Validation of an Improved Method for Detection of *Mycoplasma bovis* in Milk

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Introduction

M. bovis and several other *Mycoplasma spp* cause intramammary, respiratory and joint infections that have serious economic consequences for the dairy industry (González RN *et al*, 1994). Mycoplasma mastitis is resistant to treatment, therefore control of mycoplasma mastitis relies primarily on early detection and culling of infected animals to reduce transmission and herd prevalence. The current method of detection with culture is less than ideal because this group of fastidious organisms requires special enrichment media, CO2 supplemented incubation chambers with a positive or negative determination requiring an average of 10 days. Previously published molecular methods for the detection of *Mycoplasma spp* nucleic acids in milk samples have been demonstrated to have similar sensitivity to conventional culture methodology (Cai *et al*, 2005; Bashiruddin *et al*, 2005; McAuliffe *et al*, 2005). Such assays present several challenges beginning with the extraction of DNA from milk samples to the DNA amplification procedures which are technically complex. The goal of our research was to develop and validate a simple, efficient extraction process and couple it with a sensitive real-time PCR assay to detect *M. bovis* in bulk tank samples.

Materials and Methods

A ten-fold dilution series was made from banked *M. bovis* organisms by suspending 1 mL of the culture in 9 mL of DNA free water. Equal volumes of the diluted culture were extracted with the Ambion MagMAX AI/ND Viral Isolation kit and the Qiagen DNeasy kit following the manufacturer's recommendations. The extracted DNA was then amplified using primers modified from those identified by Baird *et al* (1999) on a 96 well plate in triplicate. The amplified DNA was detected and quantified through use of SYBR Green dye system (Applied Biosystems). An additional dilution series was constructed and tested using bulk tank milk that was negative by standard culture techniques. This dilution series was also extracted with both kits and subjected to the same real-time PCR protocol. Finally, the quality and quantity of the extracted DNA was determined by spectrophotometry and gel electrophoresis.

Results

With the same real-time PCR method, the MagMAX extraction is equal to the Qiagen extraction in the efficiency of concentrating the *M. bovis* DNA from the sample. In comparison to the Qiagen extracted material, the MagMAX extracted material had a reduced amount of substances that interfered with the PCR. This was evident in the melt and dissociation curves, and the electrophoresis of the amplicons. The new primer reduced the interference of genomic DNA from milk with the assay. We were able to positively detect *M. bovis* DNA when the test culture was diluted 10-7.

Significance

The MAGMax extraction procedure and real-time PCR have a number of advantages over the current diagnostic method of culture and over the Qiagen extraction. The MAGMax extraction and PCR protocol reduces the time for a test run from seven hours to five hours allowing a realistic test turnaround of 24 hours. The MAGMax procedure costs less and requires less laboratory equipment than the Qiagen protocol. Samples in the Qiagen protocol must be pipetted individually whereas the MAGMax protocol allows the use of a multi-channel pipettor. The MAGMax extraction and PCR has acceptable sensitivity and reliability in an experimental laboratory setting. Furthermore, it will work in a high throughput automated assay system. The next step in the validation of this new protocol will be to compare it with the "gold standard" of culture in the detection of *M. bovis* in bulk tank samples. Validation of this protocol with clinical samples will provide dairy farmers and veterinarians with a better tool to manage *M. bovis* in their herds and reduce the impact of this costly disease.