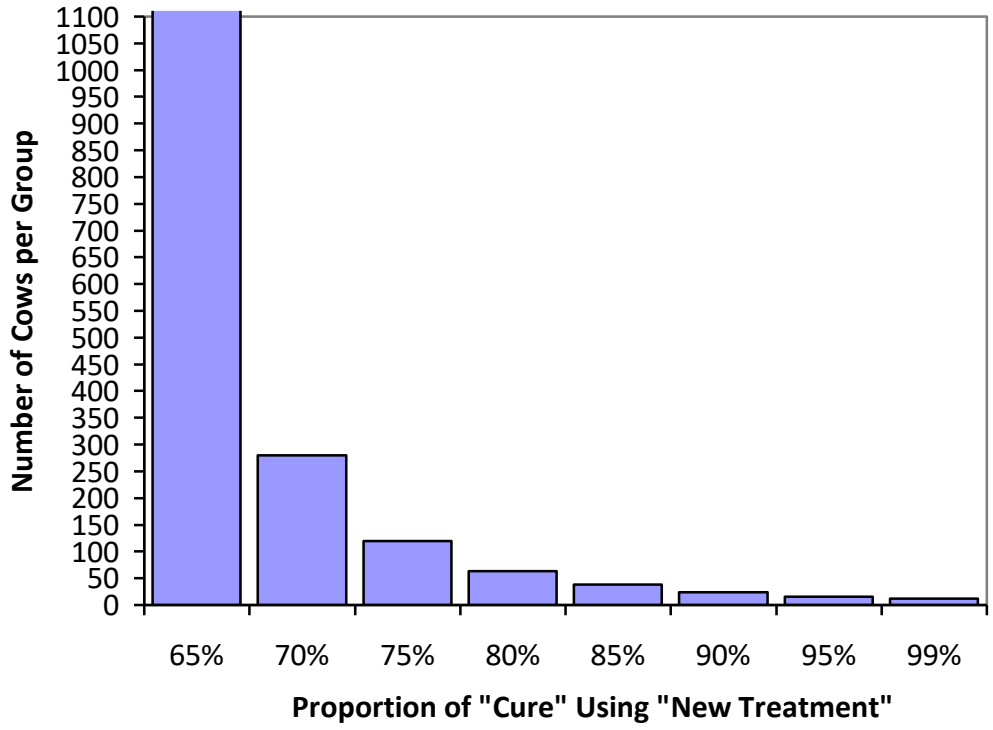

Table 1. Typical distribution of pathogens causing clinical mastitis in modern dairy herds.

Study	Cases	Strep ag[1] or Staph aureus	CNS	Env. Strep	Coliform	Other	No growth
Nash et al., 2002	686 cases in 7 herd	6%	19%	32%	17%	11%	19%
Bar et al 2007	5 herds	5%	3%	21%	40%	10%	21%
Hoe & Ruegg, 2005	217 cases in 4 herds	0%	14%	24%	25%	8%	29%
Pinzon & Ruegg, 2010	207 cases in 4 herds	2%	3%	18%	26%	9%	42%
Hohmann, 2006	1108 cases in 2 herds	0%	26%	28%	13%	6%	25%
Olde Riekerink, 2007	2850 in 106 herds	11%	6%	16%	14%	7%	46%
Kromker and other (Germany)	100 case in 1 herd	5%	3%	33%	18%	5%	36%
Tenhagen et al (Germany)	1261 cases in 10 herds	12%	24%	14%	12%	15%	23%
McDougall et al 2007 NZ	1359 quarters (single isolates)	19%	6%	44%		4%	26%
Lago et al., 2005	421 quarter cases in 8 herds	6%	10%	16%	25%	10%	32%

[1]Strep agalactiae was found only in Nash et al., 2002 & Pinzon & Ruegg, 2010 (in preparation)

Table 2. Distribution of severity scores for clinical mastitis from selected studies.

Severity Score	Clinical Symptom	Study 1[1]	Study 2[2]	Study 3[3]	Study 4[4]	Coliform cases only[5]
		N = 686	N = 169	N = 212	N = 233	N = 144
1	Abnormal milk only	75%	57%	52%	65%	48%
2	Ab.milk & abnormal udder	20%	20%	41%	27%	31%
3	Ab.milk, Ab. Udder & sick cow	5%	23%	7%	8%	22%

[1]Nash et al., 2002; [2]Oliveira, 2009; [3]Rodrigues et al., 2009; [4]Pinzon & Ruegg, 2010; [5]Wenz et al., 2001 (equivalent scoring system used)

Table 3. Most[a] results from a search of PubMed and "Google Scholar" using the terms "Bovine Mastitis Clinical Trials" only prospective trials that have occurred since 1990 were included.

