Isolation of Mycoplasma from Cows' Milk: Four-Laboratory Comparison

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Introduction

Mycoplasma species that cause mastitis in dairy cattle are often extremely contagious. In epidemics of mastitis caused by *M. bovis*, rapid and accurate diagnosis is essential for controlling the spread of this organism from cow to cow. An epidemic may be extremely costly to the producer if appropriate sampling and culture protocols are not promptly implemented. Unfortunately, few procedures for isolation of microorganisms are standardized across veterinary diagnostic laboratories. Mycoplasma culture techniques can vary greatly between laboratories. This study compared Mycoplasma culture results at four different laboratories in order to assess whether sensitivity and specificity were variable between laboratories.

Materials and Methods

Four laboratories were selected for this study based on ease of sample submission and willingness to cooperate. Techniques and media used for culture of Mycoplasma varied between laboratories. Samples collected (n=209) included mastitic milk of individual cows, pen samples, and bulk-tank samples. Samples were assigned a numeric code, split four ways, and frozen for overnight delivery to each of the four participating laboratories. Results were reported as positive, negative, or not readable (NR), indicating gross contamination of the sample. A true positive sample was defined as one reported positive by two or more laboratories. A true negative sample was defined as one reported negative by three or more laboratories. Sensitivity for each laboratory was determined by the proportion of true positives reported positive, and specificity was determined by the proportion of true negatives reported negative.

Results and Conclusions

Laboratory A correctly identified all samples, based on the above definitions of true positive and true negative (Se 100%, Sp 100%). Laboratory B identified 32/35 true positive samples as positive and 173/174 true negative samples as negative (Se 91%, Sp 99%). Laboratory C had a sensitivity of 74% and a specificity of 100%, and Laboratory D had a sensitivity of 89% and a specificity of 97%. Overall, Laboratory A provided the most accurate results. All laboratories had relatively high specificity, indicating that false positive results were unlikely. Laboratory C had a low level of sensitivity that could lead to misdiagnosis of cows as false negatives. This would be extremely costly to the producer because infected cows would not be identified and sold, but would remain in the herd as a source of infection for other cows.

When submitting samples for isolation of microorganisms, it is important to understand that all tests are not standardized among laboratories and that false negatives and false positives do occur. For this reason it is critical that practitioners and producers use common sense when reviewing test results from the laboratory. It is important to question results when they do not fit the clinical picture on the farm. At times it may be necessary to send samples to multiple laboratories if the one in question is providing results that are not consistent with those expected.