Validation of the Minnesota Easy Culture System II: Results from On-farm Bi-plate Culture versus Standard Laboratory Culture

A. Lago, LV[1]; **S. Godden,** *DVM, DVSc*[1]; **Rus Bey,** *PhD*[2], **K. Leslie,** *DVM, MSc*[2]; **R. Dingwell,** *DVM, DVSc*[2]; **P. Ruegg,** *DVM, MPVM*[3]
[1]*College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108*
[2]*Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada*
[3]*Department of Dairy Science, University of Wisconsin, Madison, WI 53706*

Introduction

Despite continued progress in mastitis control research, mastitis remains the most costly infectious disease, and the most frequent cause of antibacterial use, on commercial dairy farms. As such, research should continue on the development and validation of new management tools that will help reduce the health and economic impact of this disease, while at the same time promoting the judicious and strategic use of antimicrobials on dairy farms. Accordingly, there is increasing adoption of on-farm culture systems for selective treatment of clinical mastitis cases. Similarly, there may be an opportunity for using on-farm culture systems for the diagnosis and selective treatment of subclinical intramammary infections in fresh cows.

A multi-site, multi-herd, three-year controlled field study has been designed to validate the efficacy and quantify the cost-benefit of incorporating on-farm culture systems into both clinical and subclinical mastitis monitoring and treatment programs. This manuscript outlines the methodology followed and presents the preliminary results of one of the objectives of the project, which is validation of an on-farm culture system (Minnesota Easy Culture System II).

Materials and Methods

Farm personnel have been trained to aseptically collect milk samples from clinical mastitis quarters upon detection and from fresh cow quarters that test positive using the Californian Mastitis Test (CMT) within the first three days after calving. The fresh samples are then plated, on farm, by dipping a sterile cotton swab into the milk sample, plating it onto one-half of the on-farm culture media bi-plate, redipping the swab, and applying to the other half (Minnesota Easy Culture System II). Milk samples are then placed in the farm freezer to be transported to the laboratory. Plates are placed in an incubator overnight, and the next morning the plate is read and interpreted according to the culture system guidelines. Results are recorded as "no growth" when bacteria does not grow in either half of the bi-plate or "gram-positive" or "gram-negative", depending if growth is on the Factor or the MacConkey media half of the bi-plate respectively. A sample is considered contaminated when bacteria grow in both halves of the bi-plate. If the result is "no growth" the plate is returned to the incubator and re-read approximately 24 hours later. With "no growth" or "gram-negative" culture results, the quarter does not undergo intramammary treatment. However, with "gram-positive" culture result, intramammary therapy with cephapirin sodium (Cefa-Lak®, Fort Dodge, IA) is initiated according to the product label.

Frozen samples are cultured using identification procedures recommended by the National Mastitis Council (NMC, 1999) and standardized among university laboratories at all participating sites.

In order to describe the ability of the on-farm bi-plate culture results to differentiate a gram-positive infection (positive test result) versus a gram-negative infection or no growth (negative test result), the sensitivity, specificity and predictive values of the bi-plate results as compared to the in-laboratory culture—our gold standard—were calculated. Test characteristics were determined separately for clinical and subclinical mastitis samples. Preliminary analysis did not include cultures that were classified as contaminated on-farm or samples from which more than one bacterial isolate was isolated in the laboratory.

Results

At this stage of the study, on-farm culture results and corresponding in-laboratory results are available from 80 quarter cases of clinical mastitis and from 87 fresh cows with CMT-positive quarters.

Clinical Mastitis Quarter Cultures

Using the on-farm culture method for clinical mastitis cases, producers were able to detect 83% of the gram-positive cases (sensitivity) and classified correctly about 90% of the gram-negative cases or cases where bacteria was not present (specificity). Consequently, 83% of the treated cases were truly gram-positive (predictive value of a positive test; PV+), and 90% of the untreated cases were truly uninfected or gram-negative (predictive value of a negative test; PV-).

CMT-Positive Fresh Cow Quarter Cultures

For fresh cow CMT-positive quarters, the sensitivity of the on-farm culture to detect gram-positive quarters was 88%, and the specificity was 70%. Accordingly, 80% of the treated cases were truly gram-positive (PV+), and 81% of the untreated cases were truly uninfected or gram-negative (PV-).

Significance

Predictive values of the on-farm bi-plate versus the laboratory standard procedures are moderately high. However, the test will not be fully validated until the conclusion of the study, when the cost of missing a false negative or the cost of treating a false positive has been quantified. The bi-plate test characteristics to detect and classify bacteria growth appear not to be different between milk samples from fresh cows and clinical cases.

Relationship of Body Condition Score and Oxidant Stress on Tumor Necrosis Factor Expression in Dairy Cattle

N.J. O'Boyle, *BVSc;* **C.Corl; J.Gandy; L.M. Sordillo,** *MS, BS, PhD*
Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI

Introduction

Excessive mobilization of body fat in the dairy cow is a well-known risk factor for poor fertility, metabolic problems and increased susceptibility to a variety of infectious diseases. In humans, obese patients have an enhanced production of pro-inflammatory cytokines (such as Tumor Necrosis Factor-alpha and Interleukin-6), which has been recognized to induce a pro-inflammatory environment and facilitate oxidative damage, leading to the initiation and progression of an array of diseases. The purpose of this study was to investigate if a similar relationship exists in the dairy cow between obesity, oxidative stress, pro-inflammatory cytokines and susceptibility to disease.

Materials and Methods

Sixteen pluriparous Holstein cows in mid-lactation (150-200 DIM) were selected from a commercial herd of 3000 dairy cows based on their body condition score (BCS). Eight were selected as normal BCS (2.5-2.7) and the remainder were considered obese with a BCS of >3.5. The animals were all pregnant and balanced for milk yield (average 72 lb; 32.7 kg). They were free of any intramammary infections, lameness or concurrent disease, and this was monitored throughout the trial period. Markers of oxidative and metabolic status were measured, including non-fatty acids (NEFA), total lipid hydroperoxides, antioxidant potential of mononuclear cells, ratio of reduced to oxidised glutathione (GSH/GSSG), glutathione peroxidase activity, thioredoxin reductase activity (TrxR), LPS stimulation of whole blood and TNF-alpha levels.

Results

Obese cows had a significantly lower level of NEFAs compared to normal cows. High-BCS cows also showed indicators of oxidant stress (lower TrxR and GSH/GSSG), as well as elevated TNF-alpha levels.

Significance

Cows with a high BCS are more sensitive to oxidative stress, consistent with reports in human medicine