been recommended for use in recently fresh cows. This study examined the use of the CMT for selecting quarters in fresh cows for further bacteriological examination to identify the presence of intramammary infection. Cut-points for defining a positive CMT test and length of time post-calving were evaluated.

Materials and Methods

The study group consisted of 81 cows calving at the Kansas State University research dairy herd, and 50 cows calving at the University of Guelph dairy research herds. Quarter-milk samples were collected for standard bacteriological culture on days 1 and 3 post-calving. A positive quarter was defined as one with a bacterial mastitis pathogen present at either day 1 or day 3 post-calving. CMTs were performed cow side on each quarter at the morning milking on days 1 through 10 post-calving. Quarters were scored as negative, 1, 2, or 3, per manufacturer's recommendations. The sensitivities (specificities) of CMT for identifying positive (negative) quarters were calculated for different CMT cut-points and on different days post-calving.

Results and Conclusions

Intramammary infections were present in 36% of quarters. Quarters with intramammary infection had a higher mean CMT score throughout the first 10 days post-calving. The sensitivity of CMT for identifying positive quarters was highest when a positive CMT was defined as a score of 1 or greater. Using this criterion, a maximum sensitivity of 56.5% was found when CMT testing was performed on the third day post-calving (specificity = 56.1%). However, on day 3 post-calving the sensitivity of CMT for identifying major pathogens was 73.5% and specificity was 54.2%. Sensitivities of the CMT on day 3 post-calving for identifying quarters infected with certain pathogens were: *Escherichia coli* – 50%, *Klebsiella*-80%, *Staphylococcus aureus* – 60%, and environmental *Streptococci* – 84%. Thus, CMT used on the third day post-calving can be a useful aid for selecting quarters for milk bacteriological testing, and should be considered as one component of a fresh cow monitoring program.

Use of Eosin Methylene Blue Agar to Differentiate *Escherichia coli* from other Gram-negative Mastitis Pathogens

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Introduction

Mastitis is a continuous concern for dairy producers in the US because of its economic consequences. Coliform mastitis accounts for 20% to 80% of acute clinical mastitis cases and includes gram-negative pathogens *Escherichia coli*, *Klebsiella* and *Enterobacter* species. Rapid identification of the causative organism is essential to implement a prudent treatment plan. *Escherichia coli* can be rapidly identified with eosin methylene blue (EMB) agar based on the occurrence of a green-metallic sheen that appears on the surface of the bacterial colonies. The primary goal of this study was to evaluate EMB agar for differentiation of *E. coli* from other gram-negative mastitis pathogens. The secondary goal was to determine the time to first visible sheen.

Materials and Methods

Frozen milk samples from which gram-negative bacteria had been isolated, and gram-negative bacterial isolates from milk samples, were received from eight states. Samples were grown on 5% sheep's blood agar. Isolated colonies were then plated on EMB agar. Time

from inoculation and to first visible green-metallic sheen was recorded. Isolates were identified using (API 20E) biochemical test strips.

Results and Conclusions

Isolates and milk samples totaled 129. Nine species of gram-negative bacteria were identified using biochemical test strips. Of 63 *E. coli* isolates, 61 were EMB-positive, and of 66 non-*E. coli* gram-negative isolates, 64 were EMB-negative, for an intermethod agreement of 96.9% and a K-value of 93.7%. This indicates excellent agreement, beyond chance, between identification of *E. coli* with biochemical test strips and EMB agar.

Minimum time to first visible sheen was 3.3 hours and maximum time was 10 hours, for a mean (standard deviation) of 5.7 (1.5) hours and a median of 5.2 hours.

Rapid differentiation of *E. coli* from other gram-negative mastitis pathogens is important for initiation of an appropriate treatment plan. Cows with mild to moderate *E. coli* mastitis usually self-cure within a few days without intramammary antibiotic therapy, while mild to moderate cases of *Klebsiella spp.* mastitis tend to evolve into chronic infections and may warrant intramammary antibiotic therapy. Intramammary antibiotic therapy in most cases of mild clinical mastitis can be safely delayed until bacterial culture results are obtained. Severe cases can be treated systemically with supportive therapy (fluids, anti-inflammatories, systemic antibiotics, calcium), regardless of the causative agent, until milk culture results are obtained.

Eosin methylene blue agar is a reliable, simple and rapid method to differentiate *E. coli* from other gram-negative mastitis pathogens.

Accurate Diagnosis of the *Mycobacterium paratuberculosis* Status of Cattle Herds

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Introduction

Programs to classify cattle herds by *M. paratuberculosis* infection status must satisfy buyer's concerns that cattle from tested herds are unlikely to be infected (high negative predictive value), and seller's concerns that their herds will not be erroneously, classified as infected (high positive predictive value). Veterinarians recommending these programs need confidence that a high percentage of herds will be classified correctly. Probablistic models were used to evaluate 3 diagnostic strategies that use tests of individuals to classify cattle herds by *M. paratuberculosis* infection status: 1) enzyme-linked immunosorbent assay (ELISA) serology; 2) culture of feces; 3) ELISA serology in series with confirmatory culture of feces.

Materials and Methods

Sensitivities of the tests for individuals were empirically estimated to be, 25% for ELISA serology, 40% for culture of feces, and 20% for ELISA serology in series with culture of feces. Specificities were 99% for ELISA serology, 99.99% for culture of feces and 99.99% for ELISA serology in series with feces culture. One reactor was used to classify the herd as infected. Herd-level sensitivity and specificity was calculated for each herd-testing strategy, assuming sampling without replacement (hypergeometric probability distribution). The outcomes used to evaluate the herd-testing strategies were: 1) predictive value of a positive herd classification, 2) predictive value of a negative herd classification, and 3) percent of herds classified correctly. Each outcome was calculated for ranges of sample size (40-400), expected within-herd prevalence (1-10%), prevalence of infected herds (5-50%), and herd size (50-3200).

Results and Conclusions

The models predicted that, by ELISA serology alone, false-positive herd classifications would prevent