Study	Citation	Site	Treatments Evaluated	Neg. Control	Sig. Dif.
1	Jones, 1990	USA	SYS[b] Gentamicin or SYS Erythromycin or IMM[c] cephaparin	No	No
2	Guterbock, 1993	USA	IMM Amoxicillin or IMM cephaparin or Oxytocin	Yes[d]	No
3	Dascanio, 1995	USA	All received antimicrobials; SYS flunixin meglumine or SYS phenylbutazone	No	No
4	Wilson, 1996	USA	IMM Florfenicol or IMM cloxacillin	No	No
5	Green, 1997	UK	All received antimicrobials; Fluids & SYS flunixin, or SYS flunixin	No	No
6	Shpigel, 1997	Israel	IMM or IM Cefquinome; IMM ampicillin/cloxacillin	No	Yes
7	Morin, 1998	USA	Supportive therapy, IMM cephapirin	Yes	Yes
8	Pyorala, 1998	Finland	SYS Penicillin; SYS spiramycin; SYS enrofloxacin;	No	Yes
9	Deluyker, 1999	Europe	IMM Lincomycin/neomycin or IMM Amp/cloxacillin	No	Yes
10	Hoeben, 2000	Belgium	SYS Enrofloxacin or control	Yes	Yes
11	Sol, 2000	Holland	IMM amp/clox; oxymopen; cefazolen; rifamycin/trimethoprim; cephalothin/colistin	No	Yes
12	Erskine, 2002	USA	SYS Ceftiofur, IMM pirlimycin	No	Yes
13	Leininger, 2003	USA	Frequent milking or control	Yes	No
14	Oliver, 2003	USA	2d, 5d or 8d IMM pirlimycin	No	Yes
15	Taponen, 2003	Finland	IMM Penicillin, IMM pen. & neomycin, all SYS penicillin	No	No
16	Taponen, 2003	Finland	SYS Penicillin.; IMM penicillin/neomycin, IMM amoxicillin/clav.., SYS spiramycin	No	Yes
17	Kutila, 2004	Finland	IMM Lactoferrin , SYS Enrofloxacin	No	No
18	Roberson, 2004	USA	IMM antibiotic, frequent milking	Yes	No
19	Hektoen, 2004	Norway	Homeopathy, placebo, IMM & SYS penicillin	Yes	No
20	Oliver, 2004	USA	2d, 5d or 8d IMM Ceftiofur	No	Yes
21	Serieys, 2005	France	SYS Penethamate, IMM clox/amp.	No	No
22	Wenz, 2005	USA	IMM Pirlimycin, IMM pirlimycin & SYS Ceftiofur, IMM Cephaparin; IMM Cephapirin & SYS Ceftiofur	No	No
23	Cao, 2007	China	IMM Nisin, IMM gentamicin	No	No
24	McDougall, 2007	New Zealand	IMM Penicillin, IMM Cefuroxime, IMM Pen. & Dihydrostreptomycin	No	No
25	McDougall, 2007	New Zealand	SYS Tylosin, SYS penethamate	No	No
26	Klostermann, 2008	Ireland	IMM Lactococcus lactis, IMM antibiotic	No	No
27	Poutrel, 2008	France	SYS Danofloxacin, No treatment	Yes	Yes
28	McDougall 2009	New Zealand	SYS penethamate (all); SYS meloxicam or no meloxicam	Yes[e]	Yes
29	Shinozuka, 2009	Japan	SYS Antibiotic, Mammary flushing, SYS Antibiotic & flushing	No	Yes
30	Zhen, 2009	China	IMM antibody, IMM penicillin no treatment	Yes	Yes
31	Kromker, 2010	Germany	IMM Cefquinome & 2x/d milking or IMM Cef. & 4x/d milking	No	No
32	Suojala, 2010	Finland	SYS Enrofloxacin & IMM penicillin.; or IMM pen; SYS ketoprofen (all)	No	No

[a]vaccine trials, trials focused just on susceptibility testing, and treatments administered to dry cows are not included; [b]Systemic; [c]intramammary; [d]11 cows were given oxytocin only; [e]control group received penethamate but not meloxicam

Table 4. Pathogens and outcome variables used in studies conducted to determine outcomes of clinical mastitis (see table 3 for description of studies)

