Evaluation of the California Mastitis Test to Determine Udder Health Status of Early Lactation Dairy Cows

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Summary

Quarter milk bacteriology results of samples collected within the first week of calving were used to calculate the test characteristics of the California Mastitis Test (CMT) that estimate the udder health status of fresh dairy cows. Over 1,200 quarters were both cultured and had a CMT performed. The overall sensitivity and specificity of the CMT was 68.8% and 71.5%, respectively. Using a cutpoint of any CMT reaction as a positive test, and examining the results by various days in milk, the highest sensitivity and specificity occurred at day four (82.4% and 80.6%, respectively). The CMT has the potential to be useful tool for monitoring udder health in fresh cows.

Introduction

The dry period is a time in the lactation cycle to eliminate existing and prevent new intramammary infections (IMI). A major goal of dry cow management programs is to have as few quarters infected with mastitis-causing pathogens as possible at the next calving. Increased interest has occurred recently in novel dry cow management strategies that would help prevent new IMI from occurring in the dry period, such as external and internal teat sealers. These new strategies offer promise in helping to reduce the rate of new IMI above what can be achieved by conventional dry cow antibiotic therapy alone. Dry cow antibiotic therapy generally does not persist into the late dry period, and is ineffective against gram-negative organisms. However, whatever dry cow udder health management program is used, new IMI are still likely to occur in the dry period. Thus, emphasis should be placed on identifying infected cows early after calving as part of mastitis control in dairy herds.

Identifying and eliminating IMI in early lactation may have significant economic benefits. Preventing clinical mastitis in early lactation, decreasing the amount of discarded milk, and reducing bulk milk somatic cell count are some of the benefits. Bacteriological culture of milk samples is the standard method for identifying subclinical IMI. However, the logistic and financial considerations involved with sampling all fresh cows have precluded this technique from being widely adopted. The California Mastitis Test (CMT) is arguably the only reliable cowside screening test for subclinical mastitis that can easily be applied.

Most recent studies evaluating the CMT have looked at identifying IMI in the first 10 days of lactation, and also describing changes in somatic cell counts (SCC) during that same time. It was determined that the optimal strategy to select infected quarters for bacteriological culture was 3 days post-calving. Similar work in the Netherlands has supported those findings. These studies also have demonstrated that individual cow SCC declines more rapidly than the previously suggested 2 weeks. Furthermore, other studies have begun to evaluate specific diagnostic and treatment protocols for early fresh cows based on the results of a CMT. It seems that the CMT has the potential to be a rapid and cost-effective cowside test for fresh cows. Thus, the purpose of this study was to evaluate the ability of the CMT to determine the udder health status of dairy cows within the first week of calving, using a large number of cows in various herds.

Materials and Methods

Research herds associated with Kansas State University, Iowa State University, the State University of New York in Cobleskill, and the University of Guelph in Ontario Canada, participated in this study for a period of just over 1 year. Data were collected from 325 cows that were all starting their second or greater lactation. Upon calving, all cows had a CMT performed on each functional quarter, and an aseptic quarter milk sample was collected immediately thereafter. The CMT and culture were performed only once on each cow, and were done as close to calving as possible. However, for any given cow, these tests may have been performed on days 1 to 7 in milk.

Milk samples were sent to the microbiology laboratory associated with each university. Each laboratory had Standard Operating Procedures in place for handling samples, culture techniques, and interpretation of results consistent with recommended procedures of the National Mastitis Council. Culture results were interpreted without prior knowledge of the infection of each cow status before the dry period. Therefore, new versus persistent dry period infections were not known. All levels of growth of coagulase-negative staphylococci and *Corynebacterium bovis* were considered minor pathogens. An IMI was defined as the presence of a major mastitis organism including: *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae,* non-speciated streptococci, *Escherichia coli*, Klebsiella, Mannheimia, *Arcanobacterium pyogenes*, Pseudomonas, Yeast, Serratia, other coliforms, Enterococcus, Proteus, and Prototheca. Contaminated samples, defined as growth of three or more pathogens from the same sample, were excluded.

The CMT was performed, results interpreted, and recorded by one trained technician in each herd. The CMT

reaction of each quarter was recorded in an ordered scale as either zero, one two, or three. A score of zero indicated no reaction and one indicated a trace reaction. Various cutpoints of the CMT scores were used to define a positive CMT in the analysis.

All data were sent to the University of Guelph and stored in a database. Calculations of diagnostic test characteristics were performed using the milk bacteriological culture result as a gold standard control. The sensitivity, specificity, and the predictive values of the CMT results, compared to culture results, were calculated using standard two-by-two contingency tables. A 95% confidence interval was calculated for the sensitivity and specificity of the CMT on various days in milk.

