tant source of S. aureus for heifers. It is also apparent that contact with adult animals increases the risk of heifers calving with an IMI due to S. aureus. Application of teat dip to the udder of heifers prior to calving was associated with a decreased risk of calving with an infection caused by environmental pathogens. The significance of this observation is not clear. It is possible that application of teat dip prior to calving was a surrogate measure of a farmer's attention to detail. An individual willing to spend time dipping teats may also maintain a cleaner calving environment.

In this retrospective study, there was no good measure of environmental cleanliness and animal density, factors known to contribute to environmental pathogen IMI in lactating cows. Therefore, it was not possible to assess the contribution of these factors to the risk of heifers calving with IMI due to environmental organisms. Further investigation may be warranted to determine whether prepartum teat dipping of heifers is an effective way of minimizing IMI due to environmental pathogens.

# Use of a Cow-side Antibiotic Susceptibility Test to Predict Gram Staining Characteristics of Clinical Mastitis Pathogens of Dairy Cattle

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#### Introduction

Gram-staining characteristics of mastitis pathogens are an important consideration when developing mastitis treatment protocols. Rapid identification of clinical episodes due to gram-negative pathogens could reduce the use of intramammary mastitis (IMM) treatments with poor efficacy, while ensuring treatment of environmental streptococci infections. The objective of this study was to determine if the MASTiK<sup>®</sup> cow-side antibiotic susceptibility test could be used to predict whether a clinical mastitis episode was caused by a gram-negative pathogen, based on the susceptibility pattern obtained from the test, with the assumption that gram-negative clinical mastitis would not be treated with intramammary antibiotics.

#### **Materials and Methods**

Two milk samples were collected from the affected quarter of cows with clinical mastitis. One sample was submitted to a diagnostic laboratory for bacterial culture and the other was tested with the MASTiK<sup>®</sup> test. Briefly, 1ml of the sample was added to a 3ml reagent vial (sterile milk and pH indicator) and incubated

at 36°C (96.8°F) for 3 hours. From the preincubated sample, 50 µl were added to each of 32 wells in the test plate and incubated at 36°C (96.8° F) for 4-8 hr or until the positive control well turned positive (up to 24 hrs). MASTiK<sup>®</sup> determines susceptibility to erythromycin, oxacillin, ampicillin, penicillin, cephalothin, pirlimycin, oxytetracycline and sulfadimethoxine at varying concentrations. Wells were scored based on color change: purple indicated no bacterial growth (sensitive to the antibiotic at the given concentration), yellow indicated bacterial growth (resistant to the antibiotic at the given concentration) or intermediate. Samples were grouped based on bacteriologic culture results from the diagnostic lab as: gram-negative (GN), gram-positive (GP), mixed growth being a gram-negative and environmental streptococci (MX) or no growth (NG). Total number of resistant (yellow) wells was tallied for each sample. The sensitivity and specificity of the test for properly identifying a GN infection (GN and NG) was determined using various percent-resistant wells cutoffs. Samples with a high percentage of resistant wells were more likely to be GN, as 5 of 8 antibiotics tested have a poor GN spectrum.

## **Results and Conclusions**

A total of 77 milk samples were evaluated. Based on diagnostic lab culture results, 33 (43%), 19 (25%), 13 (17%) and 12 (15%) were GN, GP, MX and NG, respectively. The percentage of resistant wells (total wells =30/sample) for all samples in a group was 79%, GN; 33%, GP; 61%, MX and 36% NG. Assuming an infection identified as GN would not be treated, calling an infection GN when it is truly GP or MX should be avoided as studies have shown untoward effects of not treating environmental streptococci infections.<sup>1-4</sup> Thus a desirable percent-resistant wells cutoff would be one with 100% specificity. A cutoff of 94% or greater resistant wells was required to avoid misclassifying a MX infection as GN and would identify 11% of GN infections in this study. A deficiency of the test used in this manner is its inability to identify MX infections. With MX infections, it is likely that either the GN or GP organism predominates and is represented on the MASTiK® test based on relative bacterial numbers in the sample. When MX infections are excluded, a cutoff of 74% or greater resistant wells had a sensitivity of 68% and specificity of 100% for identifying a GN infection. The MASTiK<sup>®</sup> test may be more sensitive for identifying infection, as a 1ml sample is used, compared to 10-100 µl used in routine bacteriologic culture of milk. Thus 12 NG samples from the lab had growth on the MASTiK<sup>®</sup>. Many NG samples are presumed to be GN infections, however, the resistance patterns of NG samples were more similar to GP than GN in this study.

The MASTiK<sup>®</sup> test can provide useful information to make treatment decisions for individual mastitis cases,<sup>3</sup> however, the presence of MX infections may preclude its use for reliably identifying GN infections that would not receive intramammary antibiotics.

## Acknowledgements

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# Effect of Estradiol Cypionate in Postparturient Dairy Cattle at Increased Risk for Metritis

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#### Introduction

Metritis is a post-parturient uterine disease that affects fertility, milk production and health of dairy cattle. To reduce the effects of metritis, some dairymen have adopted fresh cow programs that often include the administration of 4 mg estradiol cypionate (ECP) to postparturient cows with retained fetal membranes. Estradiol is reported to have positive effects on uterine immune function. This study was conducted in order to determine if the administration of 4 mg of ECP to cows at high risk for metritis is efficacious in decreasing metritis in the first 10 days postpartum.

#### **Materials and Methods**

A clinical trial utilizing cows experiencing hypocalcemia, retained fetal membranes, dystocia, stillbirth, or twins was conducted in one California dairy. Animals were assigned into treatment (4 mg ECP) or control (2 ml vegetable oil) groups at calving. Metritis was classified as mild (fever never reached  $103.5^{\circ}$ F) or severe (fever  $\geq 103.5^{\circ}$ F). Cows with severe metritis were treated with 30 ml Excenel® once daily for a minimum of three days. Logistic regression was used to assess the effect of ECP treatment on the occurrence of metritis, controlling for confounders.