DEVELOPMENT OF A DNA AMPLIFICATION ASSAY FOR DETECTION OF MYCOPLASMA SPECIES IN BULK TANK MILK

M.M. Birchen, C.W. Maddox MS, PhD, R.L. Wallace DVM, MS

TAKE HOME MESSAGES

- Mycoplasma mastitis is a contagious and costly disease that cannot effectively be treated with antibiotics.
- Using DNA amplification to detect Mycoplasma is the quickest method of detection, and should be used to prevent Mycoplasma from being introduced to dairy herds.

Mycoplasma is an emerging and extremely contagious mastitis pathogen in the Midwest. Less than 100 bacteria can cause clinical mastitis, and one milliliter (1cc) of mastitic milk can infect more than 100,000 cows.1 Cows infected with Mycoplasma have abnormal milk classically described as sand-like clots in a tan, whey fluid.2 Mycoplasma mastitis increases somatic cell count, causes inflammation of affected quarters, and dramatically reduces milk production, but generally does not cause fever or other systemic signs of toxic mastitis.3

First discovered in New York in 1962, Mycoplasma spread to California and Florida quickly.4 Increasing herd sizes and movement of cattle has contributed to a rapid increase of Mycoplasma mastitis outbreaks in the Midwest. A Wisconsin study showed an increase in new Mycoplasma mastitis outbreaks from 2 herds in 1992 to 83 herds in 2000.¹ (figure 1)

Economically, Mycoplasma is one of the costliest mastitis pathogens. Loss of profit from reduced milk yield is approximately $450 per case.⁵ This figure does not include increased costs of culling or labor required to manage Mycoplasma outbreaks. In comparison to other mastitis pathogens, the loss per case for Staph aureus was $185, Strep. ag $390, environmental strep $140, E. coli $130, and A. pyogenes $350.⁵

Treatment of Mycoplasma is frustrating because antibiotics are nearly useless. Mycoplasmas lack cell walls, the main target for penicillin and many commonly approved antibiotics. Thus Mycoplasma is completely resistant and non-responsive to antibiotic mastitis treatments. Mycoplasmas also evade the cow's immune system by constantly changing the protein-targets on the surface of the organism.³ Thus, Mycoplasma infections become chronic. A cow that clears a case of clinical mastitis will continue to serve as a Mycoplasma reservoir to infect other cows. For this reason, separation of Mycoplasma-infected animals into a do-not-breed, do-not-treat pen
is advisable. For small farms where complete separation is difficult, culling is the most economical decision.\(^2\)

Young calves become infected by drinking *Mycoplasma*-contaminated milk.\(^4\) Conditions seen in calves infected with *Mycoplasma* include inner ear and respiratory infections, swollen joints and generalized septicemia. Heifers that were infected as calves can break with *Mycoplasma* mastitis during the stress of calving and early lactation due to a persistent infection. Pasteurization will kill *Mycoplasma*. If waste milk is fed to calves, a stringent quality-monitoring program should be used to prevent accidents that could infect an entire calf crop.

The purchase of new animals is generally how *Mycoplasma* is introduced to uninfected herds.\(^4\) Therefore it is highly recommended to test all animals prior to purchasing. Bulk tank milk is an easy and cost-effective way to test an entire herd. Traditional culture requires at least 10 days for results whereas DNA amplification (PCR) takes only a few hours. Since purchased animals are not always quarantined, quick results are necessary to prevent *Mycoplasma* epidemics. The objective of this project was to develop a rapid, yet accurate test to detect *Mycoplasma* in bulk tank milk.

**MATERIALS AND METHODS**

Single bulk tank milk samples were collected by producers, frozen, and shipped to the University of Illinois Veterinary Diagnostic Laboratory (UI VDL). One mL of milk centrifuged at high speed rinsed and centrifuged again before resuspending in *Mycoplasma* enrichment broth. Samples were incubated for 24 hours at 37 C under nine percent carbon dioxide. Samples were centrifuged, boiled for ten minutes to break cell membranes, then frozen (-20 C) for ten minutes to release DNA. Samples were centrifuged and the liquid portion was used as the template for DNA amplification. After 40 cycles of DNA amplification (the *Mycoplasma* DNA in the tube was doubled 40 times), the template was analyzed by electrophoresis (pulling of negatively charged DNA toward a positive electrode). A positive *Mycoplasma* sample would show a band of DNA at approximately 360 base pairs when viewed with ultraviolet light.

**RESULTS**

Of the 41 milk producers who sent samples, the average herd size was 160 cows, average BTSCC was 312,000, and the average RHA was 21,052 pounds.

Only one bulk tank milk sample was positive by both PCR and culture for *Mycoplasma*. The species was identified as *M. bovis*, the most common mastitis-causing *Mycoplasma* species. This assay is genus specific, detecting any *Mycoplasma* species, but does not cross react with any other microorganisms. Using this test we determined that we could detect approximately 1000 *Mycoplasma* per mL of milk. Recently two more Illinois dairies have been diagnosed with *Mycoplasma*.

**DISCUSSION**

By developing Mycoplasma PCR, UI VDL is now able to routinely test every bulk tank milk sample for *Mycoplasma*. Now, *Mycoplasma* results can be generated rapidly and inexpensively. Along with bulk tank samples, the UI VDL will continue to offer routine bacterial culture of
individual cow milk samples. For information on receiving mastitis culture kits contact Karel Earl, Veterinary CEPS/Extension, at (217) 333-2907 or kearl@cvm.uiuc.edu.

REFERENCES