# Treatment of Clinical Mastitis: Historic and New Perspectives

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## Introduction

Mastitis continues to be of great importance to the US dairy industry. When diseases on dairy farms were recently evaluated according to effects on productivity, international trade, animal welfare, and zoonotic risk, mastitis ranked highest, above salmonellosis, paratuberculosis, and bovine virus diarrhea.[1] Subclinical mastitis caused by contagious mastitis pathogens still causes substantial economic loss on some US dairy farms, but it can readily be controlled by dry cow antibiotic therapy, hygiene practices in the milking parlor, and selective culling. In herds that have achieved contagious mastitis control, clinical mastitis caused by opportunistic pathogens in the environment or on teat skin is the predominant form of the disease.[2,3] Because these pathogens are ubiquitous, eradication of clinical mastitis is an unreasonable goal. Rather, dairy producers must strive to reduce the incidence of clinical mastitis by instituting hygienic practices that minimize exposure of teats to pathogens and by optimizing immune function through proper nutrition and vaccination. When clinical mastitis develops, as it does at an annual incidence rate of < 5% to > 50%,[4] veterinarians and dairy producers must make appropriate treatment decisions.

Despite decades of treating clinical mastitis, controversy still exists about when and how to treat it, particularly in herds that have controlled contagious pathogens. The predominant mastitis-causing pathogens in those herds are coliform bacteria, *Streptococcus* spp. other than *Streptococcus agalactiae* (environmental streptococci), and *Staphylococcus* spp. other than *Staphylococcus aureus*.[2,3] An ideal treatment regimen would be one that is safe, reduces pain and suffering, is efficacious (resulting in rapid clinical and bacteriological cure), causes short milk and slaughter withholding times, avoids unnecessary drug use or excessive labor, positively impacts milk production and survivability, and is economical. Almost certainly the ideal regimen would differ for mild, moderate, and severe episodes of clinical mastitis and episodes caused by different pathogens. Hundreds of different treatments for clinical mastitis are used daily on US dairy farms, but, in general, even the most common treatments have not been studied in controlled clinical trials to determine if they are better than no treatment at all. In the remainder of this paper, I will discuss some of the common treatments for clinical mastitis in herds that have controlled contagious mastitis, and suggest areas for future study.

## Role of the Immune System

A cow's health and the functional status of her immune system at the time of mastitis development influence the severity of clinical mastitis and the efficacy of treatment.[5,6] Therefore, a good nutritional program that maintains cows in proper body condition, provides adequate vitamin E, selenium, and other vitamins and minerals, and minimizes the incidence of ketosis and other periparturient diseases should be considered part of a clinical mastitis treatment program. Vaccination of cows against lipopolysaccharide core antigens reduces the severity of clinical coliform mastitis episodes and should increase the likelihood of treatment success.[7,8] These and other management practices that promote good immune function (such as avoidance of heat stress) not only assist in prevention of clinical mastitis, but also in its treatment.

## Antibiotics

The most controversial issue regarding treatment of clinical mastitis in recent years has been whether or not to use antibiotics. The controversy has been driven by concerns about the cost of antibiotic treatment (especially discarded milk), the risk of antibiotic residues in milk and meat, and the efficacy of available products for pathogens other than *Streptococcus agalactiae* or *Staphylococcus aureus*. Many producers have adopted a policy of no antibiotic use for all cases or all mild cases of clinical mastitis, or for documented or suspected cases of coliform mastitis.[9-11]

### Intramammary Antibiotics

Eight antibiotics are labelled and commercially available for intramammary (IMM) use in lactating dairy cows in the United States. They are procaine penicillin G, with or without novobiocin; amoxicillin, hetacillin, cloxacillin, cephapirin, erythromycin and pirlimycin. Two or 3 doses, 12 or 24 hours apart are recommended. The milk withholding period ranges from 36 to 96 hours. For a cow producing 30 lb of milk/milking and a milk price of $14.00/cwt, the cost of discarded milk ranges from $25.20 to $46.20. Adding the cost of the tubes ($1-$2.50 each), these costs are substantial.

The IMM products containing amoxicillin, cloxacillin, and cephapirin are labelled for mastitis caused by *Streptococcus agalactiae* and *Staphylococcus aureus*, so their use in herds that have controlled contagious pathogens constitutes extra-label use. All the remaining products are labelled for *Streptococcus dysgalactiae* and 3 are labelled for *Streptococcus uberis*. Only the hetacillin-containing product is labelled for mastitis caused by *Escherichia coli*, and none are labelled for mastitis caused by other coliform or enterococcal bacteria. Extra-label drug use results when any of these products is used at an increased frequency or duration other than is stated on the label.