	Number				Bacteriological Cure[c]	
Study[a]	Cows[b]	Groups	Predominant Pathogens	Primary clinical outcome	Treated Groups	Control
1	86	3	Sick cows, Gram -;80% E.coli	Clinical signs, death	ND[d]	NC[e]
2	254	3	Gram + & -; 25% no growth	Appearance of milk at 9th milking	44%, 55%	49%
3	45	2	"toxic mastitis"	Milk yield, death, symptoms	ND	NC
4	156	2	35% Staph aureus	Bacteriological cure	24%, 34%	NC
5	54	3	"toxic mastitis" 24% no growth	Clinical signs, death	ND	NC
6	47	4	Induced E.coli	Clinical signs, mastitis scores	83%, 95%, 83%, 55%	NC
7	124	2	Gram + & -; 30% no growth	Appearance of milk at 10th milking	67%	46%
8	487		Gram + & -	Clinical signs, NAGase	28-71%[f]	NC
9	232	2	35% Strep spp; 25% Staph aureus	Appearance of milk at 8-10th milking, SCC	63%, 47%	NC
10	12	2	Induced E.coli	Milk composition, clinical signs, chemiluminescence	100%	100%
11	159	5	Staph aureus	Clinical signs, SCC	50%, 46%, 67%, 56%, 57%	NC
12	104	2	"Severe mastitis"; 20% no growth	Survival, treatment failure	ND	NC
13	16	4	Induced E.coli	Clinical signs, serum lactalbumin, SCC	100%	100%
14	68	3	Induced Strep uberis	SCC	58%,69%,80%	NC
15	117	2	Gram + penicillin susceptible only	Clinical signs, CMT	73%, 79%	NC
16	118	2	Staph aureus	Clinical signs, NAGase	56%, 79%	NC
17	6	2	Induced E. coli	Clinical signs, SCC, yield	100%	NC
18	70	4	Gram + & -	Clinical signs, CMT, relapse	67%, 45%, 53%	55%
19	57	3	Gram + & -; 33% Staph aureus	Clinical signs, mastitis scores	ND	ND
20	23	3	Induced Strep uberis	Bacteriological cure	43%,88%, 100%	NC
21	184	2	Gram + & -	Clinical signs, SCC	54%, 46%	NC
22	144	4	Gram + & -; 69% Gram -	Survival, recurrence	27%,45%,33%,52%	NC
23	93	2	45% Strep ag; 19% Staph aureus	Clinical signs; SCC	61%, 45%	NC
24	1476	3	Gram +; 32% Strep uberis	Clinical signs, recurrence	75%, 70%, 76%	NC
25	595	2	Gram +	Clinical signs, recurrence	81%, 84%	NC
26	48	2	Gram + & -; 30% no growth	Clinical signs	28%, 36%	NC
27	23	2	Induced E.coli	Clinical signs, SCC	100%, d14	100%
28	727	2	Gram + & -; 45% Strep uberis	Clinical signs, recurrence, SCC, milk yield, culling	ND	ND
29	57	3	Acute Gram negative	Clinical signs, milk yield	ND	NC
30	36	3	Induced & natural S aureus	Appearance of milk, SCC	83%, 67%	0%
31	93	2	Gram + & -; 35% no growth	Clinical cure, milk yield	57%, 63%	NC
32	132	2	Acute E.coli	Clinical signs, survival	91%,87%	NC

[a]from Table 3; [b]number of cases may have been greater because of multiple cases in cows, a few studies include clinical and subclinical cases; [c]varying days to sample collection were used; [d]not determined in the study; [e]no non-treated group [f]cure was determined for specific pathogens and varied among pathogens

Table 5. Results of an informal survey of veterinarians[a] from several countries responding to a query of the 3-5 most popular intramammary antimicrobials (ranked within country) used to treat clinical mastitis during lactation.

Active Ingredients	>1 antibiotic	Contains Anti-inflammatory	Argentina	Brazil	Canada	Holland	Italy	Spain	USA
Amoxicillin	No	No							X
Amoxicillin clavulanic acid Prednisone	Yes	Yes	X			X		X	
Ampicillin Cloxacillin	Yes	No	X	X					
Cefalexin Kanamycin	Yes	No						X	
Cefacetrile rifaximin	Yes	No					X		
Ceftiofur	No	No		X	X				X
Cefoperazone	No	No				X		X	
Cefquinome	No	No				X	X		
Cephapirin	No	No			X				X
Cephapirin prednisolone	Yes	Yes					X		
Gentamycin	No	No		X					
Penethamate streptomycin framacetin prednisolone	Yes	Yes	X					X	
Penicillin naficillin streptomycin	Yes	No	X						
Penicillin streptomycin novobiocin polymyxin b cortisone	Yes	Yes			X				
Pirlimycin	No	No			X				X
Spiramycin neomycin flumethasone	Yes	Yes	X						
Tetracycline neomycin prednisolone	Yes	Yes		X					

[a]personal communication with Martin Pol (Argentina), Fernanda Hoe (Brazil), Greg Keefe (Canada), Otlis Sampimon (Holland), Alfonso Zecconi (Italy), Luis Miguel (Spain) and PLR (USA, author)