Results and Discussion

A total of 1,283 quarter CMT and bacteriology results were available for analysis. Overall, the prevalence of IMI in early lactation was 10% of quarters. The predominant major mastitis-causing organisms isolated were environmental streptococci spp. and *E. coli*. The proportion of specific pathogens cultured of *Strep. uberis*, *Strep. dysgalactiae*, *E. coli*, Klebsiella, *Staph. aureus*, and all other major pathogens were 10.2%, 3.9% 15.6%, 8.6%, 14.8%, and 17.9%, respectively.

The calculated sensitivity, specificity and predictive values of the CMT are shown in Table 1. When any level of a positive CMT was defined to be an IMI, the sensitivity and specificity were 68.8% and 71.5%, respectively. By increasing the cutpoint at which a CMT was considered positive, to those reactions that were greater than a score of one, the sensitivity decreased (55.0%) and the specificity increased (86.5%). Based on the prevalence of IMI in these data, and using the same cutpoint of CMT reaction of greater than one, the positive predictive value of the CMT increased, and the negative predictive value decreased (Table 1). Stratifying the sensitivity and specificity of the CMT on the day in milk in which it was performed, a general increase of the sensitivity occurred up to day 4. The highest specificity was also calculated on day 4.

The sensitivity of the CMT reflects its ability to detect an IMI. It is the proportion of quarters testing positive for the CMT in which an IMI was detected. The specificity of the CMT is its ability to detect quarters that did not have an IMI. It is calculated as the proportion of noninfected quarters that had a negative CMT. In combination, these two test characteristics describe how well the CMT can discriminate between infected and noninfected quarters. The predictive values of the CMT reflect the way that the test results are used in the field. The positive predictive value indicates the likelihood that a quarter with a positive CMT is indeed infected. Conversely, the negative predictive value indicates the likelihood that a quarter with a negative CMT is not infected. It is not appropriate to select a test based on predictive values, as the predictive value of any test is influenced both by the sensitivity and specificity, but also by the prevalence of disease in a population. For example, the predictive value of a CMT at a given sensitivity/specificity would be different in a herd with a very low number of new IMI at calving compared to a herd with many cows with infected quarters.

As a screening test to detect IMI in early lactating cows, a high sensitivity would be ideal. This would enable the CMT to detect the majority of quarters that had an IMI, and then these quarters could be sampled further for bacteriology. However, it is also desirable to limit the amount of false-positive reactions by having a high specificity to the CMT, or else the test would be no better than sampling all fresh cows anyway. With a calculated sensitivity and specificity of 82% and 80%, respectively, it would appear that the CMT may be a useful test to use on the fourth day of lactation. This finding is consistent with previous reports. With a relatively high and consistent negative predictive value, when a CMT was scored as having no reaction, there was a high likelihood that no IMI was present. An important consideration of this study is that all CMT reactions were scored by technicians in research dairy herds. The performance of the CMT may be very different when used on commercial dairy farms.

A valuable addition to a fresh cow program would be a rapid, cost-effective cow-side test that would identify IMI. Identifying and treating IMI before the milk becomes colostrum-free and saleable, and before the cow reaches high production, could have great economic benefits for the herd. Furthermore, identifying infected quarters and cows for further attention or to enroll them in the herd's udder health protocol immediately following calving would be beneficial to decrease the overall herd bulk milk somatic cell count. The results of the current study confirm conclusions made from other trials that the CMT has the potential to be a rapid and accurate test for fresh cows. A definite need exists for a cow-side test that would identify those specific pathogens in CMT positive quarters.

Table 1.   **Calculated Test Characteristics of the California Mastitis Test within the First Week of Calving to Determine the Udder Health Status of Dairy Cows. Bacteriological culture made from the same 1,283 quarters were used as the gold standard control.**

CMT cutpoint[1]	No. of quarters	Prevalence[2] (%)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value	Negative predictive value
> Zero	1283	10.0	68.8 (60-76)	71.5 (70-72)	21.1	95.4
> One	1283		55.0 (47-63)	86.5 (85-87)	31.4	94.6
> Zero						
Days in milk						
1	311	9.6	73.3 (55-87)	66.5 (64-68)	23.2	94.8
2	348	8.6	76.7 (58-89)	70.8 (69-72)	19.8	96.8
3	244	7.4	77.8 (53-93)	62.4 (60-64)	14.1	97.2
4	115	15.6	82.4 (58-95)	80.6 (76-83)	32.6	96.3
5	100	7	71.0 (31-95)	79.6 (76-81)	20.8	97.4

[1]CMT scored as: zero (no reaction), one (trace reaction), two, or three.
[2]Prevalence of intramammary infection defined as growth of a major mastitis-causing pathogen.