Added to the cost of the drugs and discarded milk is the potential for great economic loss associated with violative residues in bulk milk, particularly when drugs are used in an extra-label manner or farms lack a good cow identification system. Use of on-farm test kits to detect residues in milk from treated cows can prolong the milk-discard time as a result of false positive reactions.[12] In summary, the substantial costs associated with IMM antibiotic therapy must be justified.

Clinical trials that fully evaluate the efficacy and cost-effectiveness of commercially-available IMM antibiotics for clinical mastitis, using non-treated cows for comparison, are not available. Therefore, we must rely on in vitro susceptibility data, results of experimental inoculation studies, results of a few field trials, and personal experience to determine the benefits of IMM antibiotics.

### Susceptibility Testing of Pathogens

Results of in vitro susceptibility testing, using the disk diffusion and/or minimum inhibitory concentration (MIC) methods, indicate that most (80%-100%) environmental streptococci are susceptible to the antibiotics in commercially available mastitis tubes, while *Enterococcus faecalis* is frequently resistant (< 50% susceptibility).[13-16] Susceptibility varies among *Staphylococcus* species.[14-16] Coliform bacteria are generally resistant to these antibiotics, with the exception of cephapirin to which there is low to moderate susceptibility.[14-17] Antibiotic resistance in vitro is likely to equate with inefficacy in vivo, but antibiotic susceptibility in vitro cannot be assumed to predict efficacy in vivo. With the exception of pirlimycin and penicillin-novobiocin, interpretation of zone size on the disk diffusion assay is based on serum concentrations achieved with dosage regimens used in human medicine.[14,18] Also, IMM antibiotics may be distributed unevenly in an inflamed gland,[19] and milk may reduce the bactericidal activity of the drug.[20] Erythromycin, penicillin, penicillin-novobiocin, and cephapirin alter neutrophil morphology or function in vitro,[21-23] and could potentially inhibit somatic cell clearance of bacteria in vivo.

### Field Trials Using Labelled IMM Antibiotics

With the exception of pirlimycin, efficacy data for commercially available IMM antibiotics are not readily available regarding treatment of clinical mastitis. When cows with naturally occurring environmental streptococcal mastitis were treated with pirlimycin IMM, clinical and bacteriological cure rate 10 days later (70%) was higher than for cows receiving no antibiotics (32%). However, when clinical mastitis was caused by coliform bacteria, clinical and bacteriological cure rate was similar when cows received no antibiotics (80%) or pirlimycin (72%).[24]

Financial data concerning use of commercially-available mastitis tubes to treat mild clinical mastitis caused by environmental pathogens was provided by Van Eenennaam, et al.[25] Cows with mild clinical mastitis were treated with cephapirin or amoxicillin IMM at labelled dosages or with 100 units of oxytocin IM every 12 hours for 2 or 3 milkings. There was no difference in clinical cure by the 9th milking or bacteriological cure by day 21 among treatment groups for cows with environmental streptococcal or coliform infections.[26] However, oxytocin-treated cows had more relapses and additional infections, particularly due to environmental streptococci. The net result was no difference in days of discarded milk, milk yield, or time to removal from the herd when antibiotics were used to treat clinical mastitis.[25]

### Antibiotic Treatment of Environmental Streptococcal Mastitis

From the above in vitro and in vivo data, it appears that commercially available IMM antibiotics, used according to the label, are effective for treating infections caused by environmental streptococci and reducing recurrent clinical episodes. Without treatment, *Streptococcus* spp. can persist for long periods in the mammary gland (even throughout the dry period), with periodic clinical flare-ups.[27] Indeed, an economically important outbreak of *Streptococcus uberis* mastitis followed cessation of antibiotic treatment of mild clinical mastitis in 1 herd.[10] A report on the pathology of experi-

mental *Streptococcus uberis* mastitis indicated that intramammary infection incited a rapid and marked influx of neutrophils into the mammary gland, but those neutrophils did not contribute substantially to phagocytosis and bacterial removal. By 6 days post-infection, the bacteria had invaded into the subepithelium, septal tissues, lymphatics and lymph nodes, and early stages of involution and fibrosis were evident, along with focal alveolar necrosis.[28] These findings suggest early treatment of clinical mastitis caused by *Streptococcus uberis* is likely to be more successful than waiting to see if signs resolve on their own before treating. Also, rapid treatment and resolution of environmental streptococcal mastitis is likely to reduce the duration of high SCC. This will become increasingly important as the SCC limit in the United States is gradually lowered.

More research on the optimal timing of antibiotic treatment for environmental streptococcal mastitis is needed. In a recent study in Great Britain, cows were experimentally infected with *S. uberis*. Antibiotic treatment was begun when milk electrical conductivity increased, but prior to onset of clinical signs in the cows. These early-treated cows required fewer doses of antibiotic and fewer days of discarded milk than did cows that went untreated until clinical signs were apparent.[29] In the same study, oxytocin administration and stripping of infected glands prevented development of clinical mastitis in only 25% of cows, with the remainder requiring prolonged antibiotic treatment. Because commercially-available IMM antibiotics are labelled for short duration of treatment, field trials also are needed to compare the outcome (efficacy and economics) of labelled vs prolonged duration of IMM treatment for environmental streptococcal mastitis.

### Antibiotic Treatment of Coliform Mastitis

The in vitro and in vivo data presented above do not support use of commercially available IMM antibiotics at labelled dosages for treatment of coliform mastitis. However, this should not lead to the conclusion that antibiotic treatment of coliform mastitis is never beneficial or indicated. It is true that most coliform infections (experimental or natural) resolve within 7 to 14 days without antibiotic treatment, often before resolution of clinical signs.[30,31] However, in a recent study using DNA fingerprinting, 5% of cows with naturally-occurring clinical *E. coli* mastitis experienced at least one subsequent episode of clinical mastitis in the same gland caused by the same isolate; this indicates that infection persisted in the gland. In 3% of cows, identical *E. coli* isolates caused clinical mastitis in > 1 quarter at different times, suggesting that infection was spread from quarter to quarter.[32] Appropriate antibiotic therapy might have prevented these occurrences.

The concentration of coliform bacteria in the milk at the time of clinical mastitis diagnosis appears to affect the severity and outcome of the disease.[31,33] When low numbers of coliform bacteria (< 142 cfu/cm$^2$ )were isolated from milk, mastitis resolved quickly and without long-term effect on milk production when supportive treatment was administered without antibiotics.[31] However, when higher numbers of coliform bacteria were isolated, only 1/3 of cows responded similarly; the remainder experienced persistent clinical or bacterial infection, destruction of the quarter, or permanently decreased milk production. Early detection and antibiotic therapy might have minimized these consequences.

Lastly, an unknown proportion of cows with coliform mastitis develop bacteremia which requires antibiotic treatment to reduce suffering and prevent long-term sequelae. In one study, 32% of cows with severe or protracted coliform mastitis were diagnosed with bacteremia.[34] The proportion of milder coliform mastitis episodes leading to bacteremia is probably much lower.[35]

Since there appear to be several potential indications for administering antibiotics to cows with coliform mastitis, the next question is are any IMM or systemic antibiotics effective? Several studies found no benefit from antibiotic treatment, even when the antibiotics were chosen because of high in vitro susceptibility. For example, when cows with experimental *E. coli* mastitis were treated with 4 IMM infusions of gentamicin, peak milk bacteria count, duration of infection, and severity of infection were not different than in untreated control cows.[36] Florfenicol given IMM to cows with naturally occurring clinical mastitis caused by *E. coli* or *Klebsiella* sp. resulted in a bacteriological cure in only 40%-50% of cows at 28 days and was no more effective than cloxacillin.[37] Colistin sulfate given IMM at three 12-hour intervals after experimental *E. coli* mastitis did not lower milk bacteria count or endotoxin level, reduce clinical signs, or speed elimination of infection compared with cows receiving no antibiotics.[38] Similarly poor results were found using parenteral administration of gentamicin (no difference in outcome of clinical coliform mastitis compared with erythromycin treatment or no treatment)[39] or enrofloxacin (no improvement in bacteriologic cure rate compared with oxytocin administration).[40]

Despite these negative findings, some antibiotics have shown promise for treatment of coliform mastitis. Intramammary (75 mg, q 12 h, for 3 treatments) or intramuscular (1 mg/kg, q 24 h, for 2 treatments) cefquinome (a 4th-generation cephalosporin) resulted in improved clinical mastitis scores. Intramuscular administration resulted in improved return to milk production in cows with experimentally induced *E. coli* mastitis, compared with IMM administration of ampicillin and cloxacillin.[41] Also, the recovery rate (return to

≥ 75% milk production) for cows with naturally occurring clinical coliform mastitis that were treated with sulfonamide and trimethoprim was higher (89%) when the organism was susceptible to these antibiotics in vitro than when it was resistant (75%). The odds ratio for recovery of cows with susceptible bacteria was 2.75 (95% confidence interval (CI) = 1.25-5.85).[42]

Antibiotics available for parenteral use against coliform mastitis in the United States are limited. Coliform bacteria are resistant to macrolides such as erythromycin and tilmicosin. Trimethoprim-sulfa is not available for parenteral use, and enrofloxacin is banned for use in dairy cows. Other parenteral antibiotic choices, based on good distribution into the milk, would include oxytetracycline and florfenicol. Sulfonamides, penicillins, aminoglycosides and ceftiofur do not penetrate as well. The apparent lack of efficacy of aminoglycosides, their persistence in kidney tissue, and the voluntary ban on their use make them an unreasonable choice. Ceftiofur is a logical choice for treatment of bacteremia, but concentrations achieved in the milk would not be expected to inhibit coliform bacteria.[43]

In a study at the University of Illinois,[17] cows with clinical mastitis were treated with antibiotics plus supportive measures or with supportive measures alone. Antibiotic-treated cows received cephapirin IMM twice daily until 24 hours after clinical mastitis (CM) had resolved, and oxytetracycline IV once daily if systemic signs of disease were present or the mammary gland was visibly inflamed. Supportive measures included oxytocin administration and stripping of the gland (twice a day, 3 times a day, or every 3 hours depending on the severity of disease), flunixin meglumine IM every 8 hours if systemic signs of disease were present, and fluid therapy if the cow appeared dehydrated. Most episodes of mastitis were mild. Cows with clinical coliform mastitis that were treated with antibiotics had a significantly higher clinical cure rate by the 10th milking than cows not treated with antibiotics, as was true for cows with *Streptococcus* spp. mastitis. When all cases of clinical mastitis were considered, antibiotic treatment resulted in a higher bacteriological cure rate by 14 days, fewer subsequent CM events in the original glands, a tendency for fewer subsequent CM events in additional glands, and reduced severity of clinical disease. These findings suggest that cephapirin IMM to effect and parenteral administration of oxytetracycline are reasonable, albeit extra-label, choices for treatment of clinical coliform mastitis until better drug choices become available.

Further study is needed to determine which coliform mastitis episodes warrant antibiotic treatment, which drugs or drug combinations are most effective, and how frequently and how long antibiotics should be administered. For example, antibiotic treatment would probably be more beneficial for periparturient cows than cows in mid or late lactation, because *Escherichia coli* grows more rapidly in milk of periparturient cows.[44] It also is possible that agents will be identified that augment the activity of antibiotics against coliform mastitis, and that these agents will be developed into viable treatments. For example, lactoferrin has been found to greatly increase the bactericidal activity of novobiocin against *E. coli* in vitro.[45]

### Extra-label Antibiotic Usage

When antibiotics are used in an extra-label manner in the United States, Animal Medicinal Drug Use Clarification Act (AMDUCA) regulations must be followed.[46] Until more scientific data become available to assess the efficacy and cost-effectiveness of antibiotic treatment protocols for clinical coliform mastitis (or clinical mastitis in general), veterinarians and producers should monitor the efficacy of treatment protocols they establish by keeping records of clinical mastitis cases: eg, cow and gland(s) affected, date of episode, culture results (if available), treatment(s) administered, days (or weight) of discarded milk, and cost of treatment(s). A variety of recording sheets are available for data collection and analysis, and some computer software programs allow more detailed analyses, such as somatic cell count changes in treated cows.

### Making Antibiotic Treatment Decisions on the Farm

Identifying the pathogen responsible for clinical mastitis in an individual cow assists the veterinarian or producer in making the most effective treatment (or culling) decision for that cow. Identifying the predominant pathogens causing clinical mastitis in a herd assists the veterinarian in establishing routine treatment protocols and appropriate mastitis control measures. Some mastitis experts have recommended selective antibiotic treatment based on the clinical mastitis pathogen: antibiotics are administered to cows with gram-positive (streptococcal or staphylococcal) mastitis but not to cows with coliform mastitis. Unfortunately, there is no accurate way to predict the cause of mastitis without culturing the milk. Even when performed by experienced veterinarians, clinical diagnosis of coliform mastitis had 0.64 sensitivity and 0.61 specificity (overall accuracy 62%).[47] The accuracy of clinical prediction can be improved using algorithms, discriminant equations, or logistic regression models designed to distinguish gram-negative (coliform) mastitis from clinical mastitis caused by gram-positive bacteria. However, such models can be cumbersome, and if one assumes that half of all clinical mastitis cases in a herd are caused by coliform bacteria, the positive predictive values of

the published models are ≤ 0.75.[47-49] Despite poor accuracy of clinical diagnosis, it is probably the most common way treatment is determined in the field.

Milk yield and milk composition at or before the onset of clinical mastitis do not allow prediction of the causative pathogen.[50] A number of reports describe fair to excellent performance of Enzyme-linked immunosorbent assay (ELISA) or Limulus amoebate lysate-based tests that detect endotoxin in milk for differentiating coliform from other types of mastitis in the laboratory or at cow-side.[31,51,52] However, such tests are not currently used for mastitis diagnosis in the United States.

A definitive diagnosis of mastitis pathogens requires bacteriologic culture of milk. The time delay between sample collection and results, the cost of culturing, and the time/skill required to properly collect, store, and transport the samples are among the reasons that it is not performed on a routine basis. When it is performed, treatment often is initiated before culture results are available, or samples are frozen and cultured only if treatment is unsuccessful. In either case, clinical diagnosis, not culture results, is used to make the initial treatment decision. Delaying antibiotic treatment until culture results are available has been recommended, but the effect of this delay on the outcome of clinical mastitis has not been reported.

The HyMast® test is a rapid bacteriological test system that can be used at the farm or in a veterinary clinic/truck in place of, or in conjunction with, traditional bacteriologic culturing. Gram-negative (coliform) and gram-positive (streptococcal/staphylococcal) bacteria can be distinguished by the presence of growth on selective media, and growth may be observed by as early as 8 hours. Using traditional culturing as the gold standard, in one report this system identified coliform bacteria from mastitic milk with 0.60 sensitivity (similar to the sensitivity of clinical prediction) and 0.98 specificity.[53] More recently, the system was shown to be insensitive for detecting gram-positive mastitis at 12 hours and most sensitive at 36 hours (no shorter than culture). Moreover, reading at 36 hours would result in unintentional treatment of some gram-negative mastitis episodes.[54] Further, the system was unreliable for identifying specific species of gram-positive bacteria.[54]

Another frustration associated with bacteriologic culturing or the HyMast test is that milk samples from approximately 15% to 40% of cows with clinical mastitis are bacteriologically negative. Coliform bacteria probably cause a majority of these episodes in most herds,[55] but other, often untreatable, agents such as *Mycoplasma* spp. may be the cause.

Given all these difficulties, I still recommend routine antibiotic treatment for clinical mastitis. Avoidance of antibiotic treatment is clearly not indicated because of the beneficial effects of antibiotics for environmental streptococcal mastitis. Avoidance of antibiotics for coliform mastitis could potentially result in worsening of the severity and outcome. The lack of a simple, accurate on-farm diagnostic scheme for differentiating mild coliform mastitis (which may not need to be treated) from environmental streptococcal mastitis leads me to prefer routine antibiotic treatment.

### Anti-inflammatory Agents

A variety of non-steroidal (eg, aspirin, phenylbutazone, flunixin meglumine, ibuprofen, carprofen, ketoprofen) and steroidal anti-inflammatory agents have been experimentally administered to cows with clinical coliform or endotoxin-induced mastitis, and their use in the field is frequent. In general, they cause a reduction in fever and in the glandular signs of inflammation. In a recent study,[56] cows with naturally occurring clinical mastitis given ketoprofen + systemic antibiotics were more likely to return to ≥ 75% of previous milk production than cows given antibiotics only. No non-steroidal anti-inflammatory drugs are labelled for use in dairy cows in the United States. Recommended milk and slaughter withholding times for those commonly used in an extra-label manner are published.[57] Dexamethasone and isoflupredone acetate are labelled for use in lactating dairy cows, and should be considered as alternatives to non-steroidal anti-inflammatory drugs in nonpregnant cows. The efficacy of steroidal or non-steroidal agents administered early in the course of clinical mastitis for prevention of fetal loss or estrus cycle alteration is uncertain.

### Other Treatments

Administration of oxytocin and/or frequent milkout of the gland or cow are commonly recommended treatments for clinical mastitis, but documentation of efficacy is lacking. In a small field trial, oxytocin administration and frequent milkout of the cow were detrimental to the outcome of mild clinical mastitis episodes, compared with no treatment.[58] Calcium is a logical treatment to administer (IV, SC, or PO), since hypocalcemia is documented in cows with clinical coliform mastitis, and oral administration of potassium chloride may benefit cows that are inappetant. In cows with signs of shock, treatment with isotonic or hypertonic saline solution IV is indicated.[59,60] Treatment with vitamin E is not indicated,[61] and no cytokines are available for treatment of clinical mastitis. As discussed for antibiotic therapy, extra-label use of drugs must follow AMDUCA regulations, and the efficacy of treatment protocols established on farms should be evaluated by the producer and veterinarian